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Determination of Vitamins, Minerals, Amino Acids and Organic Acids On Rivea Hypocrateriformis (Desr.) Choisy.

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ABSTRACT

Medicinal plants play a significant part in natural wealth. The use of plant raw materials is one of the areas of modern pharmaceutical science in the production of herbal medicines. Vitamins, minerals, amino acids and organic acids are essential substances that our bodies need to develop and function normally. The selected plant Rivea hypocrateriformis (Desr.) Choisy. (Convolvulaceae), a large climber and is considered as the botanical source of Phanji, a classical drug of Ayurveda. Researchers show that its stem contains two new bergenin derivatives; stem possess anti-inflammatory activity and aerial parts possess antioxidant property, Hepatoprotective and anti-implantation. Hence, the present study was undertaken to determine the amino acids, vitamins, organic acids and minerals on R. hypocrateriformis. Vitamins are organic compounds present in minute amounts in natural foodstuffs. Vitamin analysis shows the presence of vitamins like vitamin D_2 (Ergocalciferol), vitamin E (Tocophenol), vitamin K_1 (Phylloquinone), vitamin A (β- Carotene) and vitamin C in the whole plant extract of R. hypocrateriformis. Minerals are the naturally occurring inorganic element or compound having an orderly internal structure and characteristic chemical composition, crystal form and physical properties. Mineral analysis shows the presence of calcium (Ca), iron (Fe), magnesium (Mg), zinc (Zn), sodium (Na), phosphorous (P) and potassioum (K). Aminoacids are the building blocks of protein. This analysis shows the presence of various aminoacids like glycine, lysine, valine, alanine, tyrosine, cysteine, isoleucine, leucine, proline, serine, aspartic acid, glutamic acid, arginine and phenylalanine. Organic acids are the organic compounds having acidic properties. Organic acid analysis of the selected plant indicates the presence of oxalic acid, tartaric acid, malic acid, pyruvic acid, citric acid and succinic acid. However, further studies are needed to determine and isolate compounds responsible for the specific therapeutic properties of the plant.

KEY WORDS: Amino acid, Organic acid, phylloquinone, Rivea hypocrateriformis and vitamins.

I. INTRODUCTION

Importance of mineral nutrition is known since ancient times. The tenet "Let food be thy medicine and medicine be thy food", exposed by Hippocrates nearly 2,500 years ago, is receiving renewed interest. Woodward in1699 for the first time observed that plants grow better in muddy than in rain water. De Saussure (1804) confirmed the inorganic mineral elemrnt of the plants ash are obtained from all the soil. He also recognised that mineral elements present in the soil are important for the growth and development of plants.

The family Convolvulaceae is a fairly large and homogenous group comprising about 50 genera and nearly 1,700 species (Okereke et al., 2015). Rivea hypocrateriformis. is a robust woody climing shrub belonging to the family Convolvulaceae and is found in subtropical forests of India, Nepal, Sri Lanka, Pakistan, Bangladesh, Myanmar and Thailand (Salehi et al., 2020). Leaves of R. hypocrateriformis were evaluated for its nutritive value and antioxidant potential (Sneha et al., 2015).

Plants have great importance due to their nutritive value and continue to be a major source of medicines as they have been found throughout human history (Balick et al., 1996). Vitamins are organic compounds required as vital nutrients in tiny amounts by an organism. Vitamins serve as biocatalysts in many chemical reactions as well as

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precursors to various body factors and also required for a variety of biological processes such as mental alertness eg., niacin; resistance to infections eg., vitamin C. vitamin A is necessary in vision (Mcguire and Beerman, 2007; Combs, 2008), gene transcription (Combs, 2008; Duester, 2008), immunity, dermatology (Nelson et al., 2008), growth and development (Solomon, 2001) and so on.

