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Evaluation of Nutritional and Mineral Composition of Morning glory leaves

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ABSTRACT

Rivea hypocrateriformis (Desr.) Choisy, a member of the Convolvulaceae family and commonly known as 'Morning Glory,' is a woody climbing shrub widely employed in Ayurvedic medicine. In traditional medicine, various parts of this plant, including its bark, stems, and leaves, have been employed to address a range of health concerns, such as malaria, cancer, mental disorders, and pain relief. In spite of its numerous medicinal attributes, no published work is available till date on nutritional values and elemental analysis of leaves. The nutrient and antinutrient compositions of the leaves were investigated. The result of proximate analysis shows that crude lipid, crude fibre, crude protein, and carbohydrate in the leaves were $2.33 \pm 0.51\%$, $21.34 \pm 0.17\%$, 11.32 ± 0.22 and $48.19 \pm 0.21\%$. The antinutrient parameters evaluated in the leaves were phytic acid (2.32 ± 0.16) %, saponin content (1.36 ± 0.25 %), alkaloidal contents (0.27 ± 0.06) and oxalate content (0.23 ± 0.10) %. The predominant mineral elements in the leaf powder according to ICP-MS were Ca (45.26 mg/100g), Mg (15.80 mg/100g), and Na (13.31 mg/100g). Lead and arsenic contents were not detected in the leaves *Rivea*. Hence the outcome of this study revealed that *R. hypocrateriformis* leaves could be a valuable Nutraceuticals supplement and a cheapest source of essential nutrients to the human diet.

Keywords: Macronutrients; proximate analysis; elemental analysis; oxalate content and ICP-MS.

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INTRODUCTION

Plants have long been recognized as valuable sources of medicine and have a significant impact on the survival and well-being of ethnic and tribal communities worldwide. These medicinal plants are utilized across the globe to treat various human and animal ailments. Scientists have been drawn to explore these indigenous plant resources for their ethnomedicinal and nutritional potential, searching for bioactive compounds that can offer therapeutic benefits¹⁻⁴.

Medicinal plants not only provide essential medicinal compounds but also contain important dietary components like carbohydrates, proteins, and fats. These nutritional elements play a crucial role in meeting the body's physiological, metabolic, and morphological requirements⁵⁻⁷. Natural products derived from plants find applications in medications, dietary supplements, and various healthcare products. Moreover, plants serve as valuable resources for the discovery of new medicinal compounds with properties such as antioxidants, hypoglycemic agents, and hypolipidemic agents. Plants are a rich source of medicinal remedies, and numerous pharmaceuticals have their origins in plant-derived compounds, either directly or indirectly⁸⁻¹⁰.

Ancient Indian literature, particularly the Rigveda and the Atharvaveda, contains numerous references to the healing properties of various herbs. The Atharvaveda, in particular, laid the foundation for Ayurveda, the traditional Indian healthcare system, where "ayus" signifies life and "veda" signifies knowledge, essentially meaning the "science of life." Detailed information about herbs can be found in two important treatises, the Charak Samhita and the Shusruta Samhita, which serve as the basis for Ayurvedic medicine¹¹⁻¹³. Recognizing the significance of herbs, a six-volume work titled "A Compendium of Indian Medicinal Plants" has been published.

In modern times, herbs have gained renewed importance, especially as the detrimental effects of food processing and excessive medication have become increasingly concerning. Herbs are now being utilized in various ways, including cosmetics, food products, and alternative medicine. In addition to the macronutrients like lipids, proteins, and carbohydrates necessary for human growth, the supply of precise quantities of inorganic micronutrients is also crucial. Micronutrients such as chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) constitute a small fraction of our diet but play vital roles in metabolic processes. An imbalance, either an excess or deficiency of these micronutrients, can disrupt the normal biochemical functions of the body^{14,15}.

