

Phytochemical Analysis and Evaluation of Antioxidant Activity of *Vitex Negundo* Seed Extract

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ABSTRACT

Objective: Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and in the present study, chief phytoconstituents of the *Vitex Negundo* seed extract were identified in order to relate their presence with bioactivities of the plant extract and the antioxidant activity of the extract was studied. **Methods:** Screening of *V. negundo* seed extract was performed for the presence of various primary and secondary metabolites using standard methods. Then the *V. negundo* seed extract was subjected to antioxidant assay using In vitro inhibition of lipid peroxidation (Ohkawa et al Method). **Results:** Phytochemical analysis of the crude extracts revealed the presence of carbohydrate, steroids, flavonoids, glycosides, alkaloids, proteins, tannins and phenolic compounds. After the *V. negundo* seed extract was subjected to antioxidant assay the IC50 value of *V. negundo* seed extract was found to be 46.75 µg/ml and that of ascorbic acid 38.42 µg/ml. **Conclusion:** It is evident from the study that *V. negundo* seed extract possesses majority of phytochemical classes of compounds and has antioxidant activity comparable to that of ascorbic acid.

Key words: nirgundi, *Vitex Negundo* seed extract, lipid peroxidation, Ohkawa et al Method, Phytochemical analysis

INTRODUCTION

Mankind has been using plants as therapeutic agent for thousands of years and continues to rely on them for health care. The medicinal value of most of the plants lie in some chemical substances that they produce as secondary metabolites which has a physiological action on the human body. These ingredients have been proved to be useful in the treatment of chronic as well as infectious diseases. Plants and plant based medicines are the basis of many of the modern pharmaceuticals we use today for various ailments.

For the present study, seeds of *Vitex Negundo* were selected. *V. negundo* belongs to family Verbenaceae which comprises of 75 genera and nearly 2500 species^{1,2}. The whole part of *V. negundo* is detected to have medicinal properties and is used for various ailments in indigenous system of medicine. Every part of this plant is valuable in medicine and various preparation of plant has been mentioned in indigenous system of medicine for various skin diseases³ and as nerve sedative⁴. Activities such as antibacterial⁵ anti-fungal⁶ anti-inflammatory, antiandrogenic⁷ etc. have been reported from the plant. They are of high value as constituents of Ayurvedic preparations such as 'Vishagarphathaila', which is widely used to treat rheumatism in India⁸. The chloroform extracts of detached seeds of *V. negundo* showed anti-inflammatory activity, mosquito repelling activity⁹ and antitumor activity¹⁰. It has hepato protective action against CCl₄ which induces liver damage, and has analgesic activity¹¹. Plants produce various antioxidant compounds to counteract reactive oxygen species (ROS) in order to

survive¹²⁻¹⁴. These ROS's are exacerbating factors in cellular injury and aging process¹⁵. Literature revealed that plant parts extracted with methanol has considerable antioxidant property¹⁶. Hence, the hydroalcoholic extract of the plants was subjected to antioxidant assay using In vitro inhibition of lipid peroxidation (Ohkawa et al Method).

MATERIAL AND METHODS

Collection

Vitex Negundo Linn. (Chaste tree) seeds were collected from the local market Borivali, Mumbai, Maharashtra in the month of June 2013. Authentication of the collected material was carried out at Blatter Herbarium St. Xavier's College, Mumbai by Dr. Rajendra D. Shinde, and its identity was confirmed to be *Vitex Negundo* Linn. (Chaste tree), family Verbenaceae, with herbarium accession no. 2888.

Processing of Nirgundi Seeds

V. negundo seeds were garbled and unwanted plant parts, was remove. Seeds were then allowed to dry at room temperature. Dried seeds were ground to powder in mixer and sieved through 20# to get seed powder.

Extraction

Extraction of seeds of *V. negundo* was carried in soxhlet extractor using ethanol and water (70:30) as solvent.

