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Phytoconstituents, GC-MS Characterization of Omega Fatty Acids, and Antioxidant Potential of Less-Known Plant *Rivina humilis* L.

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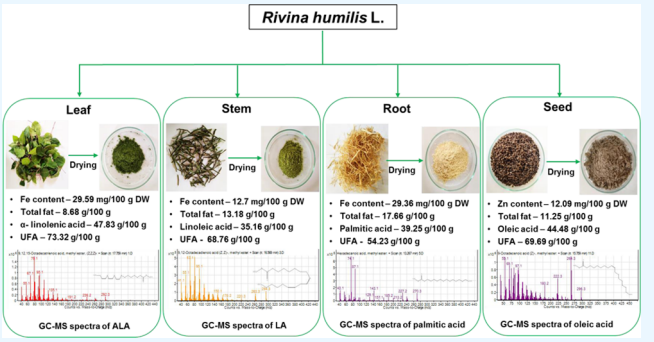
ABSTRACT: *Rivina humilis* L. (Petiveriaceae), commonly known as the pigeon berry, accumulates betalains in berries. The present study was focused on identifying the phytoconstituents, mineral content, fatty acid composition, phenolics, flavonoids, antinutritional factors, and antioxidant activities of different plant parts (leaf, stem, root, and seeds), which are otherwise not well explored. Phytoconstituent analysis revealed seeds as a potential source of carbohydrates (50.15 g/100 g), proteins (10.96 g/100 g), and fats (11.25 g/100 g). Roots showed the highest fat (17.66 g/100 g) and dietary fiber (81.49 g/100 g). Leaves and roots contain more iron (29.59 and 29.39 mg/100 g), whereas seed has high zinc content (12.09 mg/100 g). Leaf oil showed 47.83 g/100 g of omega-3-fatty acid, confirmed by GC-MS analysis. Seed oil showed 22.23 g/100, 44.48 g/100, and 24.04 g/100 g of palmitic, oleic, and linoleic acids, respectively. The leaf extract has the highest TPC (597.55 mg/100 g), followed by the seed (421.68 mg/100 g). The leaf's 80% ethanolic extract had high TFC (2442.19 mg/100 g), followed by 70% methanolic extract (1566.25 mg/100 g). The antinutritional profile indicated significant phytic acid and oxalates in the leaf (9.3 g/100 and 2.07 g/100 g) and stem (6.9 and 1.58 g/100 g) and low tannin content (<0.5 g/100 g). The leaf's 80% ethanolic extract exhibited double the TAA than 70% methanolic extract (1.52 g/100 g). The leaf with an 80% ethanolic extract had the lowest DPPH and ABTS radical scavenging EC_{50} (2.22 and 0.37 mg/mL). The leaf with an 80% ethanolic extract (479.73 mg/100 g) and seed (391.14 mg/100 g) had the highest FRAP activity. Our study proves that different parts of *R. humilis* had a good content of phytoconstituents, bioactives, and antioxidant activities. Hence, *R. humilis* leaves and seeds are a novel source of omega fatty acids and minerals reported for the first time and have potential applications in the food and pharmaceutical industries.



1. INTRODUCTION

Year in and year out, the journey of human interaction with plants has spread widely into various disciplines, such as drug development, dietary supplements or nutraceuticals, functional or medicinal food, and even the production of recombinant proteins.[1] Though 40−100,000 plant species have been frequently used for food, fiber, shelter, commercial, cultural, and medicinal purposes, only a small number of plants are widely used as food and therapeutic sources worldwide.[2] The remaining plant diversity is considered underutilized or unexplored.[2]

Rivina humilis L. comes from the family of Phytolaccaceae or Petiveriaceae and belongs to the order Caryophyllales.[3] It is commonly known as pigeon berry or blood berry. It is a perennial, ornamental, wild, diffusely branched, hairy herb that grows on various shaded soils. It grows up to a height of 120 cm (4 ft). This plant is native to the Caribbean and tropical America and is now widely naturalized in Indo-Malaysia and Pacific regions.[4] Leaf lamina is ovate, ovate-lanceolate, elliptic, or oblong, 5−14 × 2−9 cm^2, variously hairy on both surfaces and often glabrescent. Berries are magnificent in appearance as bright scarlet, glossy red, shining yellow, sweet orange, pink, or even pure white.[5] The seeds appear hairy and are sometimes described as pubescent.[6] The plant has been explored for the accumulation of betalain pigments, their safety, stabilization, and application in several food formulations with a 68% betalain retention for a 6 month shelf-life.[7−10] *R. humilis*, a perennial and quickly grown plant, could be a good source of valuable phytoconstituents and bioactives. As this plant is not much explored except its berries as a good source of betalains, there is enormous potential envisaged once its phytoconstituents are investigated to reap economic prospects.

Plant parts, such as the leaves, were used to cure wounds in Mexico.[11] In Jamaica, this herb is used for infertility, womb-related problems, and issues related to menstruation flow.[11] A decoction of stem and leaf is consumed orally against the cold

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by the Tribe Amerindians of Bocas del Toro Province in Panama.[12] In Eastern Mexico, the leaf is used against skin diseases.[13]

Studies on an unexplored plant's phytoconstituent composition and natural bioactives could open doors for its utilization in various disciplines. Each plant species has its bioactive fingerprinting and phytoconstituents that are important for the physiological functions of the human body.[14] Natural bioactive compounds play a central role in combating many human diseases. A recent study shows that most of the world's population relies on herbal medicines for their primary health care and other needs.[15] This further signifies the role of exploration of new plant sources rich in phytoconstituents and bioactive compounds.

These unexplored plant parts also supplement and accumulate essential fatty acids (omega-3, omega-6, and omega-9 FAs), glycolipids, and phytosterols with beneficial health effects.[16] The antioxidant activity of plant extracts is believed to be mediated mainly through phenolics, flavonoids, tannins, carotenoids, etc. Due to their redox properties, they could act as reducing agents, electron donors, and singlet or triplet oxygen scavengers.[17] The complex nature of phytochemicals and the involvement of multiple reaction mechanisms create difficulty in the validation of the antioxidant capacity of plant extracts by any single method. Therefore, relying on a combination of assays is recommended to provide precise information on the antioxidant properties.[18]

Accordingly, the present study focused on exploring *R. humilis* plant parts like leaf, stem, root, and seed regarding their phytoconstituents, mineral content, pigments, bioactive compounds, GC-MS profiling of fatty acids, antinutrients, and antioxidant activities for pharmaceutical applications. To the best of our knowledge, this could be the first comprehensive report on the phytoconstituents, bioactive content, omega fatty acid profiling, and antioxidant activities of *R. humilis* plant parts.

2. MATERIALS AND METHODS

2.1. Chemicals. Various chemicals of analytical grade like hydrochloric acid (HCl), sulfuric acid (H_2SO_4), nitric acid (HNO_3), phosphoric acid (H_3PO_4), sodium tungstate ($Na_2WO_4{\cdot}2H_2O$), sodium carbonate (Na_2CO_3), ammonium molybdate ($(NH_4)_6Mo_7O_{24}$), ferric chloride ($FeCl_3$), sodium chloride (NaCl), potassium ferricyanide ($K_3Fe(CN)_6$), potassium permanganate ($KMnO_4$), sodium phosphate buffer, sulfosalicylic acid, trichloroacetic acid (TCA), and potassium persulfate ($K_2S_2O_8$) were obtained from Sisco Research Laboratory (Mumbai, India). Liquified phenol, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), boron trifluoride-methanol (BF_3-methanol), tannic acid, gallic acid, rutin, aluminum chloride ($AlCl_3$), and heptadecanoic acid were purchased from Sigma-Aldrich (Bangalore, India). 2,2-Diphenyl-picrylhydrazyl (DPPH), 3,5-dinitrosalicylic acid, bovine serum albumin, methyl orange indicator, calcium chloride, phytic acid, folin-Ciocalteau's reagent, and ascorbic acid were obtained from Hi-Media Labs, Mumbai, India. Solvents like acetone, n-hexane, methanol, and absolute ethanol were procured from Merck (Mumbai, India).