Rivea hypocrateriformis (Desr.) Choisy, a woody climbing shrub belonging to the Convolvulaceae family, is widely distributed across India, Nepal, Sri Lanka, Pakistan, Bangladesh, Myanmar, and Thailand¹⁶. In traditional medicine, various parts of this plant, including its bark, stems, and leaves, have been employed to address a range of health concerns, such as malaria, cancer, mental

disorders, and pain relief. For example, indigenous communities in Pakistan's Tharparkar region use *R. hypocrateriformis* for treating malaria and alleviating pain. This plant has garnered attention for its diverse biological properties, including antioxidant, anti-implantation, antimicrobial, pregnancy interruption, anticancer, and antiarthritic properties^{17,18}. Additionally, it serves as a crucial component in the Ayurvedic formulation known as "Rasa panchaka," which is utilized for asthma treatment¹⁹. Furthermore, similar to other varieties within the related genus, like *Rivea corymbosa* Hall and *Ipomea violacea* L. found in Mexico, *R. hypocrateriformis* is also utilized as a hallucinogenic substance in India and as a psychoactive medicine in Pakistan²⁰. The current study focuses on investigating the nutritional value and content of trace elements in *R. hypocrateriformis*, shedding light on its potential health benefits.

MATERIALS AND METHOD

Collection and authentication of plant materials

Leaves of *R. hypocrateriformis* were collected from outskirts of Udaipur, Rajasthan in November 2022. The plant was identified, authenticated and certified by Botanist, Himachal Pradesh State Biodiversity Board, Shimla, Himachal Pradesh.

Reagents

The reagents employed in the experiment were sourced from Merck, Germany, and were of analytical grade quality.

Proximate analysis

The powdered leaf samples underwent a drying process in an oven at 110°C for a duration of 1 hour until a constant weight was achieved. Subsequently, the determination of moisture content, ash content, crude fiber, crude protein, and crude lipid was carried out in triplicate following the AOAC (Association of Official Analytical Chemists) method¹⁹⁻²¹.

Anti-nutrient analysis

Determination of oxalate content

To determine the oxalate content, a modified titration method was employed as described in references 24 and 25. Initially, one gram of the powdered sample was placed into a conical flask. Subsequently, 75 mL of 3M H₂SO₄ (sulfuric acid) was introduced into the flask. The mixture underwent careful stirring using a magnetic stirrer for a duration of one hour. Following the stirring, the solution was meticulously filtered through Whatman filter paper No.1 to remove any solid particulates. A measured volume of 25 mL of the resulting filtrate was then subjected to titration using a 0.1M KMnO₄ (potassium permanganate) solution. The titration continued until a light pink color, persisting for at least 15 seconds, was observed, signifying the endpoint of the

titration. The oxalate content was subsequently calculated based on the premise that 1 mL of 0.05 mol/L KMnO_4 is equivalent to 2.2 mg of oxalate^{22,23}.

Determination of phytic acid

Initially, 2.0 grams of the sample were accurately weighed and placed into a 250 mL flask. Subsequently, 100 mL of a 2% concentrated hydrochloric acid (HCl) solution was added to the flask. The mixture was allowed to stand for a duration of 3 hours before being filtered through Whatman filter paper No.1.

To further analyze the filtrate, 25 mL of it was transferred to a 250 mL conical flask. Here, 10 mL of a 0.3% ammonium thiocyanate solution was added as an indicator. The next step involved titrating the solution with a standard Iron III Chloride solution, which was known to contain 0.00195 grams of iron per milliliter. The titration reached its endpoint when a brownish-yellow color persisted for a duration of 5 minutes²⁴. The percentage phytic acid was calculated as:

$$\text{Phytic acid (\%)} = \text{titre value} \times 0.00195 \times 1.19 \times 100$$

Determination of alkaloidal content

The determination of alkaloid content followed a specific procedure. Initially, 27.5 grams of plant extract were combined with 200 mL of a 10% acetic acid solution in ethyl alcohol. This mixture was thoroughly mixed and covered, allowing it to stand for a period of 4 hours. Subsequently, the mixture was filtered using Whatman filter paper No.1.

The filtrate obtained was then concentrated to one-fourth of its original volume using a water bath. Concentrated ammonium hydroxide was incrementally added to the extract until precipitation was complete. Afterward, the solution was allowed to settle, followed by a wash with a dilute sodium hydroxide (NaOH) solution. The resulting residue was carefully collected, dried, and weighed to determine the alkaloid content^{25,26}. The alkaloid content was calculated using the equation:

$$\% \text{ alkaloidal content} = \frac{\text{Weight of precipitate}}{\text{Weight of original sample}} \times 100$$

