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Evaluation of qualitative and quantitative phytochemical screening of *Rivea hypocrateriformis*

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ABSTRACT

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 Thailand. 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. This study aimed to explore the qualitative and quantitative phytochemical analysis of *R. hypocrateriformis* leaves. Qualitative phytochemical analysis identified the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, and tannins while the quantitative phytochemical analysis showed that ethanolic extract is richest in phenolic and flavovonids content. This study on *R. hypocrateriformis* leaves serves as a crucial diagnostic tool for species identification, and the development of quality parameters. The data obtained in this study may be regarded as a reference for future research endeavors.

Keywords: *Rivea hypocrateriformis*, phenolic content, flavonoid content, qualitative analysis, qualitative analysis, etc.

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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 benefits [1-4]

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 requirements [5-7] 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 indirectly. [8-10]

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 medicine[11-13]. 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 Thailand[16] 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 treatment.[19] 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 Pakistan.[20] The current study focuses on investigating the qualitative and quantitative analysis of *R. hypocrateriformis*, and shedding light on its potential health benefits

MATERIALS AND METHOD

Plant Material

R. hypocrateriformis leaves were collected from the outskirt area of Udaipur, Rajasthan. The plant was identified, authenticated, and certified (HIMCOSTE/HPSBB/7098) by Dr. Pankaj Sharma, Himachal Pradesh State Biodiversity Board, Shimla, India.

Preparation of Extracts

Firstly, the plant leaves were washed with water to remove dirt and other foreign matters were separated and shade dried. Dried leaves were then milled to a coarse powder and then passed over sieve No. 14. The obtained dried powdered leaves of *R. hypocrateriformis* (20 g) were placed in the tube of Soxhlet apparatus in the form of a thimble and extracted with various solvents, such as ethyl acetate, methanol, and water (300 mL) at 60–65 °C for 3–4 h. The obtained extracts, respectively, ethyl acetate (EAE), ethanol (EE) and aqueous (AE) extracts, were filtered while hot and dried by evaporation using a rotary vacuum evaporator and the final dried extract samples were kept at low temperature in the fridge for further study. The residue obtained from each extract was dissolved in the same solvent for further analysis.

Qualitative Analysis of the Phytochemicals

Test for Tannins: Each leaves powder sample (0.30 g) was weighed into a test tube and boiled for 10 minutes in a water bath containing 30 cm3 of water. Filtration was carried out after boiling using number 42 (125 mm) Whatman filter paper. To 5 cm3 of the filtrate was added 3 drops of 0.1% ferric chloride. A brownish green or a blue black colouration showed positive test [13].

Test for Saponin: Distilled water (30 cm3) was added to leaves powder samples (0.30 g) and boiled for 10 minutes in water bath and filtered using Whatman filter paper number 42 (125 mm). A mixture of distilled water (5 cm3) and filtrate (10 cm3) was agitated vigorously for a stable

persistent froth. The formation of emulsion on addition of three drops of olive oil showed positive result [21].

Test for Steroid: Each sample (0.30 g) weighed into a beaker was mixed with 20 cm3 of ethanol; the component was extracted for 2 hours. To the ethanolic extract of each sample (5 cm3) was added 2 cm3 acetic anhydride followed with 2 cm3 of concentrated tetraoxosulphate (VI) acid. A violet to blue or green colour change in sample(s) indicates the presence of steroids [13].

Test for Terpenoids: Each leaves powder sample (0.30 g) was weighed into a beaker and extracted with 30 cm3 and component extracted for 2 hours. A mixture of chloroform (2 cm3) and concentrated tetraoxosulphate (VI) acid (3 cm3) was added to 5 cm3 of each extract to form a layer. The presence of a reddish-brown colouration at the interface shows positive results for the presence of terpenoids[13].

Test for Flavonoids: Each sample (0.30 g) weighed into a beaker was extracted with 30 cm3 of distilled water for 2 hours and filtered with Whatman filter paper number 42 (125 mm). To 10 cm3 of the aqueous filtrate of each leaves extract was added 5 cm3 of 1.0 M dilute ammonia solution followed by the addition of 5 cm3 of concentrated tetraoxosulphate (VI) acid. Appearance of yellow colouration which disappeared on standing shows the presence of flavonoids[13].

Test for Alkaloids: Extraction of component from 2 grams of each leaves powder sample was carried out using 5% tetraoxosulphate (VI) acid (H_2SO_4) (20 cm3) in 50% ethanol by boiling for 2 minutes and filtered through Whatman filter paper number 42 (125 mm). The filtrate was made alkaline using 5 cm3 of 28% ammonia solution (NH_3) in a separating funnel. Equal volume of chloroform (5.0 cm3) was used in further solution extraction in which chloroform solution was extracted with two 5 cm3 portions of 1.0 M dilute tetraoxosulphate (VI) acid. This final acid extract was then used to carry out the following test: 0.5 cm3 of Dragendorff ’s reagent (Bismuth potassium iodide solution) was mixed with 2 cm3 of acid extract and precipitated orange colour infers the presence of alkaloid [13].