2.2. Plant Material. The whole *Rivina humilis* L. plants were collected during August−November 2020 from shady areas of the environs of CSIR-CFTRI, Mysore (India), located geographically between 12°18′26″ North latitude and 76°38′59″ East longitude. The specimens of these plants were deposited at the herbarium center of the University of Mysore (Reference No. UOMBOT21RH07). The leaf, tender stem, and root were separated from each other. Seeds were obtained by manually deseeding the berries. The collected plant parts were washed thoroughly in running tap water, blotted well, and kept in an oven at 45 °C for drying. All of the samples were ground separately into fine powders (Philips, 750W) and stored at room temperature in polythene covers until further analysis.

2.3. Phytoconstituent Composition. The moisture content in the dried plant powders was analyzed using a moisture analyzer (Sartorius, MA 160). The phenol sulfuric acid method with the standard curve plotted against glucose was used to determine the total carbohydrate content.[19] The total reducing sugar was estimated using the DNS reagent. Proteins from the plant powders were extracted using sodium phosphate buffer (pH 6.5). Lowry's method was used to estimate the same against the standard, bovine serum albumin. The dried plant powders (5 g each) were extracted in n-hexane with a Soxhlet apparatus for 8 hours. The obtained solvent fraction was dried completely using a rotary evaporator (Hei-VAP Advantage, Heidolph Instrument GmbH & Co. KG, Schwabach, Germany). The difference in the weight of the flask was taken as the oil yield.[20] The dietary fiber content in dry-defatted plant powders was estimated using the enzymatic method.[21]

The total calorie content was determined empirically as follows[22]

$$\text{total calorie content} = 4 \times \text{percentage of proteins} + 9 \times \text{percentage of lipids} + 4 \times \text{percentage of carbohydrates}$$

A known quantity of dried plant powders was taken in crucibles (empty weight noted) and charred well before incinerating in a muffle furnace at 550 °C (8 h) for ash estimation. The weight of the ash-containing crucible was recorded after cooling to room temperature. The percentage of ash content was calculated using the formula as follows[23]

$$\text{ash content} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100\%$$

2.4. Estimation of Chlorophylls and Carotenoids. Chlorophylls and carotenoid content in various plant parts were determined following the standard method reported.[24] 0.5 g of dried plant powders were extracted with 80% acetone, and the extracts were centrifuged at 3000*g* for 10 min. The clear supernatant's absorbance was read at 450, 645, and 661.5 nm using a UV−visible spectrophotometer (Genesys 150, Thermo Scientific). The concentration of chlorophyll a (chl a), chlorophyll b (chl b), total chlorophyll (chl t), and carotenoids was calculated using the following equations[24]

$$\text{chl a} = 11.24\, A_{661.5} - 2.04\, A_{645}$$

$$\text{chl b} = 20.13\, A_{645} - 4.19 A_{661.5}$$

$$\text{chl t} = 7.05 A_{661.5} + 18.09\, A_{645}$$

$$\text{total carotenoids} = \frac{1000 \times A_{450} - [1.9 \times \text{chl a} + 63.14 \times \text{chl b}]}{214}$$

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where "A" denotes absorbance at different nanometers such as 450, 645, and 661.5 nm. The values in the equation are the constant factors to calculate the chlorophyll and carotenoid content.

2.5. Color Evaluation. Color values such as L^*, a^*, and b^* of the dried plant powders were recorded in quadruplicate using a color measuring instrument (Konica Minolta CM-5). Chroma (C^*) and hue angle (h^*) values were calculated using the following formula[25]

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

where C^*, a^*, b^*, and h^* indicate chroma, red/green, yellow/blue, and hue angle, respectively.

2.6. Elemental Analysis. Dried plant powders of approximately 5 mg were filled in a tin capsule and subjected to an elemental analyzer (Elemental Analyzer, GmbH VarioEL V3.00) to determine the carbon, hydrogen, nitrogen, and sulfur (CHNS) contents. Sulfanilic acid was used as the standard compound. The contents in the samples were expressed in g/100 g DW.

2.7. Mineral Analysis. Minerals were estimated by a microwave plasma atomic emission spectrometer (model-4210MP-AES of Agilent). The advanced valve system (AVS)-4 was fully integrated with the 4210 MP-AES instrument hardware and controlled through the MP Expert software. The emission lines for the selected minerals were set at 213.857 nm (zinc), 371.993 (iron), 324.754 nm (copper), 285.213 nm (magnesium), 588.995 nm (sodium), 766.491 nm (potassium), and 393.366 nm (calcium) using a charge-coupled device (CCD) detector view mode. 0.5 g of finely ground dried leaf, stem, root, and seed powders were incinerated in a muffle furnace for 8 h at 550 °C to ensure complete carbon burning. The ash obtained was taken in aqua regia. Zinc, iron, copper, magnesium, sodium, potassium, and calcium were analyzed after diluting with a respective acid solution (Agilent calibration mineral mix solution). The minerals were quantified using a reference standard mixture (Agilent Technologies, Mumbai, India).

2.8. Fatty Acid Estimation. Fatty acid methyl esters (FAMEs) of the extracted oil were prepared by trans-esterification.[26] Briefly, 50 μL of the oil was taken in a glass tube with heptadecanoic acid (C17:0) as the internal standard. 1 mL of BF_3 methanol was added and incubated for 30 min at 60 °C. The tubes were immediately transferred to the ice for 5 min followed by a 10 min incubation at room temperature. *n*-Hexane and distilled water (1:1) were added and vortexed vigorously. Finally, the undisturbed methyl ester layer was passed through anhydrous sodium sulfate and transferred to GC vials. GC-MS analysis was performed using an Agilent Technologies 7890B chromatograph connected directly to a 5977A inert mass spectrometer (Agilent Technologies, Milan, Italy), with GC column, DB-23 (60 m, 0.25 mm ID 0.25 mm film thickness) in splitless mode (0.5 min) with an inlet temperature at 250 °C and the carrier gas helium at a flow rate of 1 mL/min. The temperature was programmed to increase at a rate of 10 °C/min to 300 °C and then to remain isothermal at 300 °C for 5 min. The mass spectrometer detector was operated in electron ionization (EI) mode (70 eV, 200 mA), full-scan mode (m/z 40−400), and selected-ion monitoring (SIM) mode (ions at m/z 127, 140, and 256 for heptadecanoic acid as the internal standard).

2.9. Determination of Total Phenolic and Flavonoid Contents. Phenolic and flavonoid contents were estimated in three different solvent extractions. Briefly, a known quantity of the dried powders of leaf, stem, root, and seed was extracted using a mortar and pestle in three different solvent systems such as 80% ethanol (80% E), 70% methanol (70% M), and distilled water (W). The extractions were carried out until the pellet became colorless and were centrifuged at 5000 rpm for 10 min, and the supernatants were pooled together and stored at −20°C until analysis.

Folin−Ciocalteau's method was used to determine total phenolic content (TPC).[27] A known volume of the extractives was taken in a series of test tubes and made up to 3 mL using distilled water. Folin−Ciocalteau's reagent was mixed with distilled water in a 1:1 ratio, and 0.5 mL of this solution was added to each test tube, followed by incubation in the dark for 3 min. Afterward, 2 mL of 20% Na_2CO_3 was added to this mixture, vortexed thoroughly, and placed in a boiling water bath for 1 min. The absorbance of the reaction mixture was recorded at 650 nm once the solutions were brought back to room temperature. The gallic acid (0.1 mg/mL) was used as the standard compound, and the unknown samples were calculated based on the linearity curve.

