Nature's Bounty: Profiling Niacin and Riboflavin in Wild Vegetables via RP-HPLC

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Abstract

This study presents a comprehensive analysis of the vitamin composition in selected wild vegetables, namely Cassia tora, Basella alba, Rivea hypocrateriformis, and Portulaca quadreifida, utilizing Reverse Phase High-Performance Liquid Chromatography (RP-HPLC). The research aimed to identify and quantify two essential B-group vitamins, Niacin (Vitamin B3) and Riboflavin (Vitamin B2), in these indigenous plant species. Through meticulous RP-HPLC analysis and graphing techniques, the presence of Niacin was confirmed in Cassia tora and Basella alba, while Rivea hypocrateriformis and Portulaca quadreifida exhibited significant levels of Riboflavin. These findings not only highlight the nutritional richness of these wild vegetables but also emphasize their potential as natural sources of essential vitamins for human consumption. This research holds paramount significance in addressing nutritional deficiencies and promoting sustainable dietary practices. By uncovering the vitamin content in these wild vegetables, the study provides valuable insights for nutritionists, researchers, and policymakers working towards enhancing food security and public health. Furthermore, the study underscores the importance of exploring indigenous flora as reservoirs of essential nutrients, encouraging the integration of these plants into diets for improved overall health and well-being.

INTRODUCTION

According to the ancient Indian Physician **Charaka** there are no such plants which are useless, (Sharma and Ahmed, 1992). Plants which are not cultivated or domesticated are referred to as wild vegetables. Some wild plants have been used as food since ancient times and now they have become part of the human diet and traditional food system. As wild vegetables have significant contributions to a healthy lifestyle. In India there is a need to identify and explore the other wild vegetables to fulfill the demand of the growing population, wild vegetables are considered as a rich source of micronutrients and provide energy to humans. So, researchers are studying the nutritional potential of these plants.

Literature survey revealed that several wild vegetables were distributed in Maharashtra and various regions in India. On the other side, increasing urbanization and gradual exploitation of forests resulted in the disappearance of several wild species (Khilari and Sharma, 2016) Some of the residents still use them as a supplementary food material and preserve for their future needs. Due to declining popularization of wild vegetables species, special attention should be focused on maintenance and popularization of this source of food supply hence there is a need to provide scientific and systematic knowledge of wild vegetables for cultivation among people.

❖ *Cassia tora L.*

Taxonomic classification: (Pandey & Mishra, 2009)

- ➢ Division- Phanerogams
- ➢ Sub-Division- Angiosperms
- ➢ Class- Dicotyledons
- ➢ Subclass- Polypetalae
- ➢ Series- Calyciflorae
- ➢ Order- Rosales
- ➢ Family- Caesalpiniaceae
- ➢ Subfamily- Caesalpinioideae
- ➢ Genus- *Cassia*
- ➢ Species- *tora*

Vernacular name: (Bachulkar, 2016)

- ➢ English - Foetid Cassia
- ➢ Marathi - Takala
- ➢ Sanskrit - Chakramard,Prapunannat
- ➢ Hindi-Chakunda,Pamad,Panver
- ➢ Gujarati-Kuwadia
- ➢ Common name-Tarota or Tarvata

Habitat:

Very common along Roadsides & In Wastelands, sometimes Forming pure Strands Native of Australia as well as Sparsely distributed from plains to the hills; often found in association with *Cassia obtusifolia*. (Patil, 2003), (Anupama,2017).

Botanical distribution:

Cassia tora L. is a semi-wild annual herb found widely in different places (Sarwa,2014). The height of this plant is 30-90cm.*Cassia tora L.* leaves are green. The young shoots and leaves are used as a vegetable in rural areas (Kamble,2019). The leaves of this plant have subulate glands between the leaflets of the 2 lower pairs, *Cassia tora L.* The pubescent beneath are 4 cm long, 3 pairs. The leaves are this plant is thin and coriaceous. The flowers of *Cassia tora L.* have 5 sepals and these sepals are oblong to rounded. Its fruits are linear, and the size of fruit is short as well as its fruit are flat and compressed. The seed of *Cassia tora L.* is hard, thickness of seed is 3-4 mm. Its seeds are 1cm long and it also oblong or rhombohedral (Anupama, 2017). It is Suffruticose, glabrous, foetid herbs,30-60cm tall. Leaves 4-12 cm long, Rachiswith 2 glands between two lowest pairs of leaflets. Flowers yellow with reddish tinge, in sub sessile, axillary pairs. Pods 10-20*0.3cm, subtetragonous, falcate, subtroluse, beaked, shortly stiptate; seeds 20-30, oblong (Patil, 2003)