Determination of saponin content

The determination of saponin content was carried out following the method outlined by Obadoni and Ochuko. To initiate the process, 28.5 grams of the plant sample were combined with 50 mL of 20% ethyl alcohol and placed on a shaker for 30 minutes. Subsequently, the mixture was heated in a water bath at 55°C for 4 hours. After heating, the resulting mixture was filtered, and the residue was subjected to another extraction with 200 mL of 20% aqueous ethanol. The filtrates from both extractions were combined and then reduced to a volume of 40 mL in a water bath at 90°C. This concentrated solution was then transferred into a separating funnel, into which 20 mL of diethyl

ether was added and vigorously shaken. The upper ether layer was discarded, and the aqueous (bottom) layer was retained in a beaker. The retained aqueous layer was reintroduced into a separating funnel, followed by the addition of 60 mL of n-butanol, which was again shaken vigorously. The butanol extract, found in the upper layer, was retained, while the bottom layer was discarded. The butanol layer was subjected to two washes with 10 mL of 5% aqueous sodium chloride (NaCl). The resulting solution was collected and heated to evaporate the solvent in a water bath, then dried to a constant weight at 40°C in an oven for further analysis^{27,28}. The saponin content was calculated using the equation:

$$\% \text{ saponin content} = \frac{\text{Weight of residue}}{\text{Weight of Original sample}} \times 100$$

Elemental analysis

The analysis of leaf samples involved a specific procedure. First, the leaf samples were digested using concentrated nitric acid (HNO₃). After digestion, the resulting solution was carefully transferred into a 25-ml volumetric flask, and the volume was precisely adjusted to 25 ml using deionized water. To ensure accuracy in the analysis, a blank digest was also prepared following a similar procedure, but without the addition of any leaf sample. The multi-elemental analysis was then conducted using inductively coupled plasma mass spectrometry (ICP-MS), specifically utilizing the XSeries 2 instrument from Thermo Scientific. This analytical technique allowed for the comprehensive assessment of various elements within the samples.

Statistical analysis of data

The results were statistically analyzed and expressed as mean (n = 3) ± standard deviation. All experiments were performed in triplicates and the data expressed as mean ± SD using the Microsoft Excel 2010 spreadsheet.

RESULTS AND DISCUSSION

Proximate composition

The results of proximate analysis of leaves of *R. hypocrateriformis* tabulated in Table 1. The results shown that crude lipid was 2.33 ± 0.51 %, crude fibre 21.34 ± 0.17%, crude protein 11.32 ± 0.22, carbohydrate 48.19 ± 0.21% and Energy value (Kcal/100 gm) 222.29 ± 0.39.

Table 1: Proximate nutritional composition of dried *R. hypocrateriformis* leaf powder

Parameter	Composition %
Crude lipid	2.33 ± 0.51
Crude fibre	21.34 ± 0.17
Crude protein	11.32 ± 0.22
Carbohydrate	48.19 ± 0.21
Energy value (Kcal/100 gm)	222.29 ± 0.19

Values expressed as Mean \pm SD, n=3

The relatively substantial fiber content in *R. hypocrateriformis* leaves serves multiple beneficial purposes. It helps prevent constipation by promoting peristaltic movement in the digestive tract. Additionally, it aids in the absorption of certain essential minerals in the gut and assists in reducing the absorption of cholesterol^{29,30}. Such a high intake of fiber in one's diet could potentially lead to a reduced incidence of diseases linked to metabolic disorders. In contrast, the lipid content in the sample was notably low, making it the least abundant nutritional component in this study. It's worth noting that dietary lipids are essential for enhancing the taste of food and preserving its flavors. However, excessive consumption of dietary lipids has been associated with an increased risk of cardiovascular diseases, accelerated aging, and cancer. Therefore, the low crude lipid content in *R. hypocrateriformis* leaves suggests that it may help prevent certain chronic diseases related to lipids in humans. Moving on to protein content, it ranked as the third-highest proximate composition in the sample. The relatively high protein level found in *R. hypocrateriformis* leaves suggests that it could serve as a valuable dietary supplement. Dietary proteins play a crucial role in manufacturing and safeguarding various organic materials necessary for the proper functioning of the human body. They also act as enzymatic catalysts and play roles in metabolic and energy regulation. As for carbohydrates, they were the most abundant nutritional component in *R. hypocrateriformis* leaves. This high carbohydrate content makes it a rich source of energy, which can be utilized to enhance the energy content of diets³¹⁻³⁵. The overall energy content derived from the *R. hypocrateriformis* leaves sample was calculated to be 222.29 ± 0.19 kcal/100g. While this falls below the recommended daily energy value, it suggests that *R. hypocrateriformis*, as a low-energy food source, could be particularly beneficial in weight management programs, as traditionally used by practitioners.