A 2% aluminum chloride solution in methanol was prepared to estimate total flavonoid content (TFC). Subsequently, it was mixed with 1 mL of the diluted extracts, 1 mL of aluminum chloride reagent was added, incubated in the dark for 15 min, and the absorbance was measured at 430 nm.[28]

2.10. Determination of Antinutritional Factors. The tannin content was determined according to the earlier method with slight modification.[29] Briefly, 0.5 g of dried plant parts' powders were extracted with 70% methanol containing 0.1% HCl. Samples were centrifuged, and a known volume was made up to 3 mL with distilled water. 0.25 mL of the Folin−Denis reagent was added, followed by 0.5 mL of 30% sodium carbonate. The reaction mixture was incubated at room temperature for 30 min. The color developed was measured at 700 nm. Tannic acid was used as the standard.

The phytic acid content was determined based on the method reported.[30] A 2.4% HCl was added to a known amount of plant parts' powders taken in a conical flask and kept for 16 h of incubation with constant shaking. The solution was filtered, adding 1 g of sodium chloride to the filtrate. This was held for 20 min of shaking, and the supernatant was collected after centrifugation. A known volume of the supernatant obtained was diluted to 3 mL with distilled water, and 1 mL of wade reagent (0.03% $FeCl_3{\cdot}6H_2O$ + 0.3% sulfosalicylic acid) was added to this. A control was prepared without the addition of a sample. The absorbance of control, samples, and standard (phytic acid 1 mg/mL) was read at 500 nm.

The oxalate content was estimated based on the method detailed earlier.[31] In brief, 0.5 g of sample, 90 mL of distilled water, and 10 mL of 6 N HCl were refluxed for 1 h. This was made up to 150 mL, and a 50 mL aliquot was separated for further analysis. To this, 10 mL of 6 N H_2SO_4 was added, and the volume was reduced to half by heating on a mantle. The solution was filtered and washed with hot distilled water. The residue was discarded. Two (2) drops of methyl orange indicator were added to the filtrate and titrated against concentrated ammonia until the color changed to faint yellow.

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Table 1. Phytoconstituents, Pigment Content, and Color Values of Different Plant Parts of *R. humilis*[a]

	leaf	stem	root	seed
		Nutrient Composition (g/100 g DW)		
moisture (%)	6.91 ± 0.86^{ab}	7.99 ± 0.16^{b}	7.55 ± 0.10^{ab}	6.73 ± 0.86a
total carbohydrate	17.34 ± 0.21a	26.59 ± 0.22^{b}	37.25 ± 0.69^{c}	50.15 ± 1.93^{d}
total reducing sugar	2.32 ± 0.05a	1.26 ± 0.02^{b}	0.97 ± 0.01^{c}	1.73 ± 0.03^{d}
*total nonreducing sugar	15 ± 0.34a	25.33 ± 0.24^{b}	36.29 ± 0.68^{c}	48.42 ± 1.96^{d}
total protein	7.11 ± 0.12a	3.02 ± 0.06^{b}	2.34 ± 0.22^{c}	10.96 ± 0.16^{d}
total fat	8.68 ± 0.47a	13.18 ± 0.66^{b}	17.66 ± 0.45^{c}	11.25 ± 0.07^{d}
total dietary fiber	43.09 ± 1.32a	56.70 ± 0.18^{b}	81.49 ± 0.06^{c}	36.61 ± 0.20^{d}
soluble dietary fiber	4.36 ± 0.03a	5.08 ± 0.08^{b}	2.92 ± 0.08^{c}	2.62 ± 0.10^{d}
insoluble dietary fiber	38.73 ± 1.35a	51.62 ± 0.09^{b}	78.56 ± 0.02^{c}	33.99 ± 0.10^{d}
total calorie content	174.67 ± 2.74a	236.69 ± 5.25^{b}	315.53 ± 6.86^{c}	347.87 ± 9.50^{d}
ash content	17.87 ± 0.05a	14.80 ± 0.33^{b}	4.75 ± 0.17^{c}	1.87 ± 0.06^{d}
		Pigments (mg/100 g DW)		
chlorophyll a	627.3 ± 11.53a	45.43 ± 3.18^{b}	1.94 ± 0.03^{c}	0.49 ± 0.1^{c}
chlorophyll b	415.63 ± 6.46a	48.91 ± 3.95^{b}	1.24 ± 0.03^{c}	1.03 ± 0.17^{c}
total chlorophyll	1042.93 ±17.8a	94.35 ± 7.1^{b}	3.18 ± 0.06^{c}	1.52 ± 0.27^{c}
total carotenoids	152.32 ± 2.62a	19.87 ± 1.51^{b}	1.86 ± 0.13^{c}	3.24 ± 0.08^{c}
		Color Values		
L^*	49.91 ± 0.37a	65.09 ± 0.26^{b}	78.43 ± 0.36^{c}	45.67 ± 0.40^{d}
a^*	−3.17 ± 0.07a	−1.66 ± 0.04^{b}	2.71 ± 0.07^{c}	2.86 ± 0.09^{c}
b^*	18.84 ± 0.32a	20.67 ± 0.09^{b}	18.78 ± 0.39a	7.35 ± 0.29^{c}
chroma	19.10 ± 0.32a	20.73 ± 0.09^{b}	18.97 ± 0.38a	7.89 ± 0.31^{c}
hue angle	−1.40 ± 0.001a	−1.49 ± 0.002^{b}	1.43 ± 0.004^{c}	1.20 ± 0.003^{d}

[a]Note: *total nonreducing sugar content was obtained by subtracting the total reducing sugar content from the total carbohydrate content. Values represented are mean ± SD of three replicates (n = 3). Significance was tested by post hoc Duncan's test at $p < 0.05$, and values with the same superscript were not found to be significantly different from each other.

Then, the solution was heated to a boil, and 10 mL of 5% $CaCl_2$ was added upon constant stirring. After 10 min, 6 N ammonium hydroxide was added until the color changed, and the solution was kept overnight for precipitation of calcium oxalate crystals. The solution was filtered, and the precipitate was washed with hot diluted H_2SO_4. The solution was made up to 125 mL and titrated against 0.05 N $KMnO_4$. The appearance of light pink color was considered the endpoint. The oxalate content was calculated using the following formula, and the results are expressed in g/100 g DW OAEq[31]

$$\text{oxalate content} = \frac{\text{volume of } KMnO_4 \text{ consumed} \times 0.05 \times 45.02 \times 100 \times 3}{1000 \times \text{weight of sample (g)}}$$

where 0.05 indicates the normality of $KMnO_4$, 45.02 indicates the equivalent weight of oxalic acid, and 3 indicates the dilution factor.

2.11. *In Vitro* Antioxidant Assays. *2.11.1. Total Antioxidant Activity (TAA) by the Phosphomolybdenum Method.* The TAA of the prepared extracts was determined according to the phosphomolybdenum method.[32] Briefly, the reagent was prepared by combining 4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M H_2SO_4. A 3 mL of this reagent was added to 0.3 mL of each extract (80% ethanol, 70% methanol, and water) and incubated at 95 °C for 90 min. After cooling it down, the absorbance was recorded at 695 nm. Ascorbic acid was used as the standard, and the results are expressed as g/100 g DW AAEq. of the sample.

