

Estimation of Micronutrients in *Vitex negundo* L. (Karunochi) Leaves

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Abstract

The genus *Vitex* includes many species from tropical and also from temperate areas. Among these species, *Vitex negundo* possess analgesic, anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, antihistamine and anti-asthmatic properties. In the present study, analysis of crude fiber and estimation of certain minerals like calcium, iron, vitamin C and phosphorous were carried out on tender, mature and dry leaves of *Vitex negundo* (Karunochi). The results of the nutrient estimation indicates that dry leaves of *Vitex negundo* is an excellent source of calcium (15.214g), phosphorous (1.870 g), iron (16.48 mg), vitamin C (21.4 mg) and fiber (14.6 gm) when compared to tender and mature leaves.

Keywords: Calcium, Iron, *Karunochi*, *Nirgundi*, Phosphorous, *Vitex negundo*

1. Introduction

Vitex negundo is native to India and the Philippines [1]. It thrives in humid and on sandy soils [2]. A literature survey by Ahuja et al. [3], revealed that *Vitex* possesses insecticidal, anti-worm and antimicrobial properties. Phytochemical studies of *Vitex negundo* have afforded volatile oils, lignans, flavonoids, terpenes (triterpenes, diterpenes, sesquiterpenes) and steroids [4, 5]. Flavonoid glycosides from an ethanolic extract of the leaves of *Vitex negundo* are 5-hydroxy-3, 6, 7-trimethoxy-2-(3, 4-dimethoxyphenyl)-4H-chromen-4-one and 5, 7-dihydroxy-2-(3, 4-dihydroxyphenyl)-4H-chromen-4-one. The methanolic extract also contains, Negundoside, Agundoside, and Vitegnoside from bark of *Vitex negundo* *p*-hydroxybenzoic acid and β -sitosterol have been identified from the methanol and hexane extracts of *Vitex negundo*. Leaves of *V. negundo* are used as tonic, vermifuge and are used to treat catarrhal fever [6].

1.1 In Vivo Studies

Leaf oil of the plant has repellent action against stored product pests [7]. The chloroform extracts of *V. negundo* (40 mg/kg/body wt. and 135 mg/kg), exhibited cytotoxicity in a human cancer cell line panel [8]. The anti-hyperglycemic effect of leaves due to iridoid glycoside was comparable with glibenclamide. It has significant productive effect on glycoprotein metabolism [9]. Experiments have demonstrated that different parts of the plant especially leaves, fruits, roots and seeds possess anti-inflammatory and anti-arthritis activity [10–14].

Studies in albino rats by oral route revealed that the leaves of *Vitex negundo* were practically non-toxic but some histomorphological changes in the heart, liver and lungs [15]. The oral administration of the plant leaves have been claimed to have anti-inflammatory, analgesic, antihyperglycemic, anti-bacterial, anti-asthmatic and anti-implantation activity [16]. Prabhakar et al. [17]

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investigated hepatoprotective activity of Negundoside and Agundoside from *Vitex negundo*. Both compounds were used in combination with one or more pharmaceutical additives which prevent and treat hepatic diseases.

1.2 In Vitro Studies

An isolate from *V. negundo* (2-pentacyclic triterpenoids) exhibits antifeedant activity against the larvae of an agricultural pest (*Achoea janata*), and also possess antibacterial activity against *Bacillus subtilis* and *Escherichia coli* [5, 18]. A study by Sathiamoorthy et al. [4] showed that flavones glycoside, isolated from ethanolic extract of *V. negundo* leaves, exhibits significant antifungal activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans*.

The plant *Vitex negundo* and *Emblica officinalis* extracts significantly antagonized the Vipera russellii and Naja kaouthia venom induced lethal activity both *in vitro* and *in vivo* studies activities [16]. From the vast literature of this species it was clearly found that this plant has enormous medicinal properties. Little attention has been paid on the role of inorganic elements and potential nutrients in *Vitex negundo*. This study aims to estimate certain common micro-nutrients (Iron, Phosphorous, Vitamin C, Calcium and Fiber) which are very much essential to the human body. Abundant research work has been carried out on the organic constituents of the medicinal plants while little attention has been paid on the role of inorganic elements and potential nutrients in *Vitex negundo*. There is a dire need to bring light on the availability of proximate nutrients to the community and to create awareness on the medical values and importance of *Vitex negundo* on general health. This study aims to estimate certain common micro-nutrients (Iron, Phosphorous, Vitamin C, Calcium and Fiber) which are very much essential to the human body.

