Research Article

HPLC analysis of water soluble vitamin B in *Psidium guava* leaves

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Abstract

Objective: The main objective of the present work is to analyse the water soluble B vitamins present in *Psidium guava* leaves using HPLC. **Materials and Methods**: The fresh leaves of *Psidium guajava*. L collected were shade dried, grinded to fine powder and kept in dry conditions .The plant powder was mixed with water and stirred continuously until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to analysis using HPLC. **Results**: Under the described experimental conditions, the values of retention times were: 9.067min for Niacin, 12.567min for Pyridoxine, 23.358min for Thiamine and 25.867min for Riboflavin. **Conclusion:** It is concluded that *Psidium guajava* leaves are very much important for their medicinal as well as nutritional value. The leaves provide opportunities to develop as medicines, value added products and dietary supplements which possess potential health benefits. The leaves were rich sources of valuable bioactive compounds and can be explored as nutraceuticals.

Keywords: *Psidium guava* leaves, HPLC, B vitamins

Introduction

The plant *Psidium guajava* Linn is a tropical plant grows in all the tropical and subtropical areas of the world and adapts to different climatic conditions but prefers a dry climate, which comprises of numerous medicinal values. The plant is widely available in tropics and within the reach of local populace (Akanimo et al., 2016). *Psidium guajava* L., is a small tree of about 10m high with a thin, smooth, patchy and peeling bark with it leaves opposite, short - petiolate, with a prominent pinnate veins of 5-15cm long and with showy flowers that has whitish petals of up to 2cm long and numerous stamens (Josephine et al., 2017). It belongs to family Myrtaceae and originated from South America.

In traditional medicine, it has been used as a hyperglycaemic, antioxdant, hepatoprotective, anti-allergic, antimicrobial, antigenotoxic, antiplasmodial, cyototoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive agent (Kariawasam et al., 2017). Extracts and metabolites obtained from the plant, especially, from leaves and fruits acquire pharmacological activities. The leaves of *P. guajava* L. have several medicinal values. There are proven scientific evidence on antimicrobial, antidiarrhoeal , hypoglycaemic antioxidant, anticough , hepatoprotective , analgesic and anti-inflammatory (in rats and mice) , hypotensive], anti-tumor and anti-mutagenic activities of leaves of *P. guajava* L. Different preparations of leaves had scientifically validated for their phytochemical constituents , antimicrobial properties, antioxidant properties, anti-diabetic properties, antimicrobial properties, anticough properties, hepatoprotective properties, anti-diarrhoeal properties, spermatoprotective properties, antimutagenic properties, ionotropic effect,spasmolytic effect, anti-cancer, immunomodulatory activity, treatment of acne, antiproliferative activity, antipyretic, contractile affects, hypotensive, anti-malaria, CNS activity, vaginal disorders and rheumatism (Mittal et al., 2010).

India, with 1.34 billion people, is the second most populous country in the world. Over population leads to global, financial and food crisis and it opens a path to malnutrition. During malnutrition body does not get the right amount of

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vitamins, minerals and other nutrients it needs to maintain healthy tissues and organ function (Thomas et al., 2017). There are wide varieties of plants which are naturally available in our environment; various parts of these plants have nutritional values which are still unknown to us. If it consumed properly, malnutrition could be controlled up to an extent.

Materials identified as edibles are time tested for their safety for long time use within reasonable quantities. There are foods with both nutritional and medicinal. Vitamins are also vital for developing innate immunity and adaptive immunity in the body. Most of the vitamins are applied in our body to boost-up the cell-mediated immune response with the production of cytokines and T-lymphocytes. Inadequate intake and lowered nutritional status of the B vitamins lead to suppressed immunity, which predisposes to infections and aggravates malnutrition. Therefore, the intake of appropriate amounts of all such essential vitamins in our daily diet can help in supporting the cognitive power of the body leading to wellbeing of health (Aslam et al., 2017). The B vitamins are a group of water-soluble organic compounds which, while structurally diverse, all play crucial roles in the maintenance of body functions, including proper cellular function, tissue growth and development (Kennedy, 2016). The B complex vitamins play fundamental role in the maintenance of proper nervous system functions. With evidence in the literature it may be suggested that the B complex vitamins promote nerve repair, both in acceleration of nerve tissue regeneration and recovery of nerve function by a variety of mechanisms (Jiang et al., 2017).These findings and traditional applications of *P. guajava* L., leaves made a strong incentive for analysis of vitamin B complex in leaves using HPLC.

