FINAL SCRIPT: APPROVED FOR FILMING

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Title: Comparative Study on the Polysaccharide Contents and Antioxidant Activities *of Hippophae rhamnoides* subsp. *sinensis* and *Hippophae gyantsensis*

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AuthorS Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? No

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 23
Number of Shots: 57

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Chunqiao Shi:** This study compares the polysaccharide content and antioxidant activity between *Hippophae rhamnoides* subsp. *sinensis* and *Hippophae gyantsensis.* to elucidate their differences.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll: 6.1.2*

NOTE: Statements 1.2 and 1.3 have been edited

What research gap are you addressing with your protocol?

1.2. **Chunqiao Shi:** The study is methodologically sound and addresses a gap in the literature regarding species-specific differences in polysaccharide characteristics.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

1.3. **Chunqiao Shi:** T The findings of this study are significant for quality assessment and potential pharmaceutical applications.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:6.5*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Extraction and Purification of Total Polysaccharides from Sea Buckthorn Using Ethanol Defatting and Reflux Techniques

Demonstrator: Chunqiao Shi

2.1. To begin transfer 5 grams of sea buckthorn sample powder into a 250-milliliter round-bottomed flask **[1]**. Then add 50 milliliters of anhydrous ethanol and soak it at room temperature of 25 degrees Celsius for 24 hours **[2-TXT]**.

2.1.1. WIDE: Talent transferring 5 g sea buckthorn sample powder into a 250 mL RB flask.

2.1.2. Talent pouring 50 mL ethanol into the flask. **TXT: Process each sample with 3 parallel replicates**

2.2. The next day, filter the soaked sample powder using a Buchner funnel to obtain the filter residue **[1]**. Wash the filter residue three times with 50 milliliters of deionized water **[2-TXT]**.

2.2.1. Talent passing the sample through a filter placed in a Buchner funnel. NOTE: Shots 2.2.1 and 2.2.2 were consecutively shot, and the corresponding clip is C9071

2.2.2. Talent washing the residue with water. **TXT: Dry to constant weight at 40 °C**

2.3. Now, transfer the defatted filter residue into a dried 250 milliliter round-bottomed flask **[1]**. Add 150 milliliters of deionized water at a solid to liquid ratio of 1 to 30 grams per milliliter **[2]**. Perform reflux extraction in a water bath at 80 degrees Celsius for two hours twice **[3-TXT]**.

2.3.1. Talent transferring residue into 250 mL RB flask.

2.3.2. Talent adding 150 mL water to flask.

2.3.3. Talent placing flask in heated water bath. **TXT: Separate the filtrate to obtain crude polysaccharide solution**

2.4. Then, concentrate the extract using a rotary evaporator **[1-TXT]**. Store the concentrated solution in a refrigerator at 4 degrees Celsius for later use **[2]**.

2.4.1. Talent placing flask on rotary evaporator. **TXT: Concentration: 50 °C, 1.5 h**

2.4.2. Talent placing concentrated solution into refrigerator.

2.5. Next, add Sevage reagent to the crude polysaccharide solution in a one to four volume ratio and place on a magnetic stirrer **[1-TXT]**.

2.5.1. Talent adding Sevage reagent and placing mixture on a stirrer. **TXT: Stirring: 1050 rpm, 20 min**

2.6. Then transfer the mixture into a 50-milliliter centrifuge tube **[1]**. Centrifuge the suspension at 4000 *g* for 15 minutes **[2]** and collect the supernatant **[3-TXT]**.

2.6.1. Talent pipetting mixture into centrifuge tube.

2.6.2. Talent loading tube into centrifuge and setting centrifugation parameters.

2.6.3. Talent pipetting out the supernatant. **TXT: Repeat 3x until no white flocculent precipitate appears at the phase interface**

2.7. To concentrate the polysaccharide extract after protein removal, add anhydrous ethanol at a volume ratio of one to three to precipitate polysaccharides **[1].** Place the mixture at four degrees Celsius overnight **[2]**.

2.7.1. Talent adding anhydrous ethanol to the polysaccharide extract.

2.7.2. Talent placing tube in refrigerator to precipitate.

2.8. On the next day, remove the upper layer of ethanol **[1]**, transfer the mixture into a 50-milliliter centrifuge tube **[2],** and centrifuge as before **[3]**. Freeze-dry the precipitate to obtain total polysaccharides **[4]**. NOTE: The VO is edited for the additional shot

2.8.1. Talent pipetting out upper layer.

2.8.2. Talent transferring to 50 mL centrifuge tube.

Added shot: Talent placing the tube into the centrifuge. NOTE: This step is marked as 2.8.2_1, and the corresponding segment is C9091.