2. Materials and Methods

2.1 Selection of Samples

For this study, Karunochi leaves were obtained from the Oushadhavanam, near the foot hills of Western Ghats range, Amrita Vishwa Vidyapeetham, Amritanagar, Coimbatore, India during the year 2015. It was abundantly spread all over this region. A pilot study was conducted in and around the villages of the ghats with regard to the utilization of this species. From this study it was

observed that this plant was only used as a decongestant during severe cold and also the potential secret behind its therapeutic benefits remains dark. The samples were collected accordingly as tender, mature and the dry leaves of *Vitex negundo*. They were cleaned, washed and stored in air tight containers. The dried samples were powdered, sieved and stored in sealed polyethene covers. They were subsequently used for further nutrient analyses. Estimation of key nutrients such as vitamin C, calcium, phosphorous, iron and fiber were analyzed for all the above said samples using the standard procedures adapted from NIN Laboratory manual [19]. Triplicate determination of each analysis was made and average obtained was tabulated and the availability of the nutrients was compared for all the samples. The chemicals used for the study were of analytical grade. The photo electric colorimeter used for this study had a range from 400-700 nm with its resolution at 1% transmission and absorbance at zero.

2.2 Experimental Set - Up

2.2.1 Preparation of Test solution

Five grams of the sample was weighed in a crucible and was charred in a bunsen burner, followed by heating in a muffle furnace for about 4 hours, at 600° c. The crucible was cooled and it was weighed. The ash was dissolved in HCl and made up to 100 ml with distilled water. The test solution was further analysed for nutrients.

2.2.2 Estimation of Calcium

Calcium was determined by precipitating it as Calcium Oxalate and titrating the Oxalate solution with dilute Sulphuric acid against standard Potassium Permanganate solution by titrimetric method.

2.2.3 Estimation of Phosphorus

Phosphorous reacts with ammonium molybdate to form phosphomolybdic acid (Fiske and Subba Row method), which was reduced by the addition of ANSA to produce a blue color which was apparently a mixture of oxides of molybdenum.

2.2.4 Estimation of Iron

Ferric Iron reacts with ammonium thiocyanate or with Potassium thiocyanate to form ferric thiocyanate which is red in color (Wong's method). The color which is a measure of the concentration of iron was measured colorimetrically.

2.2.5 Estimation of Vitamin C

Ascorbic acid, a good reducing agent is oxidised to dehydro ascorbic acid and estimated by dye method.

2.2.6 Estimation of Crude Fiber

A known amount of the sample was weighed and its crude fiber content was estimated by acid alkali method.

3. Results and Discussion

Table 1 shows the results obtained for micro-nutrient elements and Crude fibre of *Vitex negundo* along with the standard values of *Moringa oleifera* [20, 21].

From the table it was noted that calcium was very high compared to all other micro-nutrients. Calcium concentration tremendously increased at a high rate as the leaves matured. Tender and mature leaves had calcium concentrations of 251.42 mg and 5267.13 mg respectively. It is surprising to note that the mature leaves of *Vitex negundo* contains higher quantity of calcium, when compared to *Moringa oleifera* (440mg) which was considered to be the richest source of calcium among the common green leafy vegetables. Similarly dried samples of *Vitex negundo* showed a higher quantity of calcium (15214 mg) and *Moringa oleifera* provides only 1997mg of calcium.

With regard to Phosphorus, It was observed that as the leaves get matured the phosphorus content also increases (Tender 185.2 mg, Mature 680.3 mg, Dried 1870.5 mg). It was observed that the phosphorus content present in *Vitex negundo* was comparatively high when compared to *Moringa oleifera*.

The fresh leaves of *Vitex negundo* provide 6.6mg of iron and the mature and dried leaves provides 9.4 and 16.48 mg of iron respectively, while *Moringa oleifera* leaves provides 0.85 and 28.28mg of iron respectively. Iron is needed for transporting oxygen and carbon dioxide, maintaining a healthy immune system and to provide

energy. It is vital for both physical health and mental well-being.

