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# Pharmacological and Phytochemical Evaluation of Shikakai (*Acacia concinna*) and Ritha (*Sapindus mukorossi*): Antioxidant, Antimicrobial, and Hair Growth–Promoting Activities

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Acacia concinna
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phytochemical screening
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dermal papilla cells

**ABSTRACT**

Background: Shikakai (Acacia concinna) and Ritha (Sapindus mukorossi) are two plants widely used together in traditional Ayurvedic hair care, but comparative data on their phytochemistry and bioactivity under matched experimental conditions are limited. Methods: Aqueous and ethanolic extracts of Shikakai pods and Ritha pericarp were screened qualitatively for major phytochemical classes and profiled by gas chromatography–mass spectrometry (GC–MS), then evaluated for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and total antioxidant capacity (TAC), antibacterial activity against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa (agar well diffusion and broth microdilution), antifungal activity against Candida albicans and Candida tropicalis (broth turbidity assay), cytotoxicity on L-929 fibroblasts (MTT, LDH, and neutral red uptake assays), and hair-growth-related activity on human hair follicle dermal papilla cells (BrdU incorporation, IGF-1 and VEGF secretion) together with in vitro 5α-reductase inhibition. Results: Both extracts contained saponins, flavonoids, tannins, terpenoids, coumarins, and phytosterols; GC–MS identified glycidyl oleate as a major Shikakai constituent and cis-vaccenic acid as a major Ritha constituent. Shikakai showed a marginally lower DPPH IC50 (~50 µg/mL) than Ritha (~60 µg/mL) and a marginally higher TAC (75 ± 5 vs 68 ± 4 mg ascorbic acid equivalents/g), neither difference reaching statistical significance ($p > 0.05$). Ritha produced larger inhibition zones and lower minimum inhibitory concentrations than Shikakai against all three bacterial strains (e.g., 4 vs 8 mg/mL for S. aureus) and inhibited Candida growth by 67–85% at 5 mg/mL, compared with 15–17% for Shikakai. Neither extract reduced L-929 viability below 80% at 200 µg/mL. Both extracts increased dermal papilla cell proliferation relative to control (Shikakai ~120%, Ritha ~130%; minoxidil ~150%), increased IGF-1 and VEGF secretion, and inhibited 5α-reductase activity by up to 45–50% at 200 µg/mL. Conclusion: Under these in vitro conditions, Shikakai and Ritha extracts showed antioxidant, antimicrobial, and hair-follicle-related activity with no marked L-929 cytotoxicity at the hair-assay concentrations. Ritha drove most of the antimicrobial effect, while both plants contributed comparably to antioxidant and pro-proliferative, anti-androgenic activity; topical safety and clinical efficacy remain to be established.

# التقييم الدوائي والكيميائي النباتي للشيكاكاي (*Acacia concinna*) والريثا (*Sapindus mukorossi*) : الفعاليات المضادة للأكسدة، والمضادة للميكروبات، والمحفزة لنمو الشعر

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**الملخص**

الخلفية :(Background) تُعد الشيكاكاي (*Acacia concinna*) والريثا (*Sapindus mukorossi*) من النباتات التي تُستخدم معاً على نطاق واسع في العناية التقليدية بالشعر في طب الأيورفيدا(Ayurvedic) ، ولكن البيانات المقارنة حول كيميائها النباتية ونشاطها الحيوي تحت ظروف تجريبية متطابقة لا تزال محدودة. المنهجية :(Methods)فُحصت المستخلصات المائية والإيثانولية لقرون الشيكاكاي وغلاف ثمرة الريثا نوعياً لتحديد الفئات الكيميائية النباتية الرئيسية، وحُللت باستخدام كروماتوغرافيا الغاز-مطياف الكتلة .(GC–MS) بعد ذلك، قُيِّمت نشاطية كسح الجذور الحرة لـ DPPH والسعة المضادة للأكسدة الكلية(TAC) ، والