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Research Article

Physicochemical Analysis, NMR Profiling, FTIR Analysis, Lipid and Carbohydrate Content of *Rivina humilis* Berries

La Toya Roberts, Dane Warren, Andrea Goldson-Barnaby, Raymond Reid

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Author's Affiliations

The Department of Chemistry, The University of the West Indies, Kingston 7, Jamaica

Corresponding Authors

Andrea Goldson-Barnaby

andrea.goldson03@uwimona.edu.jm

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Abstract

Rivina humilis L. belongs to the Phytolaccaceae family. The berries of the plant are underutilized. Physicochemical properties of the fruit inclusive of total soluble solids, pH, moisture, and lipid content were determined. Berry extracts were characterized utilizing Nuclear Magnetic Resonance Spectroscopy, Fourier Transform Infra Red Spectroscopy and Gas Chromatography/Mass Spectrometry. The fruit is low acid with a pH of 6.1, moisture content of 84 %, lipid content of 15 % (dry weight basis), and a total sugar content of 6.9 % (fresh weight basis). Galactose was identified as the major carbohydrate and oleic acid, the major fatty acid within the fruit. This paper provides further information regarding the physicochemical and nutritional composition of the berries, which are not utilized commercially.

Keywords

Rivina humilis; dogblood; pigeon berry; lipid; sugars.

Introduction

Rivina humilis L. belongs to the Phytolaccaceae family. The berries are bright red in colour and can be found growing wild in Jamaica. Common names of the fruit are dogblood and pigeon berry. The berries are bright red in colour (Figure 1) due to the presence of betalains. Betalains may be further classified as betacyanins and betaxanthins. Betalains possess antioxidant, antimicrobial and light absorbing properties as well as the ability to complex with metal ions.[1] By associating with other copigments various colours are observed as the light-absorbing properties of the pigment are altered.[2] Betacyanins have a red-purple coloration and a maximum absorptivity (λ_{max}) at approximately 535 nm whereas betaxanthins are yellow with a maximum absorptivity (λ_{max}) at 480 nm.[2] Other fruits containing betalains include dragon fruit and beetroot.[3-4]

Figure 1. *Rivina humilis* berries

Thirty different betalains have been identified in beetroot (18 betacyanins and 12 betaxanthins) with highest concentrations being observed in the skin. Betanin, isobetanin and vulgaxanthin I were the most abundant.[4]

A green technology that utilizes dyes to produce electricity was developed by O'Regan and Gratzel (1991).[5] This technology referred to as a Dye-sensitized solar cell (DSSC) converts visible light to electricity and consists of a photo anode, dye sensitizer, electrolyte, counter electrode, and a conductive transparent glass substrate.[6] The dye serves as a sensitizer, absorbing light and triggering its conversion to electricity.[3] Commercially, ruthenium (II) polypyridinic complexes are utilized as the sensitizer but incur significant cost. Other alternatives are being explored and include the use of natural pigments such as betalains, chlorophyll and anthocyanins. To be utilized in DSSC, the dye should possess intense absorption in the UV Vis spectrum, possess conjugated oxide (=O) or hydroxyl (OH) stretching vibration which are required to assist in the bonding of Ti (IV) sites.[7] Betalain pigments have an active carboxylic functionality. Plant extracts that have already been explored for use in this technology include *Delonix regia* flowers, *Carica papaya* leaves and curcumin dye.[3] The utilization of *R. humilis* berry extracts could also be potentially explored. Other potential applications of *R. humilis* berry extracts include its utilization in value added food products such as jams and wines or as a natural source of antioxidants and colorants.

The present research was conducted to investigate the physicochemical properties of *R. humilis* berries growing in Jamaica. We report for the first time the use of Nuclear Magnetic Resonance (NMR) spectroscopy, Fourier Transform Infra Red (FTIR) spectroscopy and Gas Chromatography/Mass Spectrometry to characterize *R. humilis* berry extracts. The carbohydrate composition and fatty acid profile of the fruit was also reported.

Materials and Methods

R. humilis berries were harvested during the period of January - March, from plants growing in the Department of Chemistry at The University of the West Indies, Kingston, Jamaica.

Juice extracts

Juice extracts were prepared by macerating mature berries with a mortar and pestle. The resulting juice was decanted and further analyzed.