Medicinal Uses:

- *Cassia tora L.* is traditionally used as vegetables by villagers and tribal people. (Supare and Patil,2011)
- The paste of the leaves can also be applied to ringworm and eczema.
- The leaf of this plant is used internally for bronchitis and asthma.
- It acts as a nerve tonic on kapha and vata dosha.
- The whole plant is used to stimulate liver, mild laxative, and heart tonic.
- It is used in treatment of skin disease (Rao and Chatterjee, 2016).
- In tribal people used the leaves for the treatment of jaundice (Choudhary et al, 2011).
- Anthelmintic, Antioxidants, Antiparasitic, Antiperiodic, Antiseptic, Depurative, Hepatoprotective, Laxative, Stomachic, Purgative, Vermifuge (Anupama, 2017).

Ayurvedic properties: (Joshi, 2003)

- Rasa:Madhura,Katu
- Virya:Usna
- Guna:Laghu,Ruksa
- Vipaka:Katu

Shaikh Rahimullah and Syed Imran Zainuddin has done proximate and phytochemical analysis of *Cassia tora L.* leaves in 2015. Sarwa Khomendra Kumar et. al in 2013 studied phyto-chemical as well as biological potential of this plant and prove that *Cassia tora L.* is medicinally effective for having anti -microbial, antioxidant hypertensive, anti-diabetic and anti-mutagenic activities for pharmacognostical properties.

The traditional uses of *Cassia tora L.* mentioned by shakyawar Y, et al in 2011. Kamble K. J and Dhage shubhangi study the physicochemical properties of *Cassia tora L.*

❖ *Basella alba L.*

Taxonomical classification: (Pandey & Mishra, 2009)

- Division: Phanerogams.
- Sub-division: Angiosperms.
- Class: Dicotyledons.
- Sub-class: Monochlamydeae or incomeletae
- Series: Curveembryae
- Family: Chenopodiaceae (Basellaceae)
- Genus: *Basella*
- Species*:alba*

Vernacular name: (Joshi, 2003)

- English: Indian spinach, Malbar nightshade.
- Marathi: Mayallu, Velbondi, Velgond
- Sanskrit: Upodaki,Apodika,Niviti.

Habitat: (Joshi, 2003)

Occurs throughout India as a weed wild or cultivated in gardens.

Botanical distribution:

Origin *Basella alba L.* is India and Indonesia. It is naturally found in tropical Asia and Africa. (Saroj et al 2012). *Basella alba L.* is extremely heat tolerant and widely cultivated in cool season. It's a cool-seasonal vegetable. This plant fruit is less stalky and purple in color (Harold, 1963). The stem of *Basella alba L.* is a big stem and its color is green. (Shade et al 2017) *Basella alba L.* is one of the wild vegetables which is rarely found in its natural habitat (Wambugu and Muthamia, 2009) but nowadays its nutritive value is good for healthier lifestyle (Varalakshmi and Devaraju, 2010). The plant has perennial and twining herbs, and its stems are 10 cm long and fleshy. Leaves are alternate and size is thick and broadly ovate or round, petioles are 1-3 cm long. The Color of the flower is reddish-purple or white. Its fruits are black or red in color (Patil, 2003).

Medicinal uses:

- Leaves are used as a vegetable in villages and used in traditional villages.
- It minimizes the burning and itching of skin.
- Leaves are used to reduce demulcent.
- Leaf-juice mixed with butter is a soothing and cooling application for burning and scals.
- They are also useful in Constipation, Pruritus, Strangury.
- Used to minimize or reduce Syphiliticuicers in nose. (Joshi,2003)
- Leaf juice is also used as a safe laxative for children.
- Leaves extracts are useful for pregnant women and also used in the urinary disease patient (Mishra et al 2006).
- Plant extract increase in the WBC count which help in the management of an Anemia and Immunity dependent disorders (Sonkar et al 2012).
- Plant leaves shows amylase activity this amylase activity help to Diagnosis of acute pancreatitis.
- Leaves are also used as anti-inflammatory analgesic (Yanadaish et al 2011).

