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IMPACT OF GROWING REGIONS ON PHYTOCHEMICAL PROPERTIES OF *VITEX NEGUNDO* L. IN KERALA

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Abstract

The present study was carried out to find the relation between the accumulation of phytochemical constituents in *Vitex negundo L.* leaf and the soil nutrients collected from two different localities of Kerala, such as Kollam and Pathanamthitta. Fresh aqueous extract of *V.negundo* leaves were prepared and subjected to qualitative and quantitative analysis. Soil collected from the two regions were also analysed for the presence of different elements. Quantative analysis revealed that the saponins were present in higher concentration in all the two localities and it was followed by tannins, flavanoids, glycossides, and carbohydrates. Glycossides was present in the least concentration in all the localities. The result obtained from the quantitative analysis show the leaves from Kollam had the higher concentration of all phytochemical constituents. From the result obtained from soil analysis it could be concluded that P, K, Fe, Mn might be necessary for the accumulation of saponins, tannins, carbohydrates, flavonoids, and glycossides. Since these were higher in soil collected from Kollam. All the phytochemicals showed a positive correlation with P, K, Fe, and Zn and a negative correlation with OC, B, S, Mn and Cu.

Keywords: Accumulation, Phytochemical constituents, Different localities

Introduction

Vitex negundo is commonly grown as wild variety and nowadays it is widely cultivated due to its medicinal importance . Antihelmintic, expectorant, carminative, digestive, anodyne, antiseptic, alterant, antipyretic, diuretic and emmenagogue, depurative, rejuvenating, ophthalmic, vulnerary, and tonic properties. Myriad medicinal properties havebeen ascribed to *Vitex* and the plant has also been extensively used in the treatment of a plethora of ailments. The leaves of Vitex are sed in traditional medicine for relieving headache, fever and catarrah and are also used for medicinal baths in fever and anaemia. The present study was aimed to find out the relation between soil properties and its effect on the accumulation of phytochemical constituents in *Vitex negundo* L. leaves collected from two localities such as, Kollam and Pathanamthitta. There were no evidence of earlier studies on relation between soil properties and accumulation of phytochemicals in the leaves of *Vitex negundo* L.

Materials and Methods

Study Area

Two regions located in Kollam and Pathanamthitta districts were selected and leaves and soil of these two regions were collected accordingly. The difference of the regions distinguished in terms of climatic conditions, annual rainfall and also altitude.

Sample Collection

The samples of *Vitex negundo* L. were collected from two different localities situated in Kollam and Pathanamthitta Districts of Kerala, India. The leaves were stored in polythene bags for qualitative and quantitative analysis.

Phytochemical Analysis

Different phytochemical constituents in plant such as Tannins, Flavonoids, Saponins, Carbohydrate and Glycosides were estimated qualitatively and quantitatively. For this, standard procedures were followed.

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Soil Analysis

Soil *p*H, Electrical Conductivity, Soil Organic Carbon, Phosphorus, Potassium, Sulphur, Iron, Manganese, Zinc, Copper and Boron in soil in two localities were estimated as per the standard procedure.

Results and Discussion

The present study was aimed to emphasis or highlight qualitative and quantitative analysis in *Vitex negundo* L. leaves. Soil study was also analysed to determine the relation between accumulation of secondary metabolites in plants and micro macro nutrients in the soil.

Phytochemical Analysis

Phytochemical constituents present in the plants show biological significance by playing crucial role in plants. These constituents provide the plant with anti-bacterial property, antifungal property, anti-inflammatory and anthelmintic properties. They also help plants in defense mechanism

Qualitative Analysis in *Vitex negundo*

Preliminary phytochemical analysis was conducted in *Vitex negundo* leaf collected from two different regions. Aqueous extract of each leaves collected from different localities were prepared. Leaf samples were tested for flavonoids, tannins, saponins, carbohydrates, and glycosides. Table 1 show that the presence of flavonoids, tannins, saponins, carbohydrates were present in two regions. This Table shows that the all phytochemical constituents analysed were present in the leaves of plants grown in two localities.

Quantitative Analysis of phytochemical constituents in *Vitex negundo*

Total quantification of phytochemicals such as flavonoids, tannins, glycosides, carbohydrates and saponins were estimated in methanolic extract of leaf of *V.negundo*.

Estimation of Flavonoids

The concentration of flavonoids was estimated in the two samples. Concentration of flavonoids was estimated as 35.20 µg/mg in Kollam and 93.67µg/mg in Pathanamthitta. Active presence of flavonoids was observed in Pathanamthitta and Kollam leaf sample showed minimum amount of flavonoids (Table 2). This results shows that percentage of flavanoid can vary depending on the climatic factors of the growing regions. Studies reveal that *Vitex negundo* leaf extract have anti oxidant properties which is attributed to richness in flavonoids. Flavonoids served as a toxicants to impart antibacterial and mosquito repellant property to the textiles (Manpreet Kaur *et al*., 2022). Flavonoids have been proven to display a wide range of biochemical and pharmacological actions such as anti-carcinogenic, anti-viral, anti-microbial, anti-thrombotic, anti-inflammatory, and antimutagenic activities. In addition, flavonoids can act as free radical scavengers and terminate the radical chains reaction that occurs during the oxidation of triglycerides in food system (Turkoglu *et al.*, 2007).