Trace elements are inorganic substances that are present in all body tissues and fluids, and their presence is vital for the maintenance of certain physicochemical processes that are essential to life. Although they donot provide energy, they have significant roles in many activities in the body (Eruvbetine et al., 2003). Generally, they are needed by the body for normal growth, development and physiology. Some minerals such as Ca, Mg, Na, K, P and S are needed in larger amounts to perform essential functions of life (Saracoglu et al., 2009). However, other minerals are required in smaller amounts, and these are referred to as trace elements, which include Fe, Cu, Mn, Co, F, I and Zn (Wood, 2000).

Aminoacids are essential in the synthesis of proteins and precursors in the formation of secondary metabolism molecules (Perez et al., 2009); that participate in cell signaling, gene expression, synthesis of hormones and antioxidant capacity (Wu, 2009).

Organic acids are important metabolites of the tricarboxylic acid cycle in plants, which is the main energy-producing cycle of the cell. Analysis of organic acids is important because of their involvement in various physiological activities of plants (Bennet-Clark, 1993). The organic acid contents in the leaves of plants vary with species and on the degree and many other factors. Since, environmental or agronomic practices may affect the expression of genes in the seeds, they may also affect the organic acid contents (Kader, 2008).

II. MATERIALS AND METHODS

Source of the plant sample:

Rivea hypocrateriformis is collected from Kottaram. It is a small village located in Kanyakumari district of Tamil Nadu. This village is used to be a resting place for the Travancore Maharajas. It is very close to Kanyakumari, Vattakottai and Marunthuvazh Malai. It is a mesmerizing beautiful place.

Vitamin Analysis:

The amount of vitamin E (Tocophenol) and vitamin C of the sample was evaluated spectrophotometrically with modification as described by (Njoku et al., 2015). The method described by Oulai et al., (2014) was used in the dtermination of vitamin A (β-carotene) and vitamin K_1 by (Fraser et al., 2000).

Mineral Analysis:

The atomic absorption spectrophotometer (AAS) was used for the analyses of Mg, Zn, Fe and P while the flame photometer was used in the analyses of K and Na. Using AAS, the ash solution, s of the plant samples were prepared by weighing 5g of each of the powdered plant samples these were washed at 550^0C in muffle furnace for 5 hrs, and the residues dissolved in 100ml of deionized water. Suitable salts of the metals were used to make their standards, lamps were fixed. The standard minerals solutions were injected to calibrate the AAS using acetylene gas. An aliquote of ash solutions were injected and the concentrations obtained from the AAS. Using the flame photometer, the diluents of sample was aspirated into the Jenway Digital flame photometer using the filter corresponding to each mineral element. All of these were carried out using the method described by (Oshodi, 1992).

Amino acid analysis (Vazquez et al., 1995):

Powdered sample of R. hypocrateriformis (3 mg) were hydrolyzed with Hcl 6M at 150^0 C during 6 hr. After hydrolysis, the acid was removed by rotary evaporation (RE500 Yamato Scientific America Inc.). Sample was resuspended on 2 ml of sodium citrate buffer Ph 2.2. Sample derivation was achieved adding α-phthaldehyde (OPA) 7.5 Mm to the sample citrate buffer (OPA reagent contains β-mercaptoethanol and Brij 35). The HPLC method precision and accuracy was evaluated using external and internal standards. The amino acids reference standard consisted on fifteen amino acids (0.05 µmoles ml^{-1}) was added to amino acid reference standard and each plant sample to normalize and quantify the amino acid content.

A gradient mobile phase of sodium acetate 0.1 M Ph 7.2 and methanol (9:1) elute sample for amino acid separation through C18 column reversed- phase octadecyl dymethylsilane particles (100 x 4.6 mm x ¼" Microsorb 100-3 C18). Fluoresence detection was realized using an excitation-emission wavelength of 360 and 455 nm respectively. Star Chromatography work station (Varian version 5.51) software was used to achieve amino acid peak integration.

Organic acid analysis (Pereira et al., 2013):

Organic acids were determined following a procedure. The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu

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Corporation, Kyoto, Japan). Separation was achieved on a SphereClone (Phenomenex, Torrance, CA, USA) reverse phase C_{18} column (5 µm, 250 mm × 4.6 mm i.d.) thermostated at 35°C. The elution was performed with sulfuric acid (3.6 mM) using a flow rate of 0.8 mL/min. Detection was carried out in a PDA (photodiode array detector), using 215 and 245 nm (for ascorbic acid) as preferred wavelengths. The organic acids found were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in mg per g of dry weight.