2.11.2. DPPH Free-Radical Scavenging Activity. The sample extracts were tested for DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity.[33] DPPH is a purple-colored stable free radical that turns yellow when scavenged. The antioxidants present in the samples can donate H^+ or e^- to DPPH and convert it into DPPH-H, which results in color change.[34] The degree of discoloration directly indicates the scavenging potential of the sample extracts. Ascorbic acid (1 mg/mL) was the standard antioxidant compound used. Briefly, 0.1 mM of DPPH stock solution was prepared in methanol. Five different volumes (10−100 μL) of samples in a concentration range of 0.5−15 mg/mL from all three extracts (80% ethanol, 70% methanol, and water) were taken for analysis. Volumes were made up to 100 μL using methanol, and 1.9 mL of DPPH stock solution was added to it. This reaction mixture was further incubated in the dark at room temperature for 15 min. The absorbance was taken at 517 nm with methanol as the blank. The percentage of the DPPH radical scavenging activity and EC_{50} values of the samples were determined using the following equation[34]

$$\text{DPPH scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

where A_{control} and A_{sample} denote the absorbance of the control and sample, respectively.

2.11.3. ABTS Free-Radical Scavenging Activity. ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) undergoes oxidation when treated with potassium persulfate, forming $ABTS^+$ free radical. Hence, the $ABTS^+$ free-radical cation was pregenerated by mixing 7.4 mM ABTS stock with 2.45 mM potassium persulfate and further incubating it at room temperature in the dark for 16 h. Upon the reaction's completion, the reagent's absorbance was adjusted to 0.7 ± 0.01 at 734 nm using methanol as the blank. The different concentrations of the extracts were treated with this solution, and the absorbance was recorded after 6 min of incubation in the dark.[35] The percentage of the $ABTS^+$ radical scavenging

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activity was determined using the following formula. The ability of the test sample to scavenge $ABTS^+$ radical cation was compared with the ascorbic acid standard, and the results were expressed in terms of mg EC_{50}/mL[36]

$$\text{ABTS scavenging activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

where A_{control} and A_{sample} are the absorbance of the control and sample, respectively.

2.11.4. Ferric-Reducing Antioxidant Power. Ferric-reducing antioxidant power of the extract prepared was assessed based on the method detailed earlier.[37] Briefly, the extracts were mixed with 0.2 M phosphate buffer of pH 6.6 and 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 30 min. Trichloroacetic acid (TCA) (10% w/v) was added after cooling and centrifuged at 8000 rpm for 10 min. From the supernatant, the upper layer was separated and mixed with an equal volume of distilled water. The absorbance was measured at 700 nm after adding 0.1% of freshly prepared ferric chloride. The FRAP activity was expressed as equivalent to the standard ascorbic acid in mg/100 g of DW AAEq.

2.12. Statistical Analysis. All values presented are mean ± SD of three analytical replicates. Data were subjected to one-way ANOVA followed by post hoc Duncan's test using SPSS 16 (SPSS Inc., Chicago, IL) for determining significant differences. A difference was considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Phytoconstituent Composition. The primary phytoconstituent composition of *R. humilis* plant parts was studied by analyzing the proximate content such as total carbohydrates, reducing sugar, nonreducing sugar, proteins, dietary fiber, and lipids (Table 1). The moisture content of different plant powders was 6.73−7.99%, which is within the acceptable limit of about 6−15% for most herbal powders to consider as Ayurveda medicine. Low moisture content reduces the activities of hydrolytic enzymes, which may destroy the active components and reduce the proliferation of microbial colonies, thereby minimizing the chance of spoilage due to microbial attack.[38] The highest total carbohydrate content was observed in seed (50.15 g/100 g DW), whereas the lowest was in leaf (17.34 g/100 g DW). A similar high carbohydrate content was reported in *Canavalia gladiata* cotyledon (53 g/100 g DW).[39]

Plant carbohydrates mainly serve as a source of energy and aid in digestion and the assimilation of other nutrients. The total reducing sugar content was calculated using the standard curve obtained for glucose. The total reducing sugar content was maximum in the leaf (2.32 g/100 g), whereas it was minimum in the root (0.97 g/100 g). The standard curve obtained for bovine serum albumin ($y = 0.0018x + 0.0096$, $R^2 = 0.993$) was used to estimate the total protein content. The highest protein content was observed in the seed (10.96 g/100 g), followed by the leaf sample (7.11 g/100 g), stem (3.02 g/100 g), and root (2.34 g/100 g). The total fat content was highest in the root (17.66 g/100 g), followed by the stem (13.18 g/100 g), seed (11.25 g/100 g), and leaf (8.68 g/100 g).

The World Health Organization (WHO) has recommended an intake of 22−23 g of fiber for every 1000 kcal of diet.[40] Dietary fiber consumption prevents hemorrhoids, hypercholesterolemia, and constipation. In addition to their nutritional benefits, dietary fibers are highly valued for their functional and technological qualities.[41] Based on the nature of dietary fiber and its health benefits, the total dietary fiber can be classified into two subcategories, i.e., insoluble (cellulose, hemicellulose, and lignin) and soluble (water-soluble mucilages, gums, and pectins). The total dietary fiber (TDF) content in *R. humilis* varied from 36.61 g/100 g (seed) to 81.49 g/100 g (root). A similar high content of TDF was reported in the forced roots of cultivars of Belgian endive (80−84 g/100 g), *Arachis hypogaea* roots (78 g/100 g), and *Ipomoea batatas* root (75.19 g/100 g).[42−44] The TDF in seed (36.61 g/100 g) was similar to that of chia seeds (30.20−34.40 g/100 g), whereas it was much higher than that of flax seeds (8.02−18.07 g/100 g).[45] The soluble dietary fiber (SDF) content was observed from 2.62 g/100 g (seed) to 5.08 g/100 g (stem). SDF and IDF complement one another in terms of health rewards. SDF binds to blood cholesterol and reduces intestinal absorption, whereas IDF regulates water absorption and intestinal control.[46]

The total ash content, which represents physiological and nonphysiological ash, is a diagnostic purity index or degree of inorganic matter.[47] The ash content was highest in the *R. humilis* leaf (17.87 g/100 g) sample. The ash content in the stem, root, and seeds of *R. humilis* was 14.80 g/100 g, 4.75 g/100 g, and 1.87 g/100 g, respectively. A similar content was observed in *Vernonia amygdalina* leaf (17.13 g/100 g).[48]

3.2. Estimation of Chlorophyll and Carotenoids. The chlorophyll and carotenoid content results estimated through the Litchenthaler method were expressed in mg/100 g DW (Table 1). Leaf showed the highest total chlorophyll content (1042.93 mg/100 g) with a significant contribution of chlorophyll a (627.3 mg/100 g). Less chlorophyll was observed in the stem (94.35 mg/100 g). Similarly, the carotenoid content was also high in the leaf (152.32 mg/100 g), followed by the stem (19.87 mg/100 g), seed (3.24 mg/100 g), and root (1.86 mg/100 g). Various studies indicated that carotenoids may prevent or inhibit certain types of cancer, atherosclerosis, age-related muscular degeneration, and other diseases. Besides that, their conjugated double-bonded structure could delocalize unpaired electrons resulting in their efficiency in antioxidant activity.[49]

3.3. Color Evaluation. Color measurement could indirectly measure other quality attributes such as flavor and pigment content because it is simpler, faster, and correlates well with different physicochemical properties.[25] The color differences of the dried powders of leaf, stem, root, and seed are presented in Table 1. The L^* values indicate the lightness of the sample. The root powder was lighter, and the values were nearer to lightness (78.43) when compared to the value of the white standard (98.79). The a^* values indicate the red (positive values) and green (negative values) color interpretations of the tested samples. The leaf sample (−3.17) was greener in color than the stem (−1.66), justifying the difference in the chlorophyll content. Similarly, yellowish samples are denoted by a positive b^* value, whereas its negative value denotes blue. The b^* values of leaf, stem, and root powders were 18.78−20.67, indicating its affinity toward yellowish color. This could be correlated with the carotenoid content in the samples. Chroma is considered the quantitative attribute of colorfulness. The higher the chroma values, the higher the color intensity of samples perceived by humans. Leaf, stem, and root powders showed a similar range of chroma