Ayurvedic properties: (Joshi, 2003)

- ➢ Rasa: Madhura
- ➢ Virya: Sita
- ➢ Guna: Snigdha, picchila
- ➢ Vipaka: Madhura

Toncgo JVV et al 2015 reported nutritional analysis Phyto-chemical screening and total phenolic content of *Basella alba L.* pharmacogenetic study of stem of *Basella alba L.* was mentioned by Saroj V et al 2010.

❖ *Portulaca quadrifida L.*

Taxonomic classification: (Pandey & Mishra,2009)

- ➢ Division-Phanerogams
- ➢ Sub-division-Angiosperms
- ➢ Class-Dicotyledons
- ➢ Subclass-Polypetalae
- ➢ Series-Thalamiflorae
- ➢ Order-Caryophyllinae
- ➢ Family-Portulaceae
- ➢ Genus-*Portulaca*
- ➢ Species-*quadrifida*

Vernacular name: (Bachulkar, 2016)

- ➢ English: Chicken weed.
- ➢ Marathi: Ranghol, Bhuchouli, Khatechaounal, Chiwli, Lahanghol
- ➢ Sanskrit: Loni

Habitat:

Original habitat is obscure, it probably arose in the tropic of Asia or Africa. (https://pfaf.org.)

Botanical distribution: (Gaikwad and Garad, 2015)

Many branched herbs, branches creeping, profusely rooting at nodes; Nodes with a whorl of dense silvery white hairs. Leaves 2 cm long, opposite: elliptic-cordate to ovate-lanceolate. Flowers yellow 0.5 cm across, solitary, terminal on an infundibular receptacle, subtended by leaves, sepal's oblong, membranous. Petals obovate; stamens 8-12 cm, style cylindrical with 3-5 arms, fruits circumcise, obovate–conical, shining, straw- yellow; seeds orbicular–reniform, dull – black many.

Ayurvedic properties: (Joshi, 2003)

- Rasa: Amla, Kasaya
- Virya: Sita
- Guna: Laghu
- Vipaka: Amla

Trupti P. Durgawale et al studied the phyto-chemical analysis of *Portulaca oleracea L.* and *Portulaca quadrifida L* extract using gas chromatography, mass spectrometry in 2018 phyto-chemical and HPLC analysis of extract of *Portulaca quadrifida L* was done by Verma S.C et al in 2016 Marathe Vishal R. and Umate Satish K. estimate the Gallic acid and Rutin and Quercetin by HPLC method in 2016.

❖ *Rivea hypocrateriformis Desr.*

Taxonomy classification: (Pandey & Mishra,2009)

- Division: Phanerogams
- Sub-division: Angiosperms
- Class: Dicotyledons
- Sub-class: Gamopetalae
- Series: Bicarpellatae
- Order: Polemoniales
- Family: Convolvulaceae
- Genus: *Rivea*
- Species: *hypocrateriformis.*

Vernacular Name: (Bachulkar,2016)

- Marathi: Phand, Phanj
- Hindi: Phanj
- Sanskrit: Phang
- Gujrathi: Phanjika

Habitat:

Rivea hypocrateriformis Desr. is found in dry subtropical forests of India, Nepal, Sri-Lanka, Pakistan, Bangladesh, Myanmar, Thailand. In India its widely found in Assam, Bihar, Maharashtra, Rajasthan, and Tamil-Nadu. (Siddiqui et al 2021).

Botanical distribution:

Rivea hypocrateriformis Desr. is robust woody climbing shrub (Saheli et al 2020) leaves are obtuse at apex, leaves color are dark green. Flowers have white fragrant. (Patil,2003).leaves are rounded-heart shaped, blunt apically, densely appraised velvet hairy below. Seeds are brown in

color, smooth or hairless, and seeds are surrounded by dry white pulp. (Sekhar et al and Sneha et al 2013.)