100gm of dried and powdered seeds were weighed and packed in filter paper, then extracted with solvent (500ml) at a temperature not exceeding 80°C in a soxhlet apparatus for 72 hrs. After completion of extraction the solvent was

Table 1: Preliminary Phytochemical Screening of *V.negundo* Seed Extract

| Sr. No. | Constituents | Test / Reagent | Observations | Inference |
|---------|------------------------------|------------------------------------|--------------|---------------------------------------|
| 1. | Carbohydrates | Molish's test | +ve | Carbohydrates present. |
| 2. | Proteins | Biuret test | +ve | Proteins present |
| 3. | Amino acids | Ninhydrin Test | +ve | Amino acids present |
| 4. | Fats and oil | Solubility Test | +ve | Fats and oil present |
| | | Staining Test | +ve | |
| | | Saponification Test | +ve | |
| 5. | Phytosterols | Salkowskii test | +ve | Phytosterols present |
| | | Liebermann burchard reaction | +ve | |
| 6. | Triterpenoids | Noller's test | +ve | Triterpenoids present |
| 7. | Alkaloids | Dragendorff's test | +ve | |
| | | Hager's test | +ve | Alkaloids present. |
| | | Mayer's test | +ve | |
| 8. | Glycosides | Borntrager's test | +ve | Glycoside present |
| | | Keller killani test | +ve | |
| | | Legal test | -ve | |
| | | Saponin foam test | -ve | |
| 9. | Tannins & phenolic Compounds | FeCl ₃ test | +ve | Tannins & phenolic compounds present. |
| | | Bromine water | +ve | |
| | | Dil. HNO ₃ | +ve | |
| | | Dil. KMNO ₄ | +ve | |
| 10. | Flavonoids | Shinoda test | +ve | Flavonoids present. |
| | | Lead acetate test | +ve | |
| | | Sodium hydroxide test | +ve | |
| 11. | Lignans | Phloroglucinol and conc. HCl (1:1) | +ve | Lignans present |

+ : Presence of constituent - : Absence of constituent

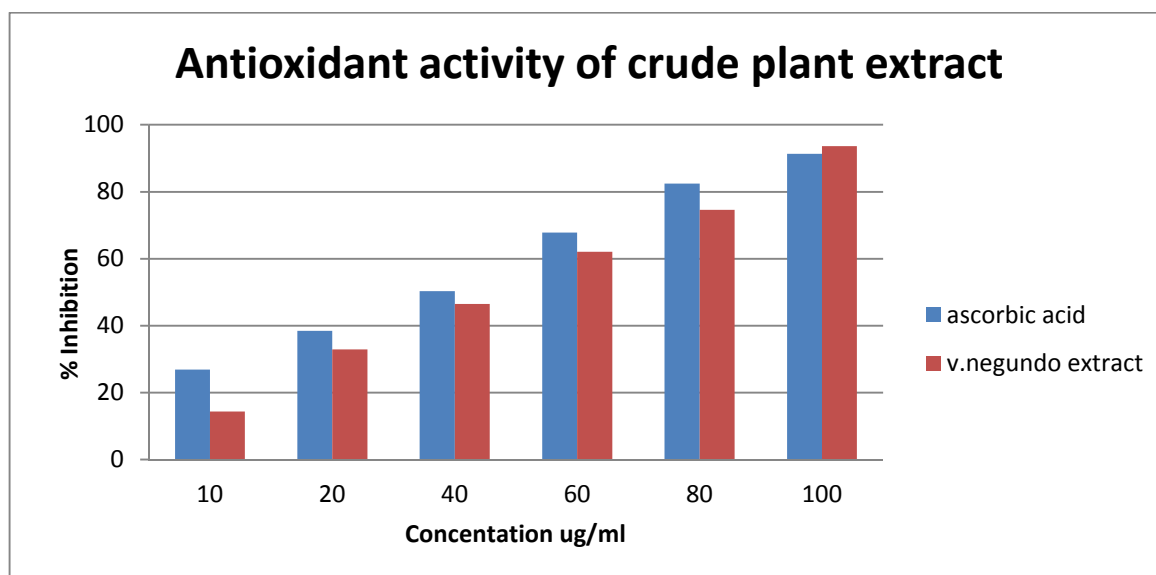


Figure 1: Results Of In Vitro Inhibition By Lipid Peroxidation Of Ascorbic Acid and *V.negundo* Seed Extract

evaporated in evaporating dish on the water bath to obtain dry extract.