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Table 2. Elemental and Mineral Analysis in Different Plant Parts of *R. humilis*[a]

	leaf	stem	root	seed
		Elemental Analysis (g/100 g DW)		
carbon	42.84 ± 0.26[a]	40.18 ± 0.92[b]	45.47 ± 0.66[c]	53.50 ± 0.89[d]
hydrogen	5.25 ± 0.11[a]	5.99 ± 0.01[b]	7.04 ± 0.06[c]	8.06 ± 0.10[d]
nitrogen	4.93 ± 0.26[a]	1.98 ± 0.03[b]	3.19 ± 0.01[c]	6.21 ± 0.13[d]
sulfur	0.50 ± 0.04[a]	0.69 ± 0.05[b]	0.46 ± 0.06[a]	0.63 ± 0.01[c]
		Mineral Analysis (mg/100 g DW)		
iron	29.59 ± 0.31[a]	12.7 ± 0.24[b]	29.36 ± 0.3[a]	11.92 ± 0.01[c]
zinc	2.83 ± 0.1[a]	0.51 ± 0.06[b]	1.38 ± 0.19[c]	12.09 ± 0.04[d]
copper	1.3 ± 0.12[a]	0.52 ± 0.01[b]	0.6 ± 0.01[c]	3.05 ± 0.03[d]
sodium	26.88 ± 1.51[a]	6.61 ± 0.46[b]	149.33 ± 2.46c	164.78 ± 9.93[d]
potassium	6556.65 ± 8.84[a]	6629 ± 66.5[b]	939.93 ± 6.97[c]	144.13 ± 7.91[d]
magnesium	815.02 ± 4.56[a]	434.41 ± 2.3[b]	221.66 ± 6.62[c]	103.43 ± 1.09[d]
calcium	3141.9 ± 46.1[a]	2628.58 ±33.2[b]	807.3 ± 10.04[c]	930.38 ± 17.68[d]

[a]Note: values represented are mean ± SD of three replicates ($n = 3$). Significance was tested by post hoc Duncan's test at $p < 0.05$, and values with the same superscript were not found to be significantly different from each other.

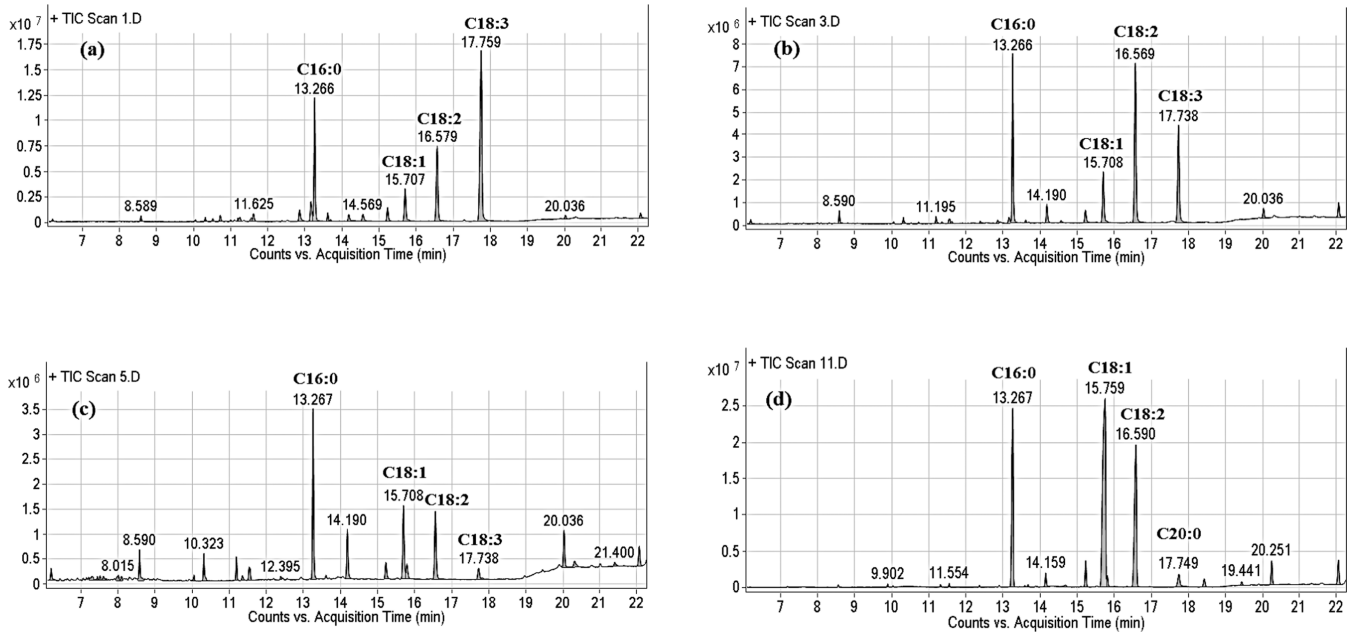

Figure 1. GC-MS quantification profile of individual fatty acid contents: (a) leaf, (b) stem, (c) root, and (d) seed.

values (18−20), whereas seed powder showed a chroma value of 7.89, which could be due to the interference of the black seed coat. The Hue angle, which considers the qualitative attribute of the color, was in the range of −1.40 to +1.43. Similar variations in color measurement values of *H. sabdariffa* leaf under different drying conditions and its pigment (chlorophyll and carotenoids) content have been reported.[37]

3.4. Elemental Analysis. Elemental analysis provides a convenient method for quantifying the weight percentage of major organic elements such as carbon, hydrogen, nitrogen, and sulfur (CHNS) contents by combustion of the sample. The CHNS content of different plant parts was analyzed using sulfanilic acid as the standard (Table 2). Seed possessed the highest carbon (53.50 g/100 g), hydrogen (8.06 g/100 g), and nitrogen (6.21 g/100 g) contents. 40.18−45.47 g/100 g of carbon content was observed in the leaf, stem, and root. There was no significant difference in the sulfur content among the different plant parts.

3.5. Mineral Analysis. Table 2 represents the micronutrient (Fe, Zn, and Cu) and the macronutrient (Ca, Mg, Na, and K) compositions of *R. humilis* plant parts. The highest iron content was obtained in leaf and root powders (29 mg/100 g) (Table 2). A similar iron content was reported in *Moringa oleifera* (29.40 mg/100 g), *Sesbania sesban* (28.57 mg/100 g), and *Achyranthes aspera* (31.61 mg/100 g) leaves.[17,50,51] The zinc content was maximum in seed powder (12.09 mg/100 g). Similarly, a very high content of zinc was reported in *Cucurbita maxima* seeds.[52] Other plant parts contained only 0.51−2.83 mg/100 g of zinc. Regardless of the biological role of zinc in humans, they are also an essential micronutrient for plants. Millions of hectares of agricultural land are affected by zinc deficiency. As chemical Zn fertilizers are being used to tackle this, exploring alternative plant-based sources for Zn has high significance.[53] In this scenario, zinc-rich seed biomass or the defatted seed cake of this plant and its various other minerals could be an excellent choice. The copper content was highest in seed (3.05 mg/100 g), whereas it was lowest in stem (0.52 mg/100 g). The calcium content was in order leaf > stem > seed > root. The magnesium content was 103.43 (seed)−815.02 (leaf) mg/100 g. The sodium content in the plant parts