Qualitative analysis

Reducing sugars

The Fehlings test was conducted to determine the presence of reducing sugars. Fehlings solution consists of Fehlings reagent A (copper sulphate) and Fehlings reagent B (alkaline solution of sodium potassium tartarate) which were mixed in equal proportions at the time of analysis. This solution (2 mL) was then added to the berry extract (5 mL) and the mixture heated (5 min). Visible colour changes within the solution were noted.[8]

Protein

The ninhydrin test was performed to detect the presence of proteins. To the berry extracts (5 mL) was added ninhydrin solution (0.1 %).[9]

Phenolics

To berry extracts (5 mL) was added ferric chloride (1M, 1 mL) to detect the presence of phenolic compounds.[8]

Anthocyanins

To berry extracts was added HCl (2 M, 2 mL) and NaOH (2 M, 2 mL).[10]

Total soluble solids (°Brix), pH

The °Brix (total soluble solids) of berry juice extracts was determined utilizing a refractometer (ExTech instruments) and the pH with a pH meter (Oakton, pH Tutor).

Moisture determination

Rivina humilis berries were oven dried (80 °C, 24 h) in a Gallenkamp Laboratory Oven OV-330, England. Moisture content was determined gravimetrically.

Preparation of ethanolic extracts

Dried, ground, *Rivina humilis* berries (4.5 g), were extracted with ethanol (80 %, 20 mL, 24 h) and concentrated *in vacuo*.

Lipid extraction

Dried, ground, *Rivina humilis* berries were Soxhlet extracted with n-hexane (reflux, 1.5 h) and concentrated *in vacuo*.

^{1}H NMR and ^{13}C NMR characterization

^{1}H NMR characterization were performed on a Bruker BioSpin 500 MHz at 500 MHz. Ethanolic extracts were analyzed in deuterated methanol whereas lipid extracts were analyzed in deuterated chloroform ($CDCl_3$) at 25 °C, with tetramethylsilane as the internal standard. In reporting ^{1}H NMR the following terms were used: singlet (s), doublet of doublet (dd), triplet (t), multiplet (m).

Fourier Transform Infrared Spectroscopy

A Bruker Vector 22 Fourier Transform Infra Red (FTIR) spectrometer was utilized to record the infrared spectra of *R. humilis* berries (powdered, lipid and aqueous extracts). FTIR spectra were recorded between 500 and 4000 cm^{-1}. Each spectrum was obtained by averaging 20 scans recorded at a resolution of 2 cm^{-1}. Spectra were baseline-corrected. OPUS software was used to acquire and manipulate the spectral data.

Silylation and Gas Chromatography/Mass Spectrometry

Ethanolic extracts of the berries (10.0 mg/mL) were silylated with N-tert-butyl-dimethylsilyl-N-methyl-triflouroacetamide (MTBSTFA, 0.1 mL). Silylated samples (1 µL) were chromatographed on an Agilent HP6890 series gas chromatograph interfaced with a HP5972 mass selective detector. Silyl derivatives were eluted with helium carrier gas (flow rate 1.2 cm^3/min) through a DB-1701 column (30 m × 0.25 mm i.d.× 0.25 µm film thickness, Agilent, Santa Clara, CA) in an oven programmed at 80 °C for 2 min and increased at a ramp rate of 20 °C/min up to 280 °C for 10 min. Samples were injected at 250 °C while the detector was maintained at 280 °C. Constituents were identified by utilizing the NBS75L library of mass spectra (match quality >90%).

Fatty Acid Methyl Ester Analysis utilizing Gas Chromatography/Mass Spectroscopy

Lipid extracts of the berries (50 µL) were trans-methylated with methanol/acetyl chloride solution.[11] The resulting fatty acid methyl esters (FAMEs) were chromatographed on an Agilent HP6890 series gas chromatograph interfaced with a HP5972 mass selective detector. Constituent FAMEs were eluted with helium carrier gas (flow rate 1 cm^3/min) through a DB-VRX column (20 m x 0.18 mm i.d. x 1.0 µm film thickness, Agilent, Santa Clara, CA) in an oven programmed at 60 °C for 3 min and increased at a ramp rate of 10 °C/min up to 250 °C for 15 min. Samples were injected at 230 °C while the detector was maintained at 250 °C. Constituents were identified by matching the mass spectra National Institute of Standards and Technology (NIST) library of mass spectra (match quality > 90%).

Data analysis

Samples were analyzed in triplicate. The mean and standard deviation was reported.