Materials and Methods

Collection of plant material: The tender leaves of the plant *Psidium guajava* were collected and the tender leaves were dried in shade. The fresh leaves of *Psidium guajava*. L collected were shade dried, grinded to fine powder and kept in dry conditions. The plant powder was mixed with water and stirred continuously until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to analysis.

Chemicals and reagents

The stock solutions (1 mg L^{-1}) of each vitamin were prepared by dissolving each of them in water. All standard solutions working standards were stored at 4-5 °C and brought to ambient temperature just prior to use. In throughout the experimental runs all the solvents, calibration and real samples were filtered through 0.45 µm nylon filter membranes. Double distilled deionized water was used throughout which was produced by a Milli-Q system.

Sample preparation

Samples were dried after separating then rinsed with distilled water. Finally, concentration of vitamins B1, B2, B3, and B6, were determined using HPLC-UV. The tests were repeated three times for sample.

Determination of water-soluble B vitamins

HPLC is one of the most convenient and accurate analytical techniques. The chromatographic parameters, *i.e.*, capacity factor, selectivity factor, resolution factor and factor symmetry, were calculated on the basis of the experimentally obtained values of retention times and width peaks for all the investigated B-complex vitamins (Akah and Onweluzo, 2014).

Originally, this HPLC intended to focus on the analysis of the four water-soluble vitamins introduced in *Psidium guajava,* including thiamine, pyridoxine, riboflavin and niacin only. However, when optimizing the HPLC methods for the analysis of those four vitamins in the samples, the interferences from other vitamins in the samples must be resolved from them. This stability study serves as a preliminary screening for the optimization of standard storage and sample extraction procedures. The separation of B vitamins using HPLC with fluorometric detectors has been applied. The use of HPLC coupled with UV detection for the study of water-soluble vitamins in food has been demonstrated to be a fast, simple, and reliable method (Sami et al., 2014).

Results and discussion

Table 1 shows the water-soluble B vitamin contents for *Psidium guajava* leaves with the retention times: 9.067 min for Niacin, 12.567 min for Pyridoxine, 23.358 min for Thiamine and 25.867 min for Riboflavin. As shown in the figure 1the concentration of b vitamins are 0.002 ug/g for Niacin, 0.002 ug/g for Pyridoxine, 0.213 ug/g for Thiamine and 0.325 ug/g for Riboflavin. Vitamin B deficiency is one of the major dietary problems in developing countries. Poor people cannot intake vitamin B from rich foods like liver, egg, meat, fish etc. They depend on vegetables and fruits for vitamins and minerals. The study will provide the vitamins B composition of *Psidium guajava* leaves. These data will also be helpful in the preparation of a complete food composition table which will then be used everywhere, e.g. for the preparation of diet therapy, for food based dietary guidelines, for nutrition education, for food security, safety and regulation, for the labeling of food in food industry, for nutritional survey and also for other research purposes. Encouraging regular intake of these *Psidium guajava* leaves containing B vitamins would remove vitamin B

Table 1. HPLC Peak list and Rt values (Detector A, 210 nm)

Peak	Retention Time	Area	Height	Concentration (ug/g)	Name
1	9.067	42	8	0.002	Niacin
2	12.567	1314	4	0.002	Pyridoxine
3	23.358	20585	4222	0.213	Thiamine
4	25.867	18473	263	0.325	Riboflavin

deficiency. Vitamin B which is present in guava leaves helps in improving blood circulation to the brain, stimulating cognitive function and relaxing the nerve.

Nowadays guava leaves widely used in hair growth as it is rich in vitamin B which nourish the follicles and aid hair growth. The medicinal properties of guava leaves still remain in the shadows in the eyes of common man. Though it is cheaply and easily available right in our surroundings, we do not make any use out of it. Out of the several uses of guava leaves, the latest research revealed as guava leaves could be used to control hair fall The merits attributed to guava leaves are so high that it must be made part of our diet.