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Table 3. GC-MS Fatty Acid (g/100 g) Profiling in *R. humilis* Plant Parts[a]

fatty acids	formula	leaf	stem	root	seed
lauric acid [C12:0]	$C_{12}H_{24}O_2$	0.25 ± 0.01[a]	0.30 ± 0.06[a]	1.19 ± 0.13[b]	0.00[c]
myristic acid [C14:0]	$C_{14}H_{28}O_2$	0.81 ± 0.04[a]	1.15 ± 0.09[b]	0.00[c]	0.28 ± 0.004[d]
palmitic acid [C16:0]	$C_{16}H_{32}O_2$	22.69 ± 0.24[a]	27.09 ± 0.10[b]	39.25 ± 0.29[c]	22.23 ± 0.31[a]
hypogeic acid [C16:0]	$C_{16}H_{30}O_2$	0.00[a]	0.00[a]	0.00[a]	0.14 ± 0.003[b]
stearic acid [C18:0]	$C_{18}H_{36}O_2$	2.93 ± 0.15[a]	2.71 ± 0.11[b]	5.33 ± 0.21[c]	2.54 ± 0.016[b]
oleic acid [C18:1]	$C_{18}H_{34}O_2$	7.84 ± 0.18[a]	11.55 ± 1.14[b]	22.17 ± 0.11[c]	44.48 ± 0.15[d]
trans-vaccenic acid [C18:1]	$C_{18}H_{34}0_2$	0.00[a]	0.00[a]	5.23 ± 0.31[b]	0.00[a]
linoleic acid [C18:2]	$C_{18}H_{32}O_2$	17.65 ± 0.17[a]	35.16 ± 0.44[b]	22.38 ± 0.06[c]	24.04 ± 0.26[d]
α Linolenic acid [C18:3]	$C_{18}H_{30}O_2$	47.83 ± 0.79[a]	22.05 ± 0.86[b]	4.46 ± 0.05[c]	0.00[d]
arachidic acid [C20:0]	$C_{20}H_{40}O_2$	0.00[a]	0.00[a]	0.00[a]	2.75 ± 0.38[b]
paullinic acid [C20:1]	$C_{20}H_{38}O_2$	0.00[a]	0.00[a]	0.00[a]	1.09 ± 0.00[b]
behenic acid [C22:0]	$C_{22}H_{44}O_2$	0.00[a]	0.00[a]	0.00[a]	2.46 ± 0.04[b]
SFA		26.68 ± 0.44[a]	31.24 ± 0.16[b]	45.77 ± 0.20[c]	30.39 ± 0.11[d]
MUFA		7.85 ± 0.18[a]	11.55 ± 1.14[b]	27.40 ± 0.20[c]	45.57 ± 0.15[d]
PUFA		65.48 ± 0.62[a]	57.21 ± 1.30[b]	26.83 ± 0.00[c]	24.12 ± 0.14[d]
UFA		73.32 ± 0.44[a]	68.76 ± 0.16[b]	54.23 ± 0.20[c]	69.69 ± 0.01[d]
SFA/UFA		1:2.7	1:2.15	1:1.13	1:2.28
PUFA/UFA		1:1.1	1:1.19	1:2	1:2.87

[a]Note: values represented are mean ± SD of three replicates (n = 3). Significance was tested by post hoc Duncan's test at $p < 0.05$, and values with the same superscript were not found to be significantly different from each other.

ranged from 6.61 (stem) to −164.78 (seed) mg/100 g. A very high content of potassium was observed in the leaf (6556 mg/100 g) and stem (6629 mg/100 g), whereas the seed (144.13 mg/100 g) had a comparatively lesser amount. Overall, leaves showed high Fe, Mg, and Ca contents, whereas seeds showed high Zn, Cu, and Na contents.

3.6. Fatty Acid Estimation. Figure 1 and Table 3 present the GC-MS chromatogram of fatty acid profiling and quantification in different plant parts. The structural elucidation of the detected fatty acid using GC-MS is presented in the Supporting data file. Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 ω-9), and linoleic acid (C18:2 ω-6, LA) are the predominant fatty acids detected in all four plant parts. Except for seed oil, α- linolenic acid (C18:3 ω-3, ALA) was also detected. Root oil contained the highest amount of palmitic (39.25 g/100 g) and stearic (5.33 g/100 g) acids. Saturated fatty acids like palmitic acid are synthesized endogenously and found in the diet. It has critical roles in the palmitoylation of proteins and the production of palmitoylated signal molecules while frequently being thought to impact adult chronic diseases negatively. It is a necessary component of cell membranes, secretory, and transport lipids.[54] The highest oleic acid content in *R. humilis* seed oil (44.48 g/100 g) was comparable with palm oil, sesame oil, safflower oil, rice bran oil, common ash oil, and desert date seed oil.[55−57]

Although oleic acid is nonessential for humans, its presence provides high oxidative stability to the oil for its usage at elevated cooking temperatures. More than 40 g/100 g of the oleic acid content in sesame oil signifies its high storage stability.[58] The essential ω-6-fatty acid, linoleic acid, LA, was observed in relatively high amounts in all parts ranging from 17.65 g/100 g (leaf oil) to 35.16 g/100 g (stem oil). Similarly, 24.04 g/100 g of LA in seed oil was more than twofold higher than that of palm oil (10 g/100 g)[7] and higher than that of flaxseed (13.94 g/100 g), chia seed (20.57 g/100 g), canola (20.12 g/100 g), and olive oil (7.01 g/100 g).[45] Also, a similar content was reported in Malabar spinach seed oil (26.02 g/100 g) recently.[59] The current consumption level of linoleic acid is approximately 6% of total dietary energy, as it is the most abundant PUFA in most food.[60] High levels of linoleic acid help prevent atherosclerosis by reducing the LDL cholesterol level in the blood.[61] A 47.83 g/100 g of ω-3-fatty acid, ALA, has been quantified in leaf oil, followed by 22.05 g/100 g in stem oil. A similar high quantity of ALA was reported in *Portulaca oleracea* leaf (49.70 g/100 g) as well as stem oil (15.62 g/100 g).[62]

In the context of research attempts of genetically modifying soy and other plants to contain higher levels of ω-3 PUFAs, finding an alternative source (with an advantage of biomass) rich in the same is relevant. Alongside, the dieticians of Canada (2013) report the required ALA level between 1.1 and 1.6 g/day depending on age and gender.[63] Additionally, seed oil contained arachidic (2.75 g/100 g) and behenic (2.46 g/100 g) acids. The saturated fatty acid (SFA) content in the plant parts was in the order root > stem > seed > leaf. The monounsaturated fatty acid (MUFA) content was detected in the range of 7.85 (leaf)−45.57 g/100 g (seed). Leaf had the highest amount of polyunsaturated fatty acid (PUFA) and total unsaturated fatty acid (UFA).

3.7. Determination of Total Phenolic and Flavonoid Contents. Phenolics are secondary metabolites associated with color, nutritional, antioxidant, and other health benefits. The redox potential of the same contributes to their effective antioxidant activity.[37] The quantity of TPC in each sample was expressed as gallic acid equivalent (mg/100 g DW GAEq., $y = 0.00151x - 0.0394$, $R^2 = 0.9971$). The highest content was in 80% ethanolic extract of the leaf (597.55 mg/100 g), followed by the seed (421.68 mg/100 g), stem (203.84 mg/100 g), and root (164.33 mg/100 g). However, the water extracts showed the lowest content of all of the plant parts analyzed (Figure 2a).