Oxidative damage and formation of carcinogens are lowered by Vitamin C. Vitamin C lowers blood pressure and decreases the risk of death and Vitamin C. It increases the absorption of iron by two to three times [22]. It was observed that the *Vitex negundo* leaves are rich in vitamin C content with 141.2mg in tender leaves and 180.9 mg in matured leaves, whereas when the leaves are dried, most of the vitamin C gets oxidized and that shows a minimal value of 21.4 mg in 100 mg of the dried sample. Figure 1-5 gives the comparison of the available proximate nutrients in *Vitex Negundo* with the reference standards (*Moringa oleifera*).

High fiber intake lowers rates of certain disorders including constipation, diverticular disease, some types of cancer, and heart disease [23]. Other potential health benefits include fiber's role in maintaining a healthy weight and glucose modulation [24]. The dried leaves of *Vitex negundo* and *Moringa oleifera* provide 14.6 and 19.9 gm of fiber per 100gm respectively. The mature leaves of both samples an equal amount of fiber.

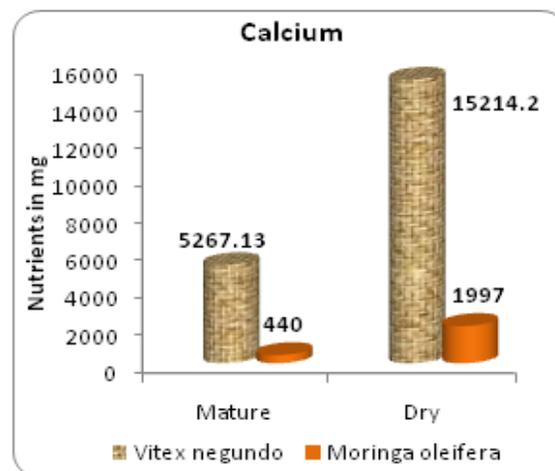


Figure 1. Comparison of Calcium in *Vitex Negundo* and *Moringa oleifera*.

Table 1. Nutrient analysis of *Vitex negundo* and *Moringa oleifera*

Nutrients/100gm	<i>Vitex negundo</i>			<i>Moringa oleifera</i>	
	Tender	Mature	Dry	Mature	Dry
Calcium (mg)	251.42	5267.13	15214.25	440	1997
Phosphorus (mg)	185.2	680.3	1870.5	70	297
Iron (mg)	6.6	9.4	16.48	0.85	28.28
Vitamin C (mg)	141.12	180.9	21.4	220	37.1
Crude Fibre (g)	0.74	0.9	14.6	0.9	19.9

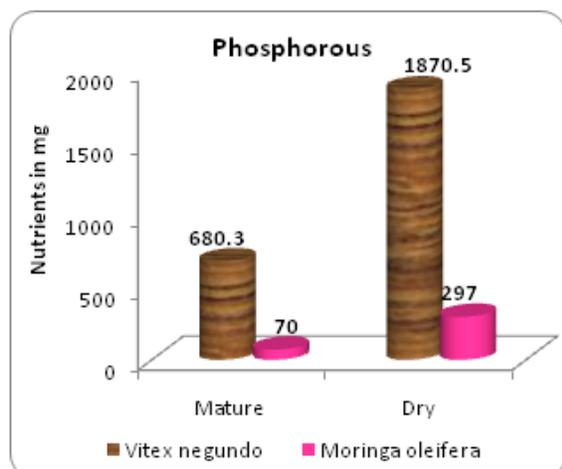


Figure 2. Comparison of Phosphorous in *Vitex negundo* and *Moringa oleifera*.

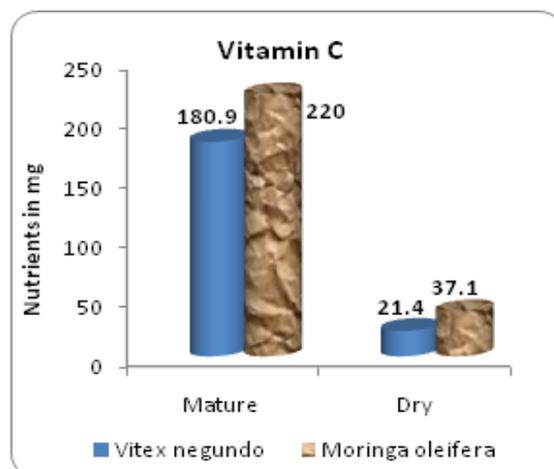


Figure 4. Comparison of Vitamin C in *Vitex negundo* and *Moringa oleifera*.