Estimation of Tannin

Total tannin content of the two localities was determined. It was found that tannin was higher in Kollam leaf sample about 71.57 µg/mg while the Pathanamthitta leaf sample contains about 60.72 µg/mg tannin.(Table 2). Tannins are believed to provide an astringent flavour which act as a natural line of defense mechanism as it reduces the appetite of ruminants or herbivorous animals and insects. In plants it also helps in the activation of nitrogen and in attracting pollinating animals towards flowers. Tannins act as a source of antioxidant and anticancer agent (Helen *et al*., 2015).

Estimation of Glycosides

The concentration of glycosides was determined in the two samples of *V.negundo* leaves. Concentration of glycosides was higher in Pathanamthitta leaf sample with about 48.40 µg/mg and Kollam leaf sample showed minimum amount of glycoside with about 26.57 µg/mg (Table 2). Studies reveals that leaves of *V. negundo* account for plant pigmentation, anticancer and antiinflammatory properties which

is attributed to richness in glycosides (Tasduq *et al*., 2008).

Estimation of total Carbohydrate

Total carbohydrate content was detrmined in the two samples of V.negundo leaves. Amount of carbohydrate was higher in Kollam leaf sample with about 41.14 µg/mg and Pathanamthitta leaf sample showed minimum amount of carbohydrate content with about 24.40 µg/mg (Table 2). In plants carbohydrates act as a vital source of energy and carbon skeltons for organic compounds and storage components in addition to this it act as signaling molecules in a manner similar to hormones (Koch., 2004). Sugars are considered as actors of a complex communication system necessary for the coordination of metabolism with growth, development and responses to environmental changes and stresses (Rolland *et al*., 2002).

Estimation of Saponin

Saponin concentration was estimated in the two samples. It was found that the saponin concentration was comparatively higher in Pathanamthitta leaf sample with about 250.35 µg/mg and Kollam leaf sample was found to have a minimum concentration of saponin with about 195.10 µg/mg (Table 2).

Soil Analysis

Total Suspended Solids, Organic Carbon *and p*H,

Total Suspended Solids (TSS) was recorded as 0.02 mS in Pathanamthitta and 0.04 mS in Kollam. TSS is anything that is captured by filtering the sample aliquot through a specific pore size filter. Suspended solids can range from particles of silt or sediment to pieces of plant material such as leaves or stems. Even insect larvae and eggs can fall in the general category of TSS. It measures a similar property to turbidity, but provides an actual weight of particulate matter for a given volume of sample (usually mg/l). Organic carbon was found to be higher in Pathanamthitta soil with 0.714% and lesser in Kollam soil with 0.63%. Soil organic carbon is the basis of soil fertility. It releases nutrients for plant growth, promotes the structure, biological and physical health of soil, and is a buffer against harmful substances. The *p*H was found to be acidic in all the samples. It was found that *p*H was higher in Kollam soil with 6.3 when compared to Pathanamthitta soil with 6.15. Hamilton *et al.* (2008) proposed a carbon -nutrient balance hypothesis. According to this hypothesis concentration of secondary metabolites in plant tissue is controlled by the availability of carbon and nitrogen in the environment.

Mineral Macronutrients: Phosphorus, Potassium and Sulphur

The amount of phosphorus showed significant variation among the two samples. Amount of phosphorus was found to be higher in Kollam soil which was 91.2 kg/Ha and in Pathanamthitta soil which was about 4.56 kg/Ha. Phosphorus in plants is key in capturing, storing, and converting the sun's energy into biomolecules, such as adenosine triphosphate (ATP), that drive biochemical reactions (e.g., photosynthesis) from germination through the formation of grain to maturity. Excess of phosphorus mostly interferes with uptake of other elements, such as iron, manganese and zinc. (Smart-fertilizer.com, 2022). Amount of potassium was found to be higher in Kollam soil with about 33.6 kg/Ha and Pathanamthitta soil was found to contain about 28kg/Ha. Potassium (K) is an essential nutrient that affects most of the biochemical and physiological processes that influence growth of the plant and its metabolism. It also contributes to the survival of plants exposed to various biotic and abiotic stresses (Wang *et al.*, 2013). Of all the macronutrients Sulphur was present in least quantity in the two localities. The amount of sulphur showed no variations among the two samples. Amount of sulphur was found to be 0.0252 ppm in both Kollam and Pathanamthitta soil. Sulfur in plants helps, develops, and activate certain important enzymes, promotes nodulation in legumes, and assists in the formation of plant proteins. It is also required for chlorophyll

formation.