Some phytochemicals are naturally present in this vegetable such as alkaloids, flavonoids tannins, saponins, phenolic compounds, glycosides, steroids, carbohydrates and amino acids (Borkar et al 2015)

Medicinal uses:

- ➢ Leaves used as vegetables and leaves also have medicinal properties.
- ➢ Juice of the branches and leaves of this plant is given with milk and sugar in Arsharoga.
- ➢ Young branches and young of this plant give various medicinal benefits.
- ➢ Rub juice of the leaves and branches are on the children's body. It helps to reduce rash.
- ➢ These vegetables help to minimize body heat (Bachulkar, 2016).
- ➢ Preventing fertility in women Ayurvedic physicians would have suggested phanj.
- ➢ Leaves juice prevents skin diseases of the hair scalp.
- ➢ The whole plant and root are used to reduce the poison of snake bites.
- ➢ The whole used to minimize heart disease and piles problems. (Siddiqui et al 2021)

Sneha D. et al studied the evaluation of phyto-chemical content nutritional value and antioxidant activity of *Rivea hypocrateriformis Desr.* Shivalingappa H. Birdar J.S. Suresh K. explained the anti-implantation activity of alcoholic extract of *Rivea hypocrateriformis Desr.* in 1999.

Materials and Methods

The following 4 wild vegetables were studied.

- *Cassia tora L.*
- *Basella alba L.*
- *Portulaca quadrifida L.*
- *Rivea hypocrateriformis Desr*.

1. Collection of plant material:

We collected *Cassia tora L.* from village Bhokar roadside, *Rivea Hypocrateriformis Desr.* From Bhambhori roadside, *Portulaca quadrifida L.* from local market of Jalgaon (Maharashtra, India), *Basella alba L.* from our home garden.

2. Identification & Authentication:

All the plant samples were Identified and Authenticated by Taxonomist Prof. Dr. S. A. Chaudhari

Estimation of Vitamins (RP-HPLC Analysis of Water- and Fat-Soluble Vitamins)

Processing of the plant's material:

Leaves of *wild vegetables* were washed thoroughly with tap water, and carefully air-dried. The dried pieces were further processed for grinding and transferred through a sieve of mesh size 40 to produce a fine powder. This powder was filled in a sealed package and stored at room temperature for further experimentation.

Determination of water-soluble vitamins:

5 grams of powder of samples were placed separately in 25 mL of H_2SO_4(0.1 N) solution and incubated for 37 min at 122°C. Then, the contents of the samples were cooled and adjusted to pH 4.5 with 2.5 M sodium acetate, and 50 mg Taka-diastase enzyme was added. The prepared solution was stored overnight at 25°C.

The mixture was then filtered through a Whatman filter paper No. 4. The filtrate was diluted with 50 ml of double distilled water and filtered again through a micropore filter (0.45 μg).

Twenty microliters of the filtrate were injected into the HPLC system (Shimadzu LC 10AS) in the triplicate. Standard stock solutions for thiamine, riboflavin, niacin, pyridoxine, cobalamin, and folic acid were used for quantification of vitamin B content of the test sample accomplished by comparison.

Instrumental condition-Reversed phase chromatographic separation was achieved on a-(RP-) HPLC column ZORBAX Eclipse Plus C18 (250 × 4.6 mm, Particle size 5 μm) through the isocratic delivery mobile phase (A/B 33/67; A: MeOH, B: 0.023 M H_3PO_4, pH = 3.54) at a flow rate of 0.5 ml/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature.

Determination of fat-soluble vitamins

Five grams powder of samples, 0.5 g of pyrogallic acid, 35 ml ethanol, and 15 ml (50%) KOH were added, mixed well separately, and refluxed for 45 min using a water bath at 47 ± 5°C. The solutions were neutralized by adding double-distilled water which then was dehydrated using anhydrous sodium sulphate. Further, the sample solutions were concentrated to approximately 2-5 ml using a water bath (47 ± 5°C), diluted to 10 ml using methanol. Then, the sample solutions were filtered using a 0.45 μg membrane and finally subjected to HPLC analysis.

Standard stock solutions for vitamin A, D3, and E were used to quantify selected fat-soluble vitamins.