Results and Discussion

Berries are known for their nutraceutical properties and are excellent sources of antioxidants, fibers, vitamins, and minerals offering various health benefits. Research conducted suggests that the phytochemical components present in berries can enhance cardiovascular risk profiles thereby decreasing the incidence of cardiovascular disease.[12] The consumption of berry has been shown to reduce low-density lipoprotein oxidation and lipid peroxidation, increase antioxidant capacity, decrease cholesterol and glucose levels as well as enhance high-density lipoprotein – cholesterol.[12] Currently there is limited scientific literature available regarding the composition of *R. humilis* berries. Most of the literature is centered around the presence of betalains within the fruit. This study further explores the chemical composition of *R. humilis* berries.

Qualitative analysis of berry extracts

R. humilis juice extracts were bright red in colour. This is due to the presence of betalains[13] and not anthocyanins as observed from the negative anthocyanin test result. Anthocyanins change colour with pH. A positive Fehlings test suggests that the major carbohydrate within the fruit is a reducing sugar. Berries have negligible quantities of protein as was seen from the negative Ninhydrin test. The presence of phenolic compounds was confirmed by the addition of ferric chloride (Table 1).

Table 1. Qualitative analysis of *Rivina humilis* berries

Class of Compound	Observation	Inference
Reducing sugars	Blue to orange-red	Reducing sugars
Proteins	No colour change	Negligible protein
Phenols	Green color	Phenolics
Anthocyanins	No colour change	No anthocyanins

Total soluble solid (TSS) and pH

The TSS is indicative of the presence of sugars. The berries had a TSS of 6.9 ± 0.2. A higher TSS (15) was reported for *R. humilis* berries grown in India.[13] The TSS of *Fragaria spp*. (strawberries) varies based on growth conditions with lower values being observed in soil grown strawberries versus those grown in hydroponic systems. Strawberries grown on farms in Finland had a TSS ranging from 5 - 11 while those grown in China on a soilless system had a TSS of 10 - 12.[14] In Mexico, values ranging from 8 - 11 have been reported for strawberries grown hydroponically.[15] *Cissus sicyoides* berries are sweeter than *R. humilis* berries with a TSS of 17.[16] The fruit is low acid with a pH of 6.1 ± 0.01 which is similar to that of *C. sicyoides* berries which have a pH of 6.[16] The moisture content of the fruit is 84.1 ± 2.5 % which is comparable to the value of 82 % reported by Khan et al., (2012).[13]

Carbohydrate and fruit acid composition

During maturation, berries go through various developmental stages producing both primary and secondary metabolites. During the first phase, organic acids and precursors of secondary metabolites develop. This is followed by a short phase referred to as veraison which marks the beginning of sugar accumulation and rapid pigmentation.[17] During verasion there is a rapid increase in the levels of glucose and fructose while organic acid composition decreased. In the third phase of development various aroma compounds are produced.[17] Goji berries contains the monosaccharides, arabinose, rhamnose, xylose, mannose, galactose, glucose and galaturonic acid.[18]

The carbohydrate composition of *R. humilis* berries was investigated utilizing GC/MS. Berry extracts were silylated prior to analysis. The major carbohydrate identified in the berries was galactose. Other carbohydrates identified included glucose, arabinose, and myo-inositol (Table 2). The presence of reducing sugars was confirmed by the Fehlings test. Galactose has been identified in other fruits and vegetables such as tomatoes, immature seedless grapes, kiwi, bell peppers and persimmon.[19-20] The major fruit acid detected was citric acid (9.65 ± 1.4 %). Khan et al. (2012), reported tartaric acid (110 mg/ 100 g fresh weight) and citric acid (37.3 mg/100 g fresh weight) as the major fruit acids present.[13] Citric acid is the major fruit acid present in blueberries (77 to 87 %).[21]

Table 2. Carbohydrates identified in aqueous extracts of *R. humilis* berries

Carbohydrates	Percentage (%)
D-Glucose	0.67 ± 0.2
Arabinose	0.75 ± 0.0
Myo-Inositol	0.98 ± 0.3
D-Galactose	12.72 ± 1.0

^{1}H NMR profiling

^{1}H NMR profiling is a rapid method of analysis that provides a metabolic fingerprint of samples analyzed. The technique has been utilized to identify metabolites in fruits, fruit juices, oils and wines, to determine sample authenticity, detect adulteration and in the differentiation of wine grape varieties.[22-24] The technique is rapid, unbiased, has high analytical precision and produces high throughput spectral data.[23] Analysis of ethanolic extracts of *R. humilis* berries utilizing ^{1}H NMR spectroscopy revealed peaks reflective of the presence of carbohydrates, fruit acids and lipids (Figure 2). Anomeric protons due to the presence of galactose were observed between δ 3 – δ 5. Protons resonating at δ 2.05 and δ 2.29 are due to the presence of citric acid. The distinctive peaks reflective of the presence of lipids were unexpected as berries are not normally known to be a significant source of lipids. Significant signals were observed at δ 0.92 (Terminal methyl group) and δ 1.31 (methylene protons) due to presence of long chain fatty acids (Table 3).