III. RESULT AND DISCUSSION

Table 1: Vitamin analysis of R. hypocrateriformis powdered whole plant:

S.No	Name	Results (mg/kg)	Ret.Time (min)	Wavelength (nm)
1	Vitamin D2 (Ergocalciferol)	ND	2.32	285
2	Vitamin E (DL-α-Tocophenol)	ND	4.13	285
3	Vitamin K1 (Phylloquinone)	ND	4.78	285
4	Vitamin A (β-Carotene)	162.80	12.93	446
5	Vitamin C	37.16	3.14	446

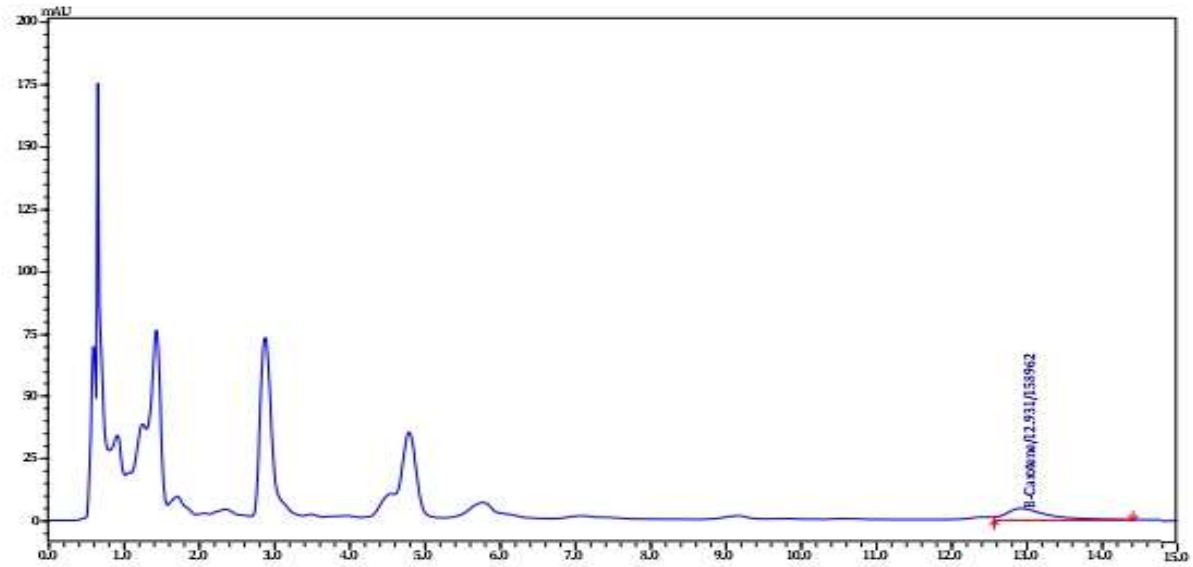

Figure 1: Chromatogram on vitamin analysis of R. hypocrateriformis powdered whole plant:

Table 2: Mineral analysis of R. hypocrateriformis powdered whole plant:

S.No	Minerals	Results (mg/kg)	Wavelength (nm)
1	Calcium (Ca)	22690.24	317.933
2	Iron (Fe)	731.46	238.204
3	Magnesium (Mg)	3195.12	285.213
4	Zinc (Zn)	2289.27	206.2
5	Sodium (Na)	1796.59	589.592
6	Potassium (K)	11729.27	766.49
7	Phosphorous (P)	1598.05	213.617