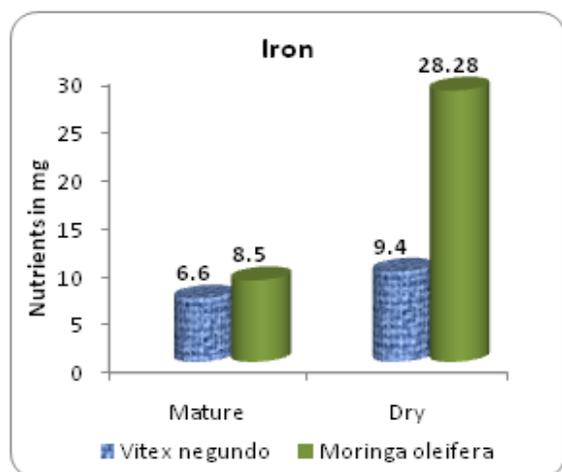


Figure 3. Comparison of Iron in *Vitex negundo* and *Moringa oleifera*.

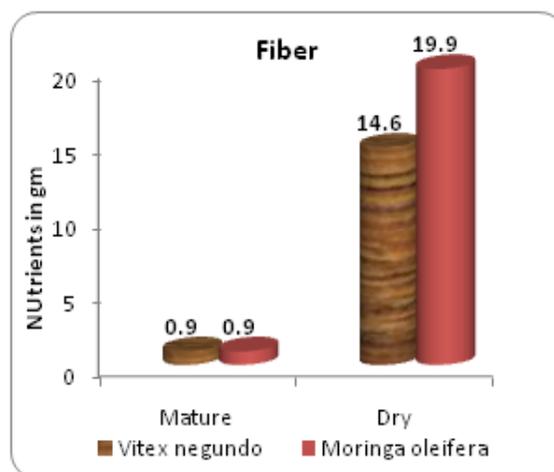


Figure 5. Comparison of Fiber in *Vitex negundo* and *Moringa oleifera*.

From the above table, it was found that all the dried samples higher nutrient density when compared to the fresh samples. The volume of fresh leaves used to dry the sample is more and the fresh leaves could not be stored for a longer duration.

4. Conclusion

Vitex negundo possess all the essential nutrients needed for the human body. Till date only the leaves and seeds were used to control pests in agriculture, but the leaves or flowers were not used for human consumption. Further studies could be recommended to analyse the other potential benefits and toxicity levels via *in vivo*,

in vitro and clinical researches and find out the possible methods to make it edible. The active compounds present in it could be isolated so as to make it available for pharmacological preparations. These studies will also promote awareness among the society on the importance of this under-utilized species to make use of it efficiently.

5. References

1. Orwa C., Mutua A., Kindt R., Jamnadass R., and Simons A., "Agro forest tree Database: a tree reference and selection guide", Version 4.0, 2009. Available: <http://www.worldagroforestry.org/af/treedb/>
2. Chowdhury N. Y., Islam W., and Khalequzzaman M., "Insecticidal activities of Nishinda (*Vitex negundo* L.,