2.8.3. Talent placing precipitate into freeze-dryer.

3. Determination of Polysaccharide Content

3.1. Transfer 3 grams of phenol into a brown volumetric flask **[1]**. Add 50 milliliters of deionized water to dissolve the phenol **[2]**. Shake until homogeneous, then label and store flask for later use **[3]**.

3.1.1. Talent transferring 3g phenol into a brown volumetric flask.

3.1.2. Shot of 50 mL deionized water being added to the flask.

3.1.3. Talent shaking flask and labelling flask.

3.2. Next, transfer 10 milligrams of anhydrous glucose into a 10-milliliter volumetric flask **[1]**. Add deionized water and shake the mixture thoroughly **[2]**. Then label the flask **[3]**.

3.2.1. Talent transferring 10 mg anhydrous glucose into a 10 mL flask.

3.2.2. Talent adding water to the flask and shaking it.

3.2.3. Talent labelling flask. NOTE: The shot has been modified

3.3. For the preparation of the test sample solution, weigh 5 milligrams of freeze-dried sea buckthorn polysaccharides into volumetric flasks **[1]**. Add deionized water to bring each to a final volume of 50 milliliters **[2]**. Sonicate each mixture for 20 minutes until completely dissolved **[3-TXT]**.

3.3.1. Talent weighing sample powders.

3.3.2. Talent adding water to flasks.

3.3.3. Talent placing flasks in sonicator. **TXT: Prepare 3 parallel replicates for each sample**

3.4. Dilute the standard glucose solution with deionized water to 0.1 milligrams per milliliter **[1]**. Then, using a micropipette, transfer 200, 300, 400, 500, 600, and 700 microliters into six clean test tubes **[2]**. Add pure water to bring each to a total volume of 1 milliliter **[3]** and gently mix each solution to ensure uniform dilution **[4]**.

3.4.1. Talent adding deionized water to the standard glucose solution.

3.4.2. Talent pipetting standards into 6 clean test tubes.

3.4.3. Talent topping up each tube to 1 mL with water.

3.4.4. Talent rotating tubes gently.

3.5. Add 1 milliliter of 6% phenol solution and 5 milliliters of sulfuric acid to each test tube and mix well **[1-TXT]**.

3.5.1. Talent adding 1 mL phenol and 5 mL sulfuric acid to a test tube. **TXT: Incubate at RT for 30 min**

3.6. Transfer the samples into cuvettes **[1].** Use pure water as blank **[2]**, and measure absorbance at 490 nanometers with a UV–visible spectrophotometer in three replicates **[3]**.

3.6.1. Talent pipetting the sample into cuvettes.

3.6.2. Talent pipetting water into a cuvette labelled blank.

3.6.3. Talent placing the sample in a UV-Vis spectrophotometer.

4. Quantitative Analysis of Sea Buckthorn Polysaccharides Using Phenol–Sulfuric Acid Colorimetry: Precision, Repeatability, Recovery, and Content Determination

4.1. For the precision test, prepare the test solution **[1]**. Perform phenol–sulfuric acid colorimetry **[2]**. Determine absorbance and repeat six times to calculate relative standard deviation **[3-TXT]**.

4.1.1. Shot of prepared JZ2 sample solution.

4.1.2. Talent performing color reaction.

4.1.3. Talent measuring absorbance times.

4.2A. For the stability test, prepare the test solution **[1]**. Perform colorimetry at 0,2, 4,6,8, and 10 hours post-preparation **[2]**. Determine absorbance at the aforementioned time points to calculate relative standard deviation **[3].** NOTE: 4.2A is an added step

4.2A.1 Shot of prepared JZ2 sample solution.

4.2A.2 Talent performing color reaction.

4.2A.3 Talent measuring absorbance times.

4.2. For the repeatability test, prepare six parallel replicates of the sample **[1]**. Conduct phenol–sulfuric acid colorimetry and determine absorbance following the established procedure **[2]**.

4.2.1. Talent preparing six replicate tubes. NOTE: Shots 4.2.1 and 4.2.2A were consecutively shot.

4.2.2. Talent conducting color reactions and measurements. NOTE: shot 4.2.2 was divided into two parts for filming: "performing reaction" and "measuring absorbance," labeled as 4.2.2A and 4.2.2B, respectively.