Health-promoting effects such as antioxidative, anti-inflammatory, and antimutagenic properties of flavonoids make them indispensable components in various nutraceutical, medicinal, and pharmaceutical applications.[64] The TFC was expressed as rutin equivalent (mg/100 g DW RutinEq., $y = 0.016x - 0.006$, $R^2 = 0.9999$). The 80% ethanolic extract of the leaf showed very high TFC (2442.19 mg/100 g), followed by 70%

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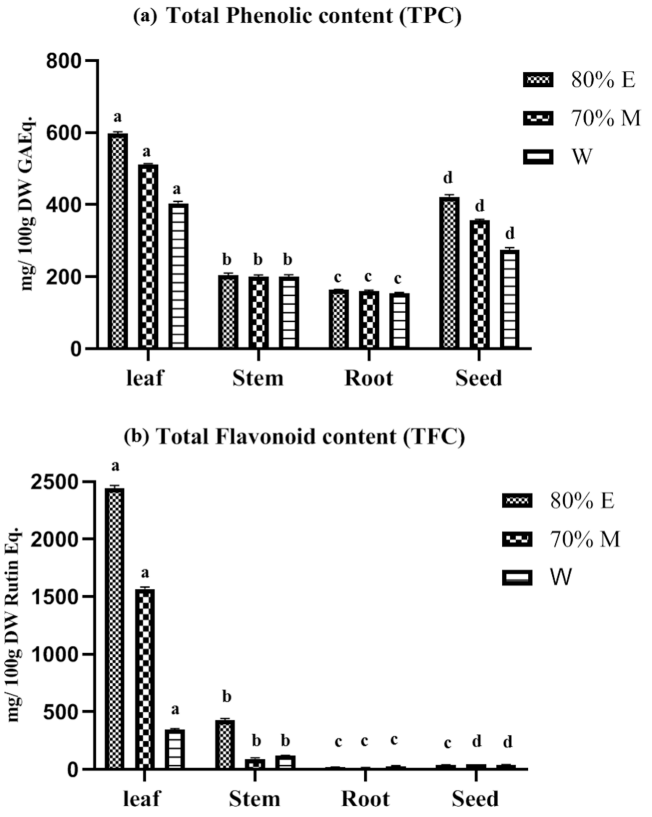

Figure 2. TPC (a) and TFC (b) of *R. humilis* plant parts. All of the values are represented as mean ± SD of three replicates, and the bars with different superscripts for different plant parts of the same extracts were significantly different from each other ($p < 0.05$).

methanolic extract (1566.25 mg/100 g). A similar content was reported earlier in *Alternanthera sessilis* (2151 mg/100 g), *Digera muricata* (1800 mg/100 g), and *Solanum nigrum* (1642 mg/100 g) leaves.[65] A sevenfold reduction in TFC through water extraction, compared to a combination of polar solvents with water, reveals the latter's effectiveness. However, the root and seed samples recorded negligible amounts compared to the leaves (Figure 2b).

3.8. Determination of Antinutritional Factors. Antinutrients are chemicals such as tannins, phytates, and oxalates that plants have developed for various biological purposes, including self-defense. They inhibit the maximal use of nutrients, particularly proteins, vitamins, and minerals, lowering the nutritional content of the food. Several of these plant compounds have been proven to be harmful to health or, if ingested in moderation, beneficial to human and animal health.[66]

Plant tannins are polyphenolic compounds with relatively high molecular weight. They form complexes with carbohydrates and proteins in aqueous solutions, which could be one of the reasons for their antinutritional effect. They are generally seen in bark, wood, fruit, leaves, and root.[67] Tannic acid with an R^2 value of 0.997 was used as the standard, and the results were expressed as mg/100 g DW TAEq. The tannin content in *R. humilis* plant parts was in the range of 116 mg/100 g (stem)−512 mg/100 g (seed) (Figure 3a). Although tannins have many adverse effects, they could be used wisely for many benefits as they are reported to possess anticarcinogenic effects, inhibitory action against gastrointestinal nematodes and deer lungworms, application in drugs because of their astringent property, and for treatment against hemorrhoids, diarrhea, etc.[68]

Phytic acid is found in plant tissues as a salt of cations such as potassium, magnesium, and calcium. It is abundant in many seeds and fruits, where it represents the storage form of phosphorus. The negatively charged phosphate groups in phytic acid chelate essential minerals in the human body and make them less available for absorption into the bloodstream.[67] The results of the phytic acid content were expressed in g/100 g DW PAEq. The maximum content of phytic acid was observed in the leaf (9.3 g/100 g) followed by the stem (6.9 g/100 g) and seed (6.4 g/100 g) and then root (1.47 g/100 g) (Figure 3b). A similar content of phytic acid was reported in wild peanut (*Plukenetia volubilis*) seeds (5.9 g/100 g).[45] Many leaves, fruits, and essentially all nuts and seeds possess oxalates. In the studied samples, the highest oxalate content was obtained in leaves (2.07 g/100 g), followed by the stem (1.58 g/100 g), root (0.77 g/100 g), and seed (0.27 g/100 g) (Figure 3c).

3.9. *In Vitro* Antioxidant Assays. *3.9.1. Total Antioxidant Assay (TAA) by the Phosphomolybdenum Method.* In TAA, the molybdenum (VI) is reduced to molybdenum (V). It forms a green phosphate/Mo(V) complex at acidic pH, showing maximum absorbance at 695 nm based on the presence of potential bioactives in plant extracts. Ascorbic acid with an R^2 value of 0.998 was used as the standard antioxidant compound, and the results were expressed as g/100 g DW AAEq.[32] The leaf showed the highest activity, followed by seed, root, and stem (Figure 4a). The leaf 80% ethanolic

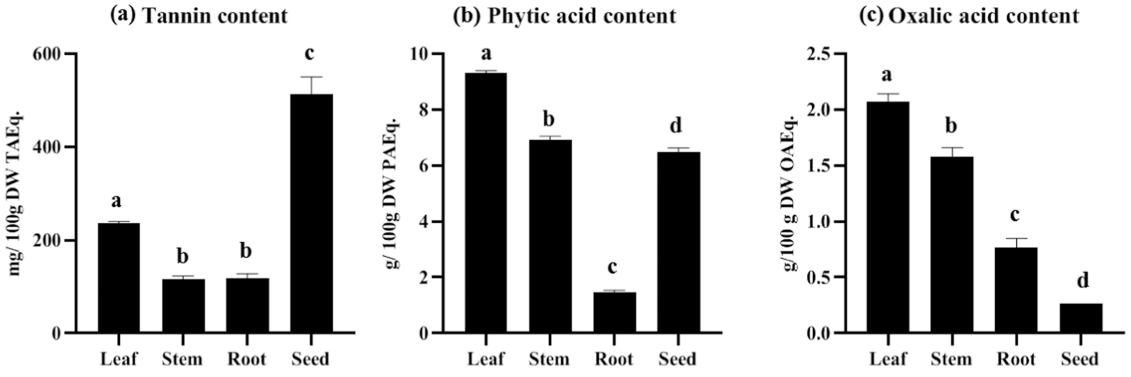

Figure 3. Antinutrient contents of *R. humilis* plant parts: (a) tannin, (b) phytic acid, and (c) oxalic acid. All of the values are represented as mean ± SD of three replicates, and the bars with different superscripts for different plant parts were significantly different from each other ($p < 0.05$).