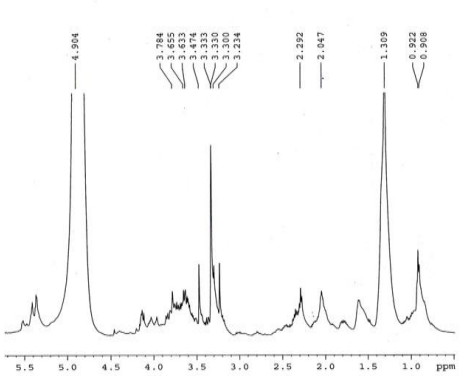

Figure 2. ^{1}H NMR of ethanolic extracts of *Rivina humilis* berries

Table 3. ^{1}H NMR analysis of ethanolic extract of *Rivina humilis* berries

Compounds	Chemical shift (δ)
Lipids	0.91 (m)
Lipids	1.31 (s)
Lipids	1.60 (m)
Lipids	1.80 (m)
Citric acid	2.05 (m)
Citric acid	2.29 (m)
Galactose	3.23 (s)
Galactose	3.30 (s)
Galactose	3.47 (s)
Galactose	3.63 (m)
Galactose	3.78 (m)
Galactose	4.10 (m)
Galactose	5.40 (m)

Lipid content and fatty acid profile

R. humilis berries had a lipid content of 15.2 ± 0.1 % on a dry weight basis. GC/MS of fatty acid methyl esters (FAME) synthesized from lipid extracts of the fruit confirmed that oleic acid (68.4 ± 1.1 %) was the major unsaturated fatty acid and palmitic acid (14.9 ± 0.5 %) the major saturated fatty acid present (Table 4). Khan et al., (2012) also reported the presence of oleic acid (23 %) and palmitic acid (30 %).[13] Trace levels of linoleic acid was detected in

berries grown in Jamaica. The ackee is an example of a fruit that is also rich in oleic acid.[25-26]

Table 4. Fatty acid profile of *Rivina humilis* berry lipid extract

Fatty acids	Trivial name	Notation	Average (%)
Tetradecanoic	Myristic	C14:0	0.2 ± 0.1
Hexadecanoic	Palmitic	C16:0	14.9 ± 0.5
Octadecanoic	Stearic	C18:0	4.3 ± 0.4
Eicosanoic	Arachidic	C20:0	1.9 ± 0.2
Docosanoic	Behenic	C22:0	2.6 ± 0.4
Tetracosanoic	Lignoceric	C24:0	0.8 ± 0.1
9-Octadecanoic	Oleic	C18:1	68.4 ± 1.1
9,11-Octadeca-dienoic	Linoleic	C18:2	0.16 ± 0.04
11-Eicosenoic	Gondoic	C20:1	1.6 ± 0.7

The ^{1}H NMR data of lipid extracts of the fruit is consistent with the presence of high levels of monounsaturated fatty acids as indicated by chemical shifts at δ 5.35 (Figure 3; Table 5). Unsaturation is also evident in the ^{13}C NMR data with down field shifts at δ 127.89, δ 128.07, δ 129.69, δ 130,00 and δ 130.19 due to the presence of oleic acid (Table 6).

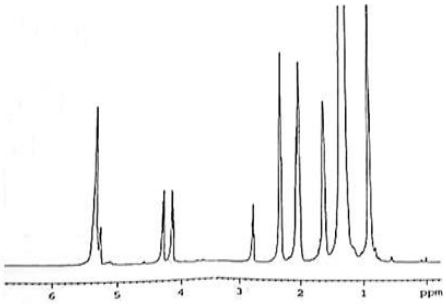

Figure 3. ^{1}H NMR of lipid extracts of *Rivina humilis* berries

FTIR analysis of *R. humilis* berry extracts

FTIR is a nondestructive method of analysis that requires small sample size and provides a rapid means of sample analysis.[27] Minimal handling of the sample is required resulting in retention of sample integrity.[28] The utilization of this technique has increased in popularity over the years. A recent report by Nag et al. (2021), utilized FTIR to study conformational changes in protein and cell structure.[28] FTIR analysis of lipid extracts of the berries revealed a peak at 727 cm^{-1} which was due to the presence of olefinic carbons (–HC=CH, cis).[25] A shoulder peak at 3013 cm^{-1} was due to the C-H stretching vibration of the olefinic group (=CH).[25] Weaker peaks observed at 871, 914 and 989 cm^{-1} were due to the presence of phenolic compounds. A sharp peak at 1746 cm^{-1} was reflective of the presence of a carbonyl functionality which is a characteristic feature of lipid extracts which are composed of triacylglycerols.