Test for Glycoside: To 2.00 g of each sample was added 20 cm^3 of water, heated for 5 minutes on a water bath and filtered through Gem filter paper (12.5 cm). The following tests were carried out with the filtrate:

(a) 0.2 cm3 of Fehling’s solutions A and B was mixed with 5 cm^3 of the filtrate until it became alkaline (tested with litmus paper). A brick-red colouration on heating showed a positive result.

(b) Instead of water, 15 cm3 of 1.0 M sulphuric acid was used to repeat the above test and the quantity of precipitate obtained compared with that of (a) above. High precipitate content indicates the presence of glycoside while low content shows the absence of glycoside [13].

Quantitative Analysis of the Phytochemicals

Total Polyphenols and Flavonoid Contents

The total phenolic content (TPC) and flavonoid (TFC) content of each *R. hypocrateriformis* leaf extracts were determined using the earlier reported method. TPC was expressed as mg of gallic acid equivalent (GAE) per 100 g of extract, while the TFC was expressed as mg of quercetin equivalents (QE) per 100 g [21].

RESULTS AND DISCUSSION

Qualitative phytochemical screening

Various phytochemical analysis tests supported that the extracts contain alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins and glycosides, recorded in Table 3. The aqueous extract was found to be negative for the presence of alkaloids as compared to methanolic and ethyl acetate extract.

Table 1: Results of phytochemical screening of different extracts of *R. hypocrateriformis* leaves extracts.

Phytoconstituents	Leaf Extracts		
	Ethyl Acetate	Ethanol	Aqueous
Alkaloids	Present	Present	Present
Glycosides	Present	Absent	Present
Flavonoids	Present	Absent	Present
Phenolic compounds	Present	Present	Present
Tannins	Present	Absent	Present
Saponins	Present	Absent	Present
Steroids	Present	Absent	Present

Qualitative phytochemical screening

Total Phenolic Content (TPC)

Total phenolic content is the method selected to determine the phenolic level in plant extracts. These phenolic compounds possess redox properties, which allow them to act as potential antioxidants. As a basis, phenolic content was measured using the Folin–Ciocalteu reagent in each extract. The results were expressed as gallic acid equivalents (GAE) per gram of dry extract weight (**Table 1**). The results indicate that the ME exhibited higher TPC comparatively to the EAE and AE, which were about 84.94±0.22 mg GAE/g for EE, 65.23±0.13 mg GAE/g for EAE, and 61.12±0.15 mg GAE/g for AE. TPC was calculated using the following linear equation, based on the calibration curve of gallic acid ($y = 0.006x + 0.459$, $R^2 = 0.981$).

Total Flavonoid Content (TFC)

Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups. As a basis, the quantitative determination of the flavonoid contents in selected plant extracts were determined using aluminum chloride in a colorimetric system. The outcomes were expressed as quercetin equivalents (QE) per gram of dry extract weight. The results showed that the EE exhibited higher TFC as compared to the EAE and AE, being approximately about 63.34 ± 0.11 mg GAE/g for EE, 44.64 ± 0.14 mg QE/g for EAE, and 33.45 ± 0.13 mg GAE/g for AE. TFC was calculated using the following linear equation, based on the calibration curve of quercetin ($y = 0.006x + 0.351$, $R^2 = 0.986$).

Table 2: Total phenolic and flavonoid content

Extracts	Phenolic Content (mg/g GAE)	Flavonoid (mg/g QE)
EE	84.94 ± 0.22^a	64.34 ± 0.11^a
EAE	65.23 ± 0.13^b	44.64 ± 0.14^b
AE	61.12 ± 0.15^c	33.45 ± 0.13^c

Ell values represent means ± SEM of three replicates. EE: Ethanolic extract; EAE: Ethyl acetate extract; AE: Aqueous extract. Statistical significance was determined at $p < 0.05$ and is indicated with different letters.

CONCLUSION

The findings of the present study, encompassing both qualitative and quantitative analyses, provide significant insights into the composition of the ethanolic extracts. This research has unveiled the presence of a wide array of secondary metabolites within these extracts, including alkaloids, phenolic compounds, glycosides, flavonoids, and tannins. In particular, the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) analyses underscore the remarkable richness of these ethanolic extracts in secondary metabolites. This implies that the ethanolic extracts contain a substantial amount of phenolic compounds and flavonoids, making them a valuable source of these bioactive substances. The ethanolic extracts are a rich source of secondary metabolites, particularly alkaloids, phenolic compounds, glycosides, flavonoids, and tannins. The high TPC and TFC values highlight their potential for various applications, emphasizing the need for further research to unlock their therapeutic and commercial potential.

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