- erbenaceae) against *Tribolium castaneum*”, *Pak Entomology*, vol. 31, p. 25–31, 2009.
3. Ahuja S. C., Uma A., and Sharma R. D., “Ayurvedic characterization of biopesticides”, *Asian Agri- History*, vol. 11, p. 103–118, 2007.
 4. Sathiamoorthy B., Gupta P., Kumar M., Chaturvedi A. K., Shukla P. K., and Maurya R., “New antifungal flavonoid glycoside from *Vitex negundo*”, *Bioorg Med Chem Lett*, vol. 17, p. 239–242, 2007.
 5. Chandramu C., Manohar R. D., Krupadanam D. G., and Dashavantha R. V., “Isolation, characterization and biological activity of betulinic acid and ursolic acid from *Vitex negundo* L”, *Phytother Res*, vol. 17, p. 129–134, 2003.
 6. Mary R. A., and Villasenor M., “Comparative anti-hyperglycemic potentials of medicinal plants”, *Journal of Ethnopharmacology*, vol. 104, p. 129–131, 2006.
 7. Gupta M., Mazumdar U. K., and Bhawal S. R., “CNS activity of *Vitex negundo* L. in mice”, *Indian J Exp Biol*. Vol. 37, p. 143–146, 1999.
 8. Diaz F., Chavez D., Lee D., Mi Q., Chai H. B., Tan G. T., Leonardus B. S. K., Riswa S., Fairchild C. R., Wild R., Farnsworth N. R., Cordell G. A., Pezzuto J. M., and Kinghorn A. D., “Cytotoxic flavones analogues of vitexicarpin, a constituent of the leaves of *Vitex negundo*”, *J Nat Prod*, vol. 66, p. 865–867, 2003.
 9. Sundaram R., Naresh R., Shanthi P., and Sachdanandam P., “Antihyperglycemic effect of iridoid glucoside, isolated from the leaves of *Vitex negundo* in streptozotocin-induced diabetic rats with special reference to glycoprotein components”, *Phytomedicine*, vol. 19, p. 211–216, 2012.
 10. Chaturvedi G. N., and Singh R. H., “Experimental studies on anti-arthritis effect of certain indigenous drugs”, *Indian J Med Res*. Vol. 53, p. 71–80, 1965.
 11. Ravishankar B., Bhaskaran N. R., and Sasikala C. K., “Pharmacology of *Vitex negundo* Linn. (Nirgundi) root”, *J Res Ayurv Siddha*, vol. 7, p. 62–77, 1986.
 12. Chawla A. S., Sharma A. K., Handa S. S., and Dhar K. L., “Chemical investigation and anti-inflammatory activity of *Vitex negundo* seeds”, *J Nat Prod*, vol. 55, p. 163–167, 1992.
 13. Tamhankar C. P., and Saraf M. N., “Anti-arthritis activity of *Vitex negundo* Linn.”, *Indian J Pharm Sci*, vol. 56, p. 158–159, 1994.
 14. Jana U., Chattopadhyay R. N., and Shaw B. P., “Preliminary studies on anti-inflammatory activity of *Zingiber officinale* Rosc, *Vitex negundo* Linn. and *Tinospora cordifolia* (Willd.) Miers. In albino rats”, *Indian J Pharmacol*, vol. 31, p. 232–233, 1999.
 15. Tandon V., and Gupta R. K., “Histomorphological changes induced by *Vitex negundo* In albino rats”, *Indian J Pharmacol*, vol. 36(3), p. 176–177, 2004.
 16. Alam M. I., and Gomes A., “Snake venom neutralization by Indian medicinal plants (*Vitex negundo* and *Emblica officinalis*) root extracts”, *Journal of Ethnopharmacology*, vol. 86, p. 75–80, 2003.
 17. Prabhakar A., Gupta B. D., Suri K. A., Satti N. K., Malhotra S., Gupta K. K., Johari R. K., Jaggi B. S., Bedi K. L., Suri O. P., and Qazi G. N., “Hepatoprotective activity of 2’-p-hydroxybenzoyl mussaenosidic acid”, Patent no. WO 2003 094946, 2003.
 18. Nagarsekar K. S., Nagarsenker M. S., and Kulkarni S. R., “Evaluation of composition and antimicrobial activity of supercritical fluid extract of leaves of *Vitex negundo*”, *Indian J Pharm Sci*, vol. 72, p. 641–643, 2010.
 19. Raghuramulu N., Nair K. M., and Kalyanasundaram S., *A Manual of Laboratory Techniques*, National Institute of Nutrition, Second Edition, Hyderabad, 2003.
 20. Janci Rani P. R., and Sarojini K. S., “Value addition to institutional diet and its effect on nutrients as well as antioxidant status in older adolescents”, [Ph.D thesis], Avinashilingam university, 2009.
 21. Nutritive value of Indian foods, *National Institute of Nutrition*, ICMR, Hyderabad, India, 2014.
 22. Walingo K. M., “Role of vitamin C (ascorbic acid) on human health-a review”, *African Journal of Food Agriculture and Nutritional Development*, vol. 5, p. 1–13, 2005.
 23. Burkitt D. P., Walker A. R., and Painter N. S., “Dietary fiber and disease”, *J Am Med Assoc*, vol. 229, p. 1068–1074, 1974.
 24. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2003–2004.