Instrumental condition-RP-HPLC analysis was performed with the HPLC system (Shimadzu LC 10AS), including a diode array detector. The column was made of stainless steel. Analysis of fat-soluble vitamins, the Agilent Eclipse XDB-C18 column was used (5 μm, 4.6 × 150 mm), the solvent was methanol, and UV detection was recorded at 318 nm for vitamin A, 262 nm for vitamin D3, and 292 nm for vitamin E. Separation of all vitamins was based on isocratic elution and the solvent flow rate was maintained at 1 ml/min. Twenty micro liters of extracted oil was directly injected into the HPLC column in triplicate.

Result and Discussion

The study aimed to determine the presence of specific vitamins in wild vegetables, focusing on Cassia tora, Basella alba, Rivea hypocrateriformis, and Portulaca quadreifida, using Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) analysis. The results obtained through HPLC analysis and corresponding graphs revealed that Cassia tora and Basella alba contain Vitamin B3 (Niacin), while Rivea hypocrateriformis and Portulaca quadreifida contain Vitamin B2 (Riboflavin).

The findings of this study hold significant importance in the realm of nutrition and natural sources of essential vitamins. Vitamins are vital micronutrients required for various physiological functions within the human body. In this study, the focus was on two B-group vitamins: Niacin (Vitamin B3) and Riboflavin (Vitamin B2).

Cassia Tora and Basella alba Contain Vitamin B_3 (Niacin):

Niacin, also known as Vitamin B3, is essential for energy metabolism and plays a crucial role in DNA repair and cell differentiation. The presence of Niacin in Cassia tora (fig. 1) and Basella alba (fig. 2) indicates that these wild vegetables could serve as natural sources of this important vitamin. Integrating these vegetables into diets, especially in regions where niacin deficiency is prevalent, can contribute significantly to addressing nutritional gaps.

Rivea hypocrateriformis and Portulaca quadreifida Contain Vitamin B_2 (Riboflavin):

Riboflavin, or Vitamin B2, is essential for the metabolism of fats, drugs, and steroids, and plays a key role in energy production. The identification of Riboflavin in Rivea hypocrateriformis (fig. 3) and Portulaca quadreifida (fig. 4) highlights these wild vegetables as potential sources of this

vitamin. Incorporating these vegetables into diets can aid in maintaining proper energy levels and supporting overall health.

Identifying natural sources of vitamins in wild vegetables is valuable for promoting sustainable nutrition and addressing dietary deficiencies, especially in regions where access to diverse foods is limited. Additionally, this study underscores the importance of exploring indigenous plants for their nutritional content, which can inform dietary recommendations and public health initiatives.