Anti-nutrient analysis

Anti-nutrient analysis refers to the assessment of compounds in food or plant materials that can interfere with the absorption or utilization of essential nutrients in the body. The results of anti-nutrient analysis tabulated in table 2.

Table 2: Anti-nutrient Composition of *R. hypocrateriformis* leaf powder

Parameters	Values %
Phytic acid	2.32 ± 0.16
Saponin	1.36 ± 0.25
Alkaloids	0.27 ± 0.06
Oxalate	0.23 ± 0.10

Values expressed as Mean \pm SD, n=3 on dry weight basis.

The saponin content in *R. hypocrateriformis* was found to be low and well within safe limits, as it was below 10%. This is important because saponin levels above this threshold can pose risks to the body. High saponin intake in human and animal diets has been associated with growth impairment, reduced nutrient bioavailability, and inhibition of biochemical reactions responsible for breaking down ingested proteins^{36,37}.

Alkaloids are potent therapeutic bioactive compounds commonly found in plants. Consumption of high levels of certain alkaloids, such as tropane alkaloids, can result in symptoms like rapid heartbeat, paralysis, and, in extreme cases, death. Similarly, excessive intake of tryptamine alkaloids can lead to a staggering gait and potential fatality. Alkaloids can also have toxic effects on the gastrointestinal tract, disrupting the mucous membrane. Fortunately, the alkaloid content recorded in this study was quite low, alleviating concerns about anti-nutrient activity^{38,39}.

The phytate content in *R. hypocrateriformis* leaves was found to be low as well. Phytate has the tendency to bind with metal ions like calcium, copper, zinc, magnesium, and iron, forming insoluble complexes that are not easily absorbed in the gastrointestinal tract. Prolonged consumption of diets high in phytate (1% - 6%) can reduce the bioavailability of zinc. On the other hand, oxalate can form chelates with toxic metals like lead and mercury and also exhibits antioxidant properties. However, the presence of oxalate in foods can lead to mouth irritation and potentially decrease calcium absorption, increasing the risk of kidney stones⁴⁰⁻⁴⁴.

It's worth noting that the concentrations of anti-nutrients, including saponin, oxalate, phytate, and alkaloids, observed in this study were all within safe limits. Therefore, consuming *R. hypocrateriformis* leaves with these levels of anti-nutrients should not result in toxic effects.

Elemental analysis

The results of elemental analysis reported in table 3. Calcium, magnesium, sodium contents were higher in the leaves. Their values were 45.26 mg/100g, 15.80 mg/100g, 13.31 mg/100g respectively. Arsenic and lead contents were not detected in the leaves.

Table 3: Measured mineral composition of *R. hypocrateriformis* leaf powder in (mg/100 g)

Minerals	mg/100 g DW
Calcium	45.26 ± 0.021
Magnesium	15.80 ± 0.027
Potassium	6.21 ± 0.011
Phosphorous	0.85 ± 0.02
Sodium	13.31 ± 0.015
Zinc	2.13 ± 0.005
Copper	0.15 ± 0.005
Manganese	3.16 ± 0.011
Iron	1.53 ± 0.005

Arsenic	ND
Lead	ND

Values expressed as Mean \pm SD, n=3 on dry weight basis, ND: not detected

Minerals play a crucial role in human nutrition, contributing to both overall physical and mental health. They are essential constituents of various bodily components, including nerve cells, bones, teeth, tissues, muscles, and blood. The mineral composition of *R. hypocrateriformis* leaves, as presented in Table 3, highlights their significance as a valuable source of both macro and micro minerals. These findings underscore the potential health benefits of incorporating *R. hypocrateriformis* leaves into one's diet, as they provide a rich array of essential minerals necessary for the proper functioning of the body. Including such mineral-rich foods in your diet can contribute to better overall health and well-being. The minerals evaluated are in the order Ca>Mg>Na> K> Mn>Zn> Fe>P >Cu.