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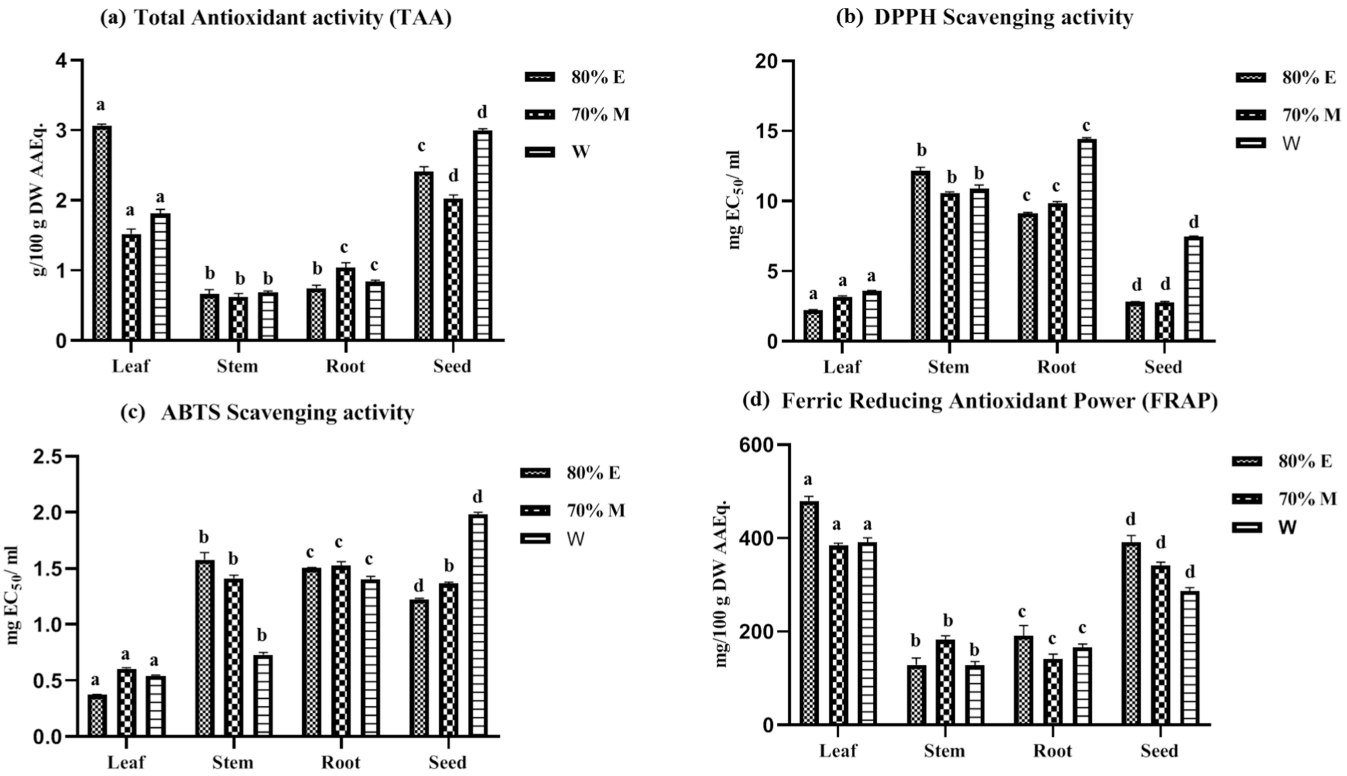

Figure 4. Antioxidant activities of *R. humilis* plant parts: (a) TAA, (b) DPPH, (c) ABTS, and (d) FRAP. All of the values are represented as mean ± SD of three replicates. The bars with different superscripts for different plant parts of the same extracts were significantly different from each other ($p < 0.05$).

extract showed double (3.07 g/100 g) the TAA when compared to the 70% methanolic extract (1.52 g/100 g). This correlates well with the high TPC and TFC of leaves in 80% ethanolic extract, as these compounds are known antioxidants. In seed, water extract showed high activity (3.00 g/100 g) followed by 80% ethanolic (2.41 g/100 g) and 70% methanolic (2.03 g/100 g) extracts. The stem showed the least activity among all of the plant parts, wherein there was no significant effect on the type of solvents used for extraction as well ($p < 0.05$).

3.9.2. DPPH Free-Radical Scavenging Activity. DPPH, a stable synthetic free radical with a characteristic absorption at 517 nm, has been used to assess the ability of the antioxidant compounds present in the extracts to transfer labile H atoms to radicals.[17] The study of atom transfer kinetics is important because free radicals in the organism are short-lived species; hence, the impact of a substance as an antioxidant depends on its fast reactivity toward free radicals. The total H atom-donating capacities are evaluated in the EC_{50} index, defined as the concentration needed to reduce a 50% DPPH radical.[69] The lower the EC_{50} value, the higher the compound's antioxidant activity. The result of the radical scavenging activity of different solvent extracts of the plant parts is shown in Figure 4b. Out of the three different solvents used for extractions, the 80% ethanolic extract showed the lowest EC_{50} value in leaf, i.e., 2.22 mg/mL. In contrast, there was not much significant difference in the 80% ethanolic (2.81 mg/mL) and 70% methanolic (2.78 mg/mL) extracts in seeds. Ascorbic acid was used as the standard antioxidant compound with an EC_{50} value of 8.51 μg/mL. This was consistent with earlier reports.[55] The stem (10.57 mg/mL in 70% methanolic extract) and root (9.12 mg/mL in 80% ethanolic extract) of *R. humilis* have comparatively less antioxidant activity. Overall, in all samples analyzed, water extract showed the highest EC_{50} values, implying its less effectiveness in bringing out the plant's antioxidant compounds than the mixture of solvents.

3.9.3. ABTS Free-Radical Scavenging Activity. Among the various *in vitro* antioxidant assays, DPPH and ABTS are extensively used to determine the antioxidant activity of different plant materials.[70] The ability of the test samples to scavenge $ABTS^+$ radical was compared to the ascorbic acid standard (EC_{50} 4.39 μg/mL). The 80% E extract of the leaf showed a significantly higher $ABTS^+$ radical scavenging activity (EC_{50} 0.37 mg/mL) than all other plant parts (Figure 4c). Unlike in the case of DPPH scavenging activity and TAA, water extract of the stem (EC_{50} 0.72 mg/mL) comes next, followed by 80% ethanolic extract of the seed (EC_{50} 1.22 mg/mL) and water extract of the root (1.40 mg/mL).

3.9.4. Ferric-Reducing Antioxidant Power (FRAP). In the FRAP assay, a key oxidant ferric ion, Fe^{3+}, is reduced to ferrous ion Fe^{2+} by the electron-donating (reductive) antioxidants in the reaction mixture that have a redox potential, under the reaction conditions employed, which is further tied to a color change. The effectiveness of dried powders of different plant parts to reduce ferric ions varied with different solvent extractions, and the ascorbic acid standard graph (R^2 = 0.996) results are expressed as mg/100 g DW AAEq. Figure 4d of FRAP assay shows that, in *R. humilis*, the reduction of ferric to ferrous ion capacity of different part extracts is leaf > seed > root > stem. The 80% ethanolic extract of the leaf (479.73 mg/100 g) and seed (391.14 mg/100 g) showed the highest FRAP activity. All three extracts of stem and root showed comparatively insignificant activity.

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4. CONCLUSIONS

The present study explored the potential phytoconstituents, GC-MS characterization of fatty acids, and antioxidant activities of different plant parts of *R. humilis*. The berries of this plant were known for their rich betalain content and other functional attributes. The presence of phytoconstituents like carbohydrates, proteins, micro- and macroelements, and fat (rich in omega fatty acids like linoleic acid and linolenic acid, which was reported for the first time), along with very high unsaturated fatty acids, is a significant finding in the plant parts such as leaf, stem, root, and seed. In view of the very high content of MUFA and PUFA, plant parts, such as leaves and seeds, could be a good choice for further exploration of their health benefits. Similarly, the micronutrient profile in leaf and seed looks optimistic and could be considered as their mineral substitute. Moreover, the good antioxidant property of the same could provide a strong rationale for developing novel therapeutic agents if this can be further validated at cell culture and animal study levels.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02883.

GC-MS spectra of the identified compounds in all of the selected plant parts of *R. humilis* (PDF)

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Notes

The authors declare no competing financial interest.

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