Mineral micronutrients: Iron, Zinc, Copper, and Boron

Micronutrients, or trace elements, are found in plant tissues in smaller amounts than macronutrients. Iron, a crucial component of enzymes and pigments, is higher in soil of Pathanamthitta and lesser in Kollam soil, assisting in plant energy production. Zinc levels in Soil of Kollam are higher at 3.0827 ppm, while in soil of Pathanamthitta it is lower at 2.4471 ppm. Zinc is crucial for plant development, enzymes, and proteins. Manganese levels in soil of Kollam were higher than soil of Pathanamthittal, with a normal requirement of 4.70 mg/kg for plant growth. Manganese is essential for chloroplast formation, photosynthesis, nitrogen metabolism, and enzyme synthesis. Copper was found in the least amount among micronutrients, with slight variations between Kollam and Pathanamthitta. Chaudhari *et al.* (2012) found that copper is essential for plant growth, activating enzymes involved in lignin synthesis, photosynthesis, respiration, and carbohydrate and protein metabolism. The soil in Pathanamthitta has higher Boron levels (1.0368 ppm), essential for cell wall synthesis, cell division, reproductive growth, pollination, fruit and seed development, and less in the soil of Kollam. The study investigated the relationship between soil nutrients and plant phytochemical accumulation. It found excess saponin concentration in samples collected in Pathanamthitta, negatively correlated with TSS, Phosphorus, Potassium, Manganese, Zinc, and Copper, and higher organic carbon, Boron, Sulphur, and Iron. Flavonoid concentration in the soil of Pathanamthitta is higher than saponin, positively correlated with organic carbon, Boron, sulphur, and iron, while negatively correlated with total suspended solids. Soil analysis revealed higher glycosides in the soil of Pathanamthitta and lesser in the soil of Kollam, with positive correlations between organic carbon, Boron, Sulphur, and Iron concentrations. The soil from Kollam contains higher concentrations of TSS, phosphorus, potassium, manganese, zinc, and copper, potentially contributing to tannin accumulation in Vitex negundo leaf, while organic carbon, boron, and iron are moderate. Carbohydrates were the least phytochemical in two samples, with higher concentrations in the soil of Kollam. Higher concentrations of macronutrients like potassium and phosphorus may contribute to carbohydrate concentration. Koirala *et al* (2020) found that methanolic and hexane extracts of *V.negundo* leaves contain phytochemicals like terpenoids, polyphenols, saponins, phenolic compounds, and flavonoids, exhibiting antimicrobial, antioxidant, and antibacterial effects.

Table 1. Qualitative analysis in *Vitex negundo* leaves collected from two localities

Sl. Number	Phytochemicals	Pathanamthitta	Kollam
1	Flavonoids	+	+
2	Tannins	+	+
3	Glycosides	+	+
4	Carbohydrates	+	+
5	Saponins	+	+

Table 2. Percentage of different phytochemicals in the leaves of *V.negundo* growing in different localities

Locality	Percentage of Phytochemicals (**µg/mg**)				
	Flavonoids	**Tannin**	**Glycosides**	**Carbohydrate**	**Saponin**
Pathanamthitta	93.67	60.72	48.40	24.40	250.35
Kollam	35.20	71.57	26.57	41.40	195.10

Conclusion

The study aimed to determine the correlation between phytochemical constituents in plant leaves and micro and macronutrients in soil of Vitex negundo growing regions, Kollam, and Pathanamthitta. The study analyzed the aqueous and methanolic extracts of Vitex negundo leaf, revealing flavonoids, tannins, glycossides, carbohydrates, and saponins. The highest concentrations were found in saponins, tannins, flavanoids, carbohydrates, and glycossides, with carbohyrates present in the least concentrations. The study found significant variations in soil nutrients between Kollam and Pathanamthitta samples, with higher electrical conductivity and organic carbon, higher macronutrients (S, P, K), and higher micronutrients (B, Fe), Mn, Zn, and Cu in Kollam soil. Pathanamthitta contains higher concentrations of saponins, flavonoids, and glycosides, possibly linked to Fe and B. Kollam has lower concentrations of P, K, Mn, Zn, and Cu, while Kollam has higher concentrations of tanins and carbohydrates. Sulphur may be a deciding factor in the accumulation of phytochemical constituents in Vitex negundo leaves. The study found that samples collected from Pathanamthitta and soil nutrients like Boron and Iron are rich in phytochemicals, including saponins, tannins, flavonoids, glycosides, and carbohydrates. These phytoconstituents have various antihelminthic, anti-inflammatory, and antioxidant properties. Increasing soil nutrient concentrations can increase the accumulation of these compounds.

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