Phytochemical screening

V.negundo seed extract was subjected to preliminary phytochemical evaluation by using standard procedures (Khandelwal, 2008)¹⁸.

In Vitro Inhibition Of Lipid Peroxidation (Ohkawa et al Method)¹⁷

V.negundo extract (test sample) was then reconstituted in water and used to make different concentrations between 10 and 100 µg/ml which were used in the experiment.

The rat was sacrificed using an overdose of ketamine. The liver was quickly removed and chilled in ice cold saline. After giving washings with ice cold saline, the liver was homogenized in 0.15M KCl to get 10% liver homogenate. Fresh liver homogenate (0.2 ml) was mixed with 0.15 M KCl (0.1 ml) and Tris buffer (0.4ml). The test sample (0.1

ml) was then added in various concentrations. Ascorbic acid was used as a positive control. *In vitro* lipid peroxidation was initiated by addition of 0.1 ml each of FeSO₄ (10µM) and Ascorbic acid (0.1 M). After incubation for 1hr at 37°C, reaction was terminated by addition of TBA reagent (2ml) and boiled at 95°C for 15 minutes for development of coloured complex. After cooling, the tubes were centrifuged at 4000 rpm for 10 minutes. The absorbance of supernatant was determined colorimetrically at 532 nm. Percentage inhibition of TBARS formation was calculated with respect to control in which no test sample was added. The inhibition of lipid peroxidation was determined by calculating the percent decrease in the formation of TBARS and IC₅₀ value was calculated.

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where,

A₀ is the absorbance of the blank.

A₁ is the absorbance in the presence of the extract or standard Ascorbic acid.

Readings were subjected to statistical analysis and IC₅₀ value was calculated by subjecting results to linear regression.

RESULTS AND DISCUSSION

Phytochemical analysis

The results of the phytochemical analysis of various fractions of the two plants are tabularized in to a table. (Table 1) The phytochemical analysis showed presence of carbohydrate, steroids, flavonoids, glycosides, alkaloids, proteins, tannins and phenolic compounds. The presence of flavanoids, phenolics, terpenoids, carbohydrates, steroids etc are found to be present in *V.negundo* seed extract.

Antioxidant assay

Oxidative stress is implicated in the pathophysiology of many diseases and conditions including diabetes, cardiovascular disorders, inflammatory conditions, liver diseases, cancer and ageing. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals and reactive oxygen species or by inhibiting the lipid peroxidation and thus preventing damage.

In the present study, the antioxidant potential of *V.negundo* seed extract was evaluated with the help of *in-vitro* antioxidant model like Lipid peroxidation.

V.negundo seed extract elicited concentration dependent inhibition of FeSO₄ induced lipid peroxidation in rat liver homogenate. The IC₅₀ value of *V.negundo* seed extract was found to be 46.75 µg/ml (Table: 7.1, Fig: 7.2) and that of ascorbic acid 38.42 µg/ml (Table: 7.2, Fig: 7.3). The correlation coefficient (R²) was calculated from graph and found to be 0.9837 for *V.negundo* seed extract and 0.9902 for ascorbic acid respectively.

Lipid peroxidation can be defined as the oxidative deterioration of lipids containing a number of carbon-carbon double bonds. Membrane lipids are particularly susceptible to lipid peroxidation. Since membranes form the basis of many cellular organelles like mitochondria, plasma membranes, endoplasmic reticulum, lysosomes, peroxisomes, etc., the damage caused by lipid peroxidation

has been implicated in the pathogenesis of various diseases. In the current method effectiveness of *V.negundo* seed extract as inhibitor of lipid peroxidation is based on its complex interaction during the peroxidation process. Initiation of lipid peroxidation by FeSO₄ take place through ferryl perferryl complex. The primary action is the prevention of lipid peroxy radicals (LOO·) production during initiation. *V.negundo* seed extract reduces the initiating perferryl radical with the formation of ubisemiquinone and H₂O₂. Additionally, it is possible *V.negundo* seed extract eliminates LOO· directly.

CONCLUSION

Our study revealed that the *V.negundo* seed extract exhibited antioxidant activity comparable with ascorbic acid. Hence further studies are needed to evaluate the *in vivo* antioxidant potential of this extract in various animal models.

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