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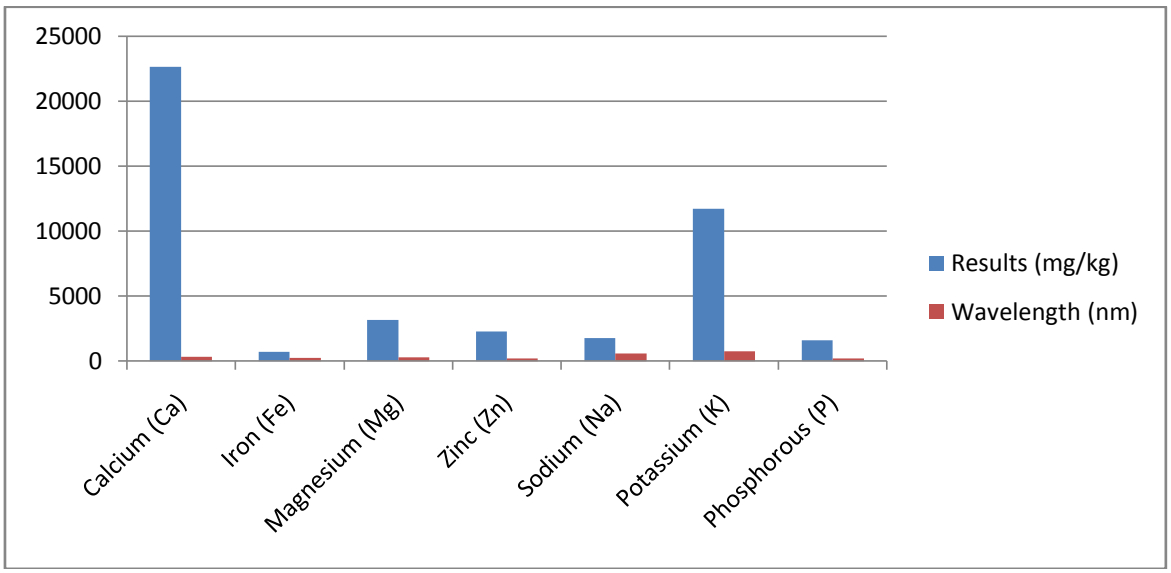

Figure 2: Mineral analysis of R. hypocrateriformis powdered whole plant.

Table 3: Aminoacid composition of R. hypocrateriformis:

S.No	Amino acids	R.T. (min)	Concentration (g/100g sample)
1	Glycine*	14.90	2.01
2	Lysine	39.39	ND
3	Valine	16.50	2.70
4	Alanine*	15.12	3.00
5	Tyrosine	26.10	3.10
6	Cysteine	12.29	2.01
7	Isoleucine	13.34	2.15
8	Leucine	17.70	5.40
9	Proline*	17.81	4.01
10	Serine*	2.24	3.10
11	Aspartic acid*	21.86	5.10
12	Glutamic acid*	22.95	8.10
13	Arginine*	31.10	3.24
14	Phenylalanine*	21.25	3.15

*= Non essential aminoacids, ND = Not detected

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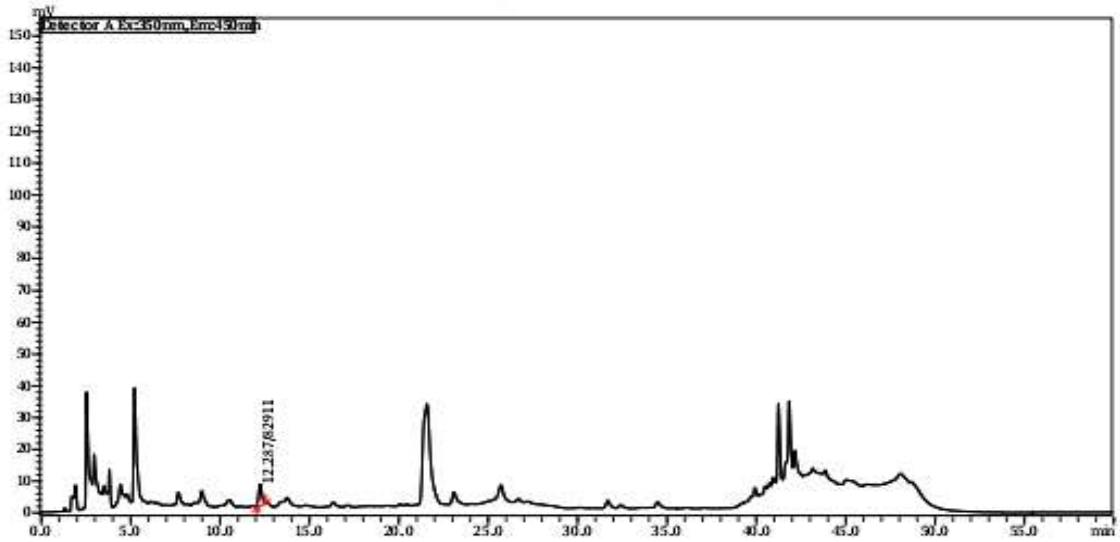