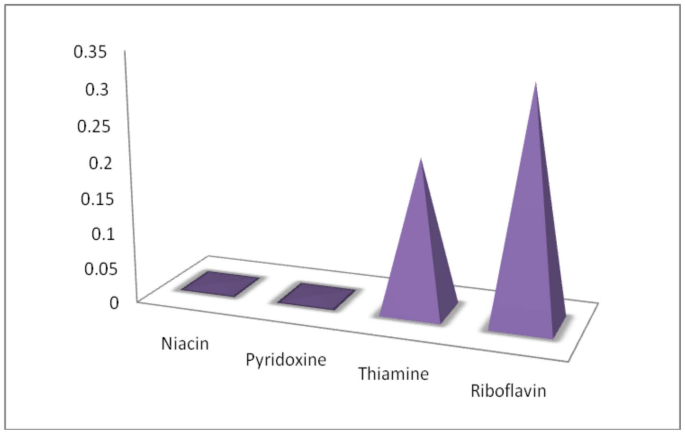

Figure 1.Concentration of vitamin B complex present in *Psidium guajava* leaves

Conclusion

Psidium guajava linn is an important nutritious plant that provides efficient amounts of nutrients that the body need for its growth and functions. Our findings has provided evidences that leaf extracts of *P.guajava L* is a potential source of vitamin B and this has justified its various therapeutic applications in forkloric medicines. The leaves were rich sources of valuable bioactive compounds and can be explored as nutraceuticals. The leaves provide opportunities to develop as medicines, value added products and dietary supplements which possess potential health benefits. It is concluded that *Psidium guajava* leaves are very much important for their medicinal as well as nutritional value.

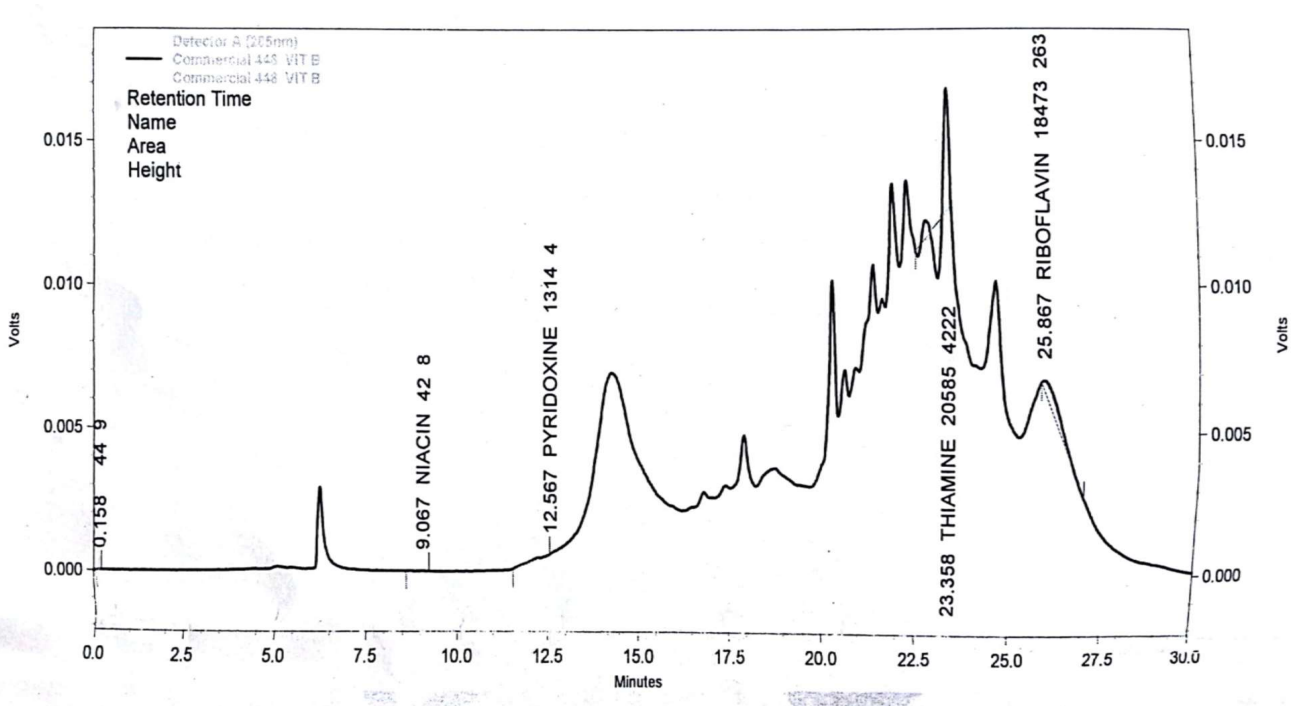

Figure 2. HPLC chromatogram of vitamin B

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