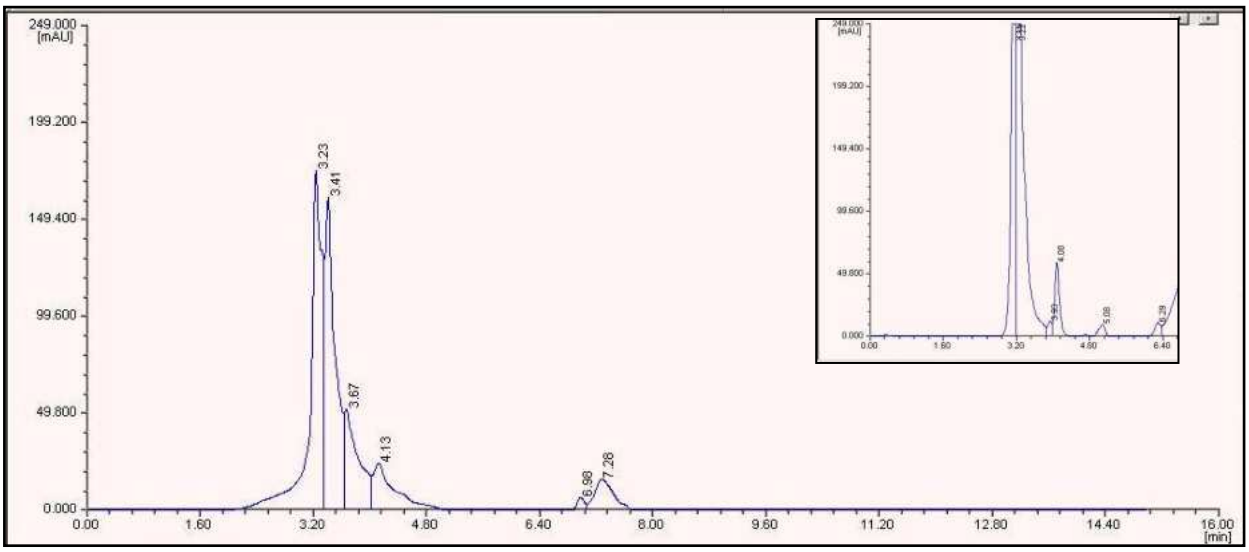

Figure 1: HPLC graph of *Cassia tora* extract (inset: HPLC graph of std. Vit. B_3)

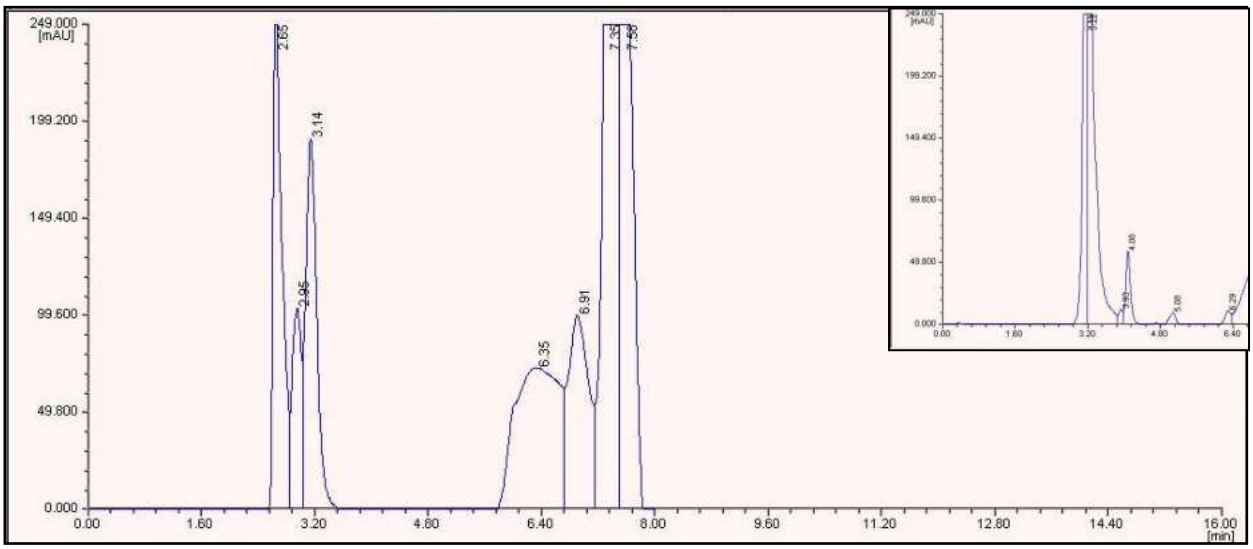

Figure 2: HPLC graph of *Basella alba* extract (inset: HPLC graph of std. Vit. B_3)