Figure 3: Chromatogram on aminoacid composition of Rivea hypocrateriformis.

Table 4: Analysis of organic acid in R. hypocrateriformis:

S.No	Name	Ret.Time (min)	Area	Conc. (mg/kg)
1	Oxalic acid	2.9	245,579	141.43
2	Tartaric acid	3.2	377,260	1188.59
3	Malic acid	4.1	93,478	55.78
4	Pyruvic acid	5.3	29,741	370.16
5	Citric acid	7.1	93,877	713.06
6	Succinic acid	9.0	48,431	703.80

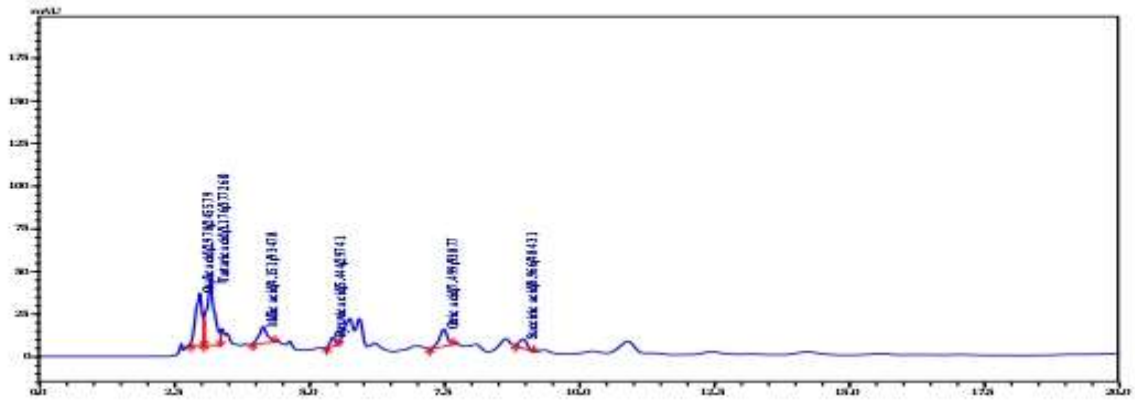

Figure 4: Chromatogram on analysis of organic acid in R. hypocrateriformis:

For normal growth and development vitamins and minerals are necessary for the human health. The vitamin analysis of R. hypocrateriformis contain copious presence of vitamins such as Vitamin A (β- Carotene) (162.80 mg/kg) and Vitamin C (37.16 mg/kg) and the absence of Vitamin D_2 (Ergocalciferol), Vitamin E (Tocophenol) and Vitamin K_1 (Phylloquinone) which is depicated in the table-1 & figure-1. The presence of Vitamin C and β-Carotene clearly demonstrate powerful antioxidant properties.

Minerals are very important for our body to stay healthy. The mineral analysis of R. hypocrateriformis shows the presence of minerals such as calcium, iron, magnesium, zinc, sodium, potassium and phosphorous (22690.24 mg/kg, 731.46 mg/kg, 3195.12 mg/kg, 2289.27 mg/kg, 1796.59 mg/kg, 11729.27 mg/kg and 1598.08 mg/kg) respectively which is representated in the table-2 & figure-2 distinctly showing the high calcium content (22690.24 mg/kg) and while, the iron content having the least value (731.46 mg/kg).