4.3. To perform the recovery test, prepare nine parallel replicates of the test solution **[1].** Add reference volumes equivalent to 80, 100, and 120 percent of total polysaccharide content **[2]**. Then perform phenol–sulfuric acid colorimetry **[3]**.

4.3.1. Shot of prepared 9 replicates.

4.3.2. Talent adding reference solution to the replicates.

4.3.3. Talent conducting color reactions and absorbance measurements. NOTE: Shot 4.3.3 was divided into two videos: 4.3.3A and 4.3.3B

4.4. For content determination, prepare the sample solution, perform phenol–sulfuric acid colorimetry following the established method **[1]**. Then determine absorbance **[2]**.

4.4.1. Talent preparing final sample solution. NOTE: Shots 4.4.1 and 4.4.2A were consecutively shot, and the corresponding clip is C9119

4.4.2. Talent performing reaction and measuring absorbance. NOTE: Shot 4.4.2 was divided into two videos: 4.4.2A and 4.4.2B.

5. Evaluation of Antioxidant Activity of Sea Buckthorn Polysaccharides Using DPPH Radical Scavenging Assay

5.1. Weigh 2.7 milligrams of DPPH into an empty 50-milliliter volumetric flask **[1]**. Add 50 milliliters of anhydrous ethanol to dissolve the DPPH and prepare a stock solution **[2-TXT]**.

5.1.1. Talent weighing DPPH powder into a 50 mL volumetric flask.

5.1.2. Talent pouring ethanol into the volumetric flask to dissolve DPPH. **TXT: Stock**

concentration: 0.054 mg/mL

5.2. Next, dissolve 5 milligrams of vitamin C in 10 milliliters of deionized water to prepare a 0.5 milligram per milliliter solution **[1]**. Dilute this solution successively to prepare vitamin C test solutions **[2-TXT]**.

5.2.1. Talent weighing and dissolving vitamin C in water. NOTE: Shot 5.2.1 was divided into two segments: 5.2.1A and 5.2.1B.

5.2.2. Talent performing serial dilutions and labeling test tubes. **TXT: Vit C Test Solutions: 0.5, 0.125, 0.03125, 0.0078125, and 0.001953 mg/mL**

5.3. Now dissolve 5 milligrams of each sea buckthorn polysaccharide sample in 10 milliliters of deionized water to prepare a 0.5 milligram per milliliter solution **[1]**. Dilute these solutions successively to obtain test concentrations **[2-TXT]**.

5.3.1. Talent dissolving each sample in water.

5.3.2. Talent performing serial dilutions. **TXT: Polysaccharide concentration: 0.5, 0.125, 0.03125, 0.0078125, and 0.001953 mg/mL**

5.4. Using the DPPH solution as the substrate, prepare the test groups in a 96-well plate **[1]**. Add 150 microliters of water and 50 microliters of DPPH for the negative control group A0 **[2]**. Add 150 microliters of the sample or vitamin C solution and 50 microliters of DPPH for the sample group A1 **[3]**.

5.4.1. Shot of a 96-well plate setup.

5.4.2. Talent pipetting water and DPPH into wells for A0.

5.4.3. Talent pipetting vitamin C or sample and DPPH into wells for A1.

5.5. For sample control group A2, add 150 microliters of sample and 50 microliters of anhydrous ethanol **[1-TXT]**. Allow the reaction to proceed in the dark for 30 minutes **[2]**, then measure the absorbance at 517 nanometers **[3]**.

5.5.1. Talent pipetting sample and ethanol into wells for A2. **TXT: Perform each setup in triplicate**

5.5.2. Talent placing plate in dark cabinet or drawer.

5.5.3. Talent using a plate reader to measure absorbance at 517 nanometers.

Results

6. Results

6.1. The standard curve of glucose showed a strong linear relationship between absorbance and concentration in the range of 0.02 to 0.07 milligrams per milliliter **[1]**. The average polysaccharide yield of *Hippophae gyantsensis* was significantly higher than that of *Hippophae rhamnoides* subsp. *sinensis* **[2]**.