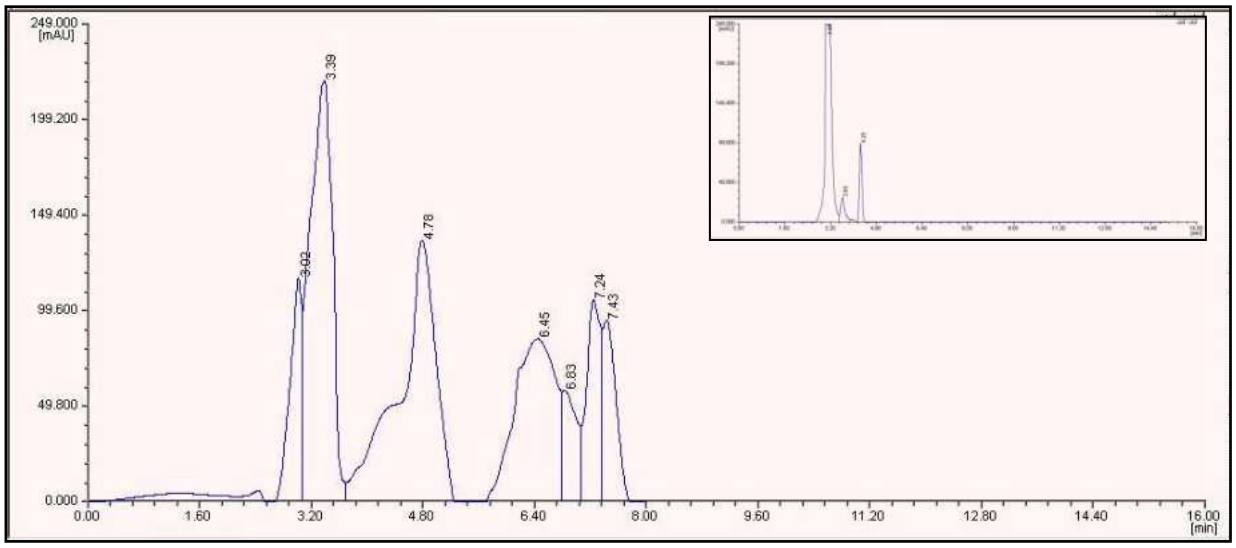

Figure 3: HPLC graph of *Rivea hypocrateriformis* extract (inset: HPLC graph of std. Vit. B_2)

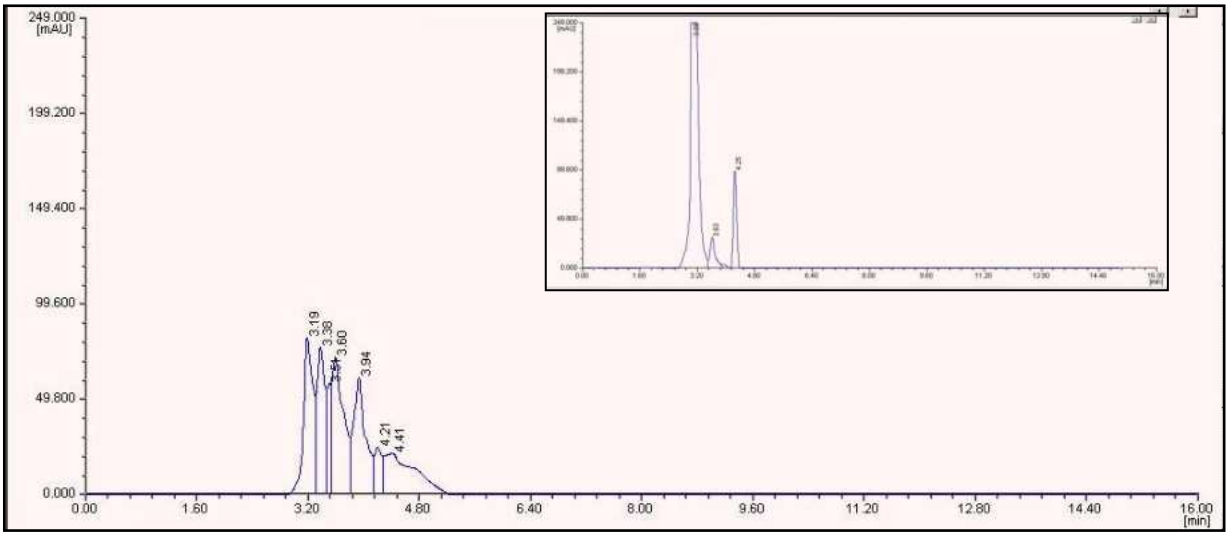

Figure 4: HPLC graph of *Portulaca quadrifida* extract (inset: HPLC graph of std. Vit. B_2)

Photo Plates

Cassia tora L.

Basella alba L.

Rivea hypocrateriformis Desr.

Portulaca quabrifida L.

Conclusion

The RP-HPLC analysis conducted in this study provides valuable insights into the vitamin composition of wild vegetables. The presence of Niacin in Cassia tora and Basella alba, as well as Riboflavin in Rivea hypocrateriformis and Portulaca quadreifida, highlights the nutritional potential of these plants. Further research and exploration of wild vegetables can lead to the development of sustainable dietary interventions, contributing to improved public health and well-being.

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Declarations

Competing interests:

The authors have no competing interests.

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Availability of data and materials

Not Applicable

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