Indeed, minerals such as calcium, magnesium, sodium, and potassium play pivotal roles in various essential bodily functions. Calcium is crucial for muscle contraction, neurological function, blood clotting, and the regulation of cell permeability. It's especially known for its role in building and maintaining strong bones and teeth^{45,46}. Osteoporosis, a condition characterized by weakened bones, can benefit from increased calcium intake, making *R. hypocrateriformis* a potential natural remedy for this condition. Magnesium is essential for enzyme activity and helps prevent heart disease. It also contributes to the formation and function of bones, muscles, nerve transmission, and immune system support. Magnesium is important for the proper functioning of β -cells, which can help prevent the onset of diabetes. Sodium is a vital mineral involved in the transmission of nerve impulses and maintaining the osmotic pressure of body fluids. Deficiency in sodium can lead to dehydration and muscle cramps. Potassium plays a key role in maintaining water and acid-base balance in the body. It's crucial for regulating cardiac rhythm, nerve action, and muscle function. A deficiency in potassium can result in muscle paralysis. Ensuring an adequate intake of these essential minerals through a balanced diet is essential for maintaining good health and preventing various health issues. *R. hypocrateriformis*, due to its mineral content, can indeed be a valuable addition to a diet aimed at supporting these functions and preventing related health conditions⁴⁷⁻⁵⁰. The sample of *R. hypocrateriformis* leaves contained four essential trace elements: Iron (Fe), Copper (Cu), Zinc (Zn), and Manganese (Mn). Among these, manganese plays a significant role in various crucial biological processes. Manganese serves as a cofactor for a variety of enzymes involved in metabolic pathways. These enzymes are essential for processes related to reproduction, growth, and skeletal development. Manganese is involved in the metabolism of various

macronutrients, including carbohydrates, proteins, and cholesterol. It plays a role in breaking down these substances, aiding in their utilization by the body. Manganese is also implicated in the formation of urea, a waste product of protein metabolism that is excreted through urine. Urea plays a vital role in maintaining the body's nitrogen balance. Overall, manganese is a trace element that contributes to the proper functioning of numerous biochemical processes in the body. Its presence in *R. hypocrateriformis* leaves highlights the potential health benefits of including these leaves in one's diet, as they can provide a natural source of this essential mineral to support various metabolic and physiological functions^{51,52}.

The presence of various essential trace elements in the sample, including Iron (Fe), Zinc (Zn), and Copper (Cu), highlights their potential health benefits. Iron is a critical component of hemoglobin (Hb), the molecule responsible for carrying oxygen in the blood. It also plays a role in the formation of tendons and ligaments. Iron deficiency can lead to anemia, characterized by weakness, poor resistance to infection, and, in females, infertility. Therefore, *R. hypocrateriformis* could serve as a valuable source to help improve the condition of individuals with anemia. Zinc is a vital trace element involved in the function of many enzymes. It is essential for brain development, normal growth, bone formation, wound healing, sperm production, and sexual maturation. Given these roles, the sample could potentially be used as a zinc supplement, especially for individuals facing infertility challenges. Copper is a component of various enzymes, including cytochrome oxidase, lysyl oxidase, and ceruloplasmin, which are required for the metabolism of iron in the blood. It is also involved in erythropoiesis (red blood cell production), erythrocyte function, and their survival. A deficiency in copper can lead to cardiac disorders, anemia, and neutropenia. Incorporating the sample containing these trace elements into one's diet may help address specific health concerns related to these minerals, such as anemia, infertility, and overall well-being. However, it's important to consume them in appropriate quantities as part of a balanced diet to ensure optimal health and avoid any potential adverse effects from excessive intake⁵³⁻⁵⁷.

CONCLUSION

The key discovery of this study is the recognition that *R. hypocrateriformis* leaves are a valuable reservoir of essential nutrients, including crude protein, crude fiber, crude ash, potassium, calcium, magnesium, and iron. Remarkably, the levels of anti-nutritional components in *R. hypocrateriformis* are low, and the bioavailability of calcium, iron, and zinc is high. As a result, the cultivation and consumption of *R. hypocrateriformis* are strongly encouraged as an additional source of essential minerals in the diets of indigenous populations.

Given these findings, *R. hypocrateriformis* has great potential for use in fortification, formulation, and supplementation of other food materials. Furthermore, it can be recommended as a natural remedy to combat malnutrition in the region. This versatile plant has the capacity to enhance the nutritional content of diets and contribute significantly to the overall well-being of the local population.

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