Table 6. ^{13}C Nuclear magnetic resonance spectroscopy of *R. humilis* lipid berry extract

Carbon	Assignment	δ (ppm)
α-CH_3	Acyl chains	14.12
β-CH_3	Acyl chains	22.59; 22.70
C3	Acyl chains	24.87; 25.63
C8–11 (oleyl)	Allylic	27.23
CH_{2n}	Acyl chains	29.06 - 29.78
C16	Linoleyl	31.54, 31.92
β-C2	Acyl chains	34.04, 34.19
α-CH_2O	Glycerol moiety	62.10
β-CH_2O	Glycerol moiety	68.88
β-C9	Oleyl	127.89, 128.07
β-C10	Oleyl	129.69, 130.00,130.19
α-C1	Glycerol moiety	172.84
β-C1	Glycerol moiety	173.29

FTIR analysis of ethanolic extracts of the berry revealed the presence of an active carboxyl group due to the presence of betalain pigments with carbonyl stretching vibrations occurring at 1659 cm^{-1} and an OH stretching vibration at 3402 cm^{-1} (Figure 4).[3] Other dominant peaks were observed at 1032 cm^{-1} and 1251 cm^{-1} due to the carbohydrate content of the berries.[16, 29] These peaks are due to exocyclic and endocyclic C-O vibrations in carbohydrates. In the presence of glucose or fructose, a peak at 1072 cm^{-1} is expected. Its absence provides further confirmation that these monosaccharides are not the predominant carbohydrates present in *R. humilis* berries.[30] Based on our analyses of the berries, galactose is the primary sugar present. The carbonyl functionality due to the presence of triacylglycerols was observed at 1730 cm^{-1}. This peak is noticeably absent from FTIR analysis of aqueous extracts *of C. sicyoides* berries which are low in fat.[29] Another dominant peak expected is that of the amine functionality (C-N) present in betalains, which was observed at 1458 cm^{-1}. Also resonating in this area is that of the CH_2 bending (acyl chain) and/or CH_3 deformation within fatty acid side chains. The presence of phenolic compounds was evident from smaller peaks occurring at 704, 721, 827 and 903 cm^{-}

[1]. The fruit is a natural source of antioxidants.[13] A peak at 625 cm^{-1} indicates the presence of phosphates.[31] The FTIR spectral data for the dried fruit was similar to that of ethanolic extracts. Berries are typically low in protein. Dominant vibrations due to the presence of proteins are from amide 1 (C=O stretching vibrations) and amide 2 functionalities (NH bending and CN stretching) with C=O stretching vibrations being the most pronounced and resonating between 1700–1600 cm^{-1}. The peak observed at 1659 cm^{-1} is due to the carbonyl functionality in betalain and not the presence of proteins.

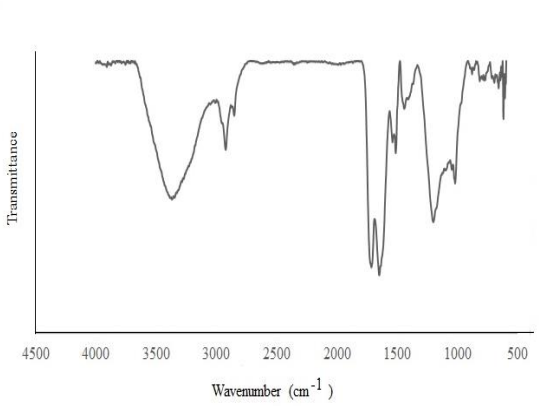

Figure 4. FTIR analysis of ethanolic extracts of *R. humilis* berries

Conclusions

R. humilis berries are an untapped source of natural colorants and antioxidants. The chemical composition of the berries appears to vary based on its geographical location. Differences were observed in the TSS, lipid content and lipid profile of berries grown in Jamaica versus that reported for berries growing in India. Oleic acid was identified as the predominant fatty acid and galactose the primary carbohydrate present. The berries may be utilized as a source of natural colourants and can be further evaluated for use in the preparation of jams, preserves, beverages and nutraceuticals.

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Conflict of interest

The authors declare that there are no conflicts of interest to the present work.

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