6.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight the diagonal line and the data points along it*

6.1.2. LAB MEDIA: Table 2. Video editor: *Highlight the value 1.18 in the column "Average (polysaccharide yields %)" and the rows corresponding to JZ1-JZ5*

6.2. The precision test demonstrated minimal variation in absorbance across six measurements and RSD of 0.21%, indicating high instrument precision **[1]**. The stability test showed that absorbance values remained consistent over a 10-hour period, with RSD value of 0.45% confirming the sample solution's stability **[2]**. The repeatability test produced consistent absorbance values across six replicates with RSD of 0.50%, validating the method's repeatability **[3]**.

6.2.1. LAB MEDIA: Table 3. *Video editor: Highlight the row "Precision test"*

6.2.2. LAB MEDIA: Table 3. *Video editor: Highlight the row "Stability test"*

6.2.3. LAB MEDIA: Table 3. *Video editor: Highlight the row "Reproducibility test"*

6.3. The recovery experiment for glucose content yielded an average recovery rate of 99.18 percent, confirming the accuracy of the method **[1]**.

6.3.1. LAB MEDIA: Table 4. *Video editor: Highlight the columns "Recoveries(%)" and "Average Recovery"*

6.4. The average polysaccharide content of *Hippophae rhamnoides* subsp. *sinensis* was significantly higher than that of *Hippophae gyantsensis* **[1]**.

6.4.1. LAB MEDIA: Table 5. *Highlight the value 352.97 ± 1.07in the column "Average (polysaccharide contentsmg/g)" and the rows corresponding to ZG1-ZG5*

6.4.2. for *H. gyantsensis*

6.5. *Hippophae gyantsensis* exhibited slightly higher antioxidant activity than *Hippophae rhamnoides* subsp. *sinensis* at all tested concentrations **[1]**.

6.5.1. LAB MEDIA: Figure 2. *Video editor: Highlight the red line (H. gyantsensis)*

6.6. At 0.5 milligrams per milliliter, the DPPH clearance rate of *Hippophae gyantsensis* reached 81.93 percent **[1]**, while *Hippophae rhamnoides* subsp. *sinensis* reached 75.17

percent **[2]**.

6.6.1. LAB MEDIA: Figure 2. *Video editor: Highlight the red data point at 0.5 mg/mL*

6.6.2. LAB MEDIA: Figure 2. *Video editor: Highlight the grey data point at 0.5 mg/mL*

1. polysaccharides
Pronunciation link:
https://www.merriam-webster.com/dictionary/polysaccharide
IPA: /ˌpɒliˌsækəˈraɪdz/
Phonetic Spelling: pol-ee-sak-uh-rydz

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2. phenol
Pronunciation link:
https://www.merriam-webster.com/dictionary/phenol
IPA: /ˈfiːnɒl/ or /ˈfiːnəl/
Phonetic Spelling: fee-nol

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3. centrifuge
Pronunciation link:
https://www.merriam-webster.com/dictionary/centrifuge
IPA: /ˈsɛntrəˌfjuːdʒ/
Phonetic Spelling: sen-truh-fyoohj

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4. reflux
Pronunciation link:
https://www.merriam-webster.com/dictionary/reflux
IPA: /ˈriːflʌks/
Phonetic Spelling: ree-fluks

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5. Sevage (as in Sevage reagent)
Pronunciation link:
No confirmed link found *(not listed in Merriam-Webster, OED, or HowToPronounce.com)*
IPA: (approximate, based on typical usage) /səˈvɑːʒ/
Phonetic Spelling: suh-vahzh

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6. spectrophotometer
Pronunciation link:
https://www.merriam-webster.com/dictionary/spectrophotometer

IPA: /ˌspɛktroʊˌfoʊˈtɒmətər/
Phonetic Spelling: spek-troh-foh-TAH-muh-ter

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7. Hippophae (genus name)
Pronunciation link:
No confirmed link found *(not commonly listed separately; might be embedded in botanical references rather than standard dictionaries)*
IPA: /ˌhɪpəˈfeɪ/
Phonetic Spelling: hip-uh-fay

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8. gyantsensis
Pronunciation link:
No confirmed link found *(specific epithet; rarely in standard dictionaries)*
IPA: /ˌdʒiːænˈtɛnsɪs/
Phonetic Spelling: gee-an-TEN-sis

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9. rhamnoides (as in *Hippophae rhamnoides*)
Pronunciation link:
No confirmed link found *(specific epithet; botanical Latin)*
IPA: /ræmˈnɔɪdiːz/
Phonetic Spelling: ram-NOY-deez