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The vitamins and minerals present in Rivea hypocrateriformis (Desr.) Choisy. clearly suggests that the selected plant is recommended for healthy diet.

Amino acid investigation of R. hypocrateriformis (Desr.) Choisy. manifest the existence of non essential and essential aminoacids. The non essential amino acids such as glycine, alanine, proline, serine, aspartic acid, glutamic acid, arginine and phenylalanine. While, lysine, valine, tyrosine, cysteine, isoleucine, leucine are the amino acids present in the methanol extract of R. hypocrateriformis (Desr.) Choisy. which are constituted in table- 3 & figure- 3.

Concerning organic acids, R. hypocrateriformis (Desr.) Choisy shows the highest value in tartaric acid (1188.59 mg / kg) followed by citric acid (713.06 mg /kg), succinic acid (703.80 mg / kg), pyruvic acid (370.16 mg / kg), oxalic acid (141.43 mg / kg) and malic acid (55.78 mg / kg) which are represented in table- 4 & figure- 4).

Vitamin C possesses an antioxidant property required for the maintenance of normal connective tissues, wound healing and also facilitates the absorption of dietary iron from the intestine (Button, 2004). Pathak and Kapil, (2004) reported that zinc is vital in protein synthesis, cellular differentiation and replication, immunity and sexual functions. Calcium is reported to be essential for blood clotting, bone and teeth formation and as a co-factor in some enzyme catalysis (Robert et al., 2003). In humans, magnesium is required in the plasma and extracellular fluid, where it helps maintain osmotic equilibrium and iron facilitates the oxidation of biomolecules to control obesity, which predisposes an individual to various diseases and also essential for haemoglobin formation (Thomos and Krishnakumari, 2015).

Moderate quantities of sodium and potassium were present in the whole plant extract of R. hypocrateriformis (Desr.) Choisy. and these are principal for extracellular and intra-cellular fluids and aid in maintaining electrolyte balance in the body (Robert et al., 2003). Phosphorous maintain blood sugar levels and normal blood contraction (Linder,1991). It is also important for normal cell growth and repair bone growth, kidney function and also maintaining the body's acid-alkaline balance (Johns and Duquette,1991).

Organic acids are important metabolites of the tricarboxylic acid cycle in plants, which is the main energy-producing cycle of the cell. Analysis of organic acids is important because of their involvement in various physiological activities of plants (Bennet-Clark, 1993). Malic acid is a dicarboxylic acid and it plays an important role in improving the performance of muscles, reducing tiredness, as well as in improving mental clarity (Carocho et al., 2013 ; Baldwin, 2002). Citric acid is mainly used as a food additive to give acidity and sour taste to food and beverages (Destandau et al., 2005).

Aspartic acid function is essential for purine, pyrimidine, asparagine and inositol synthesis. Glutamic acid and glycine participate in the synythesis of glutathione increasing the antioxidant capacity of the plant (Wu, 2009). Valine maintains the balance of branched chain amino acids, whereas alanine is involved on hepatic autophagy, gluconeogenesis and transamination. Leucine regulates the protein turn over and gene expression (Akram et al., 2011). Lysine help to maintain intestional integrity and health (Wang et al., 2009). Serine plays a major role in cellular proliferation, brain development and function, and necessary for specific functions in the central nervous system (Koning et al., 2003). Tyrosine, cysteine, isoleucine, proline, arginine and phenylalanine plays a great role in the output of carnitine, a nourisher responsible for converted fatty acids into energy and helps to reduce chooestrol (Ghoreyshi et al., 2019 ; Tufarelli et al., 2020; Slobodianiuk et al., 2021).

IV. CONCLUSION

Vitamins, minerals, organic acids and amino acids are nutrients that our body needs to grow and develop. From the results of the present study concluded the plant holds a terrific promise in providing nutrient supply that could promote good health, which justifies and confirms the Rivea hypocrateriformis is an excellent nutrient source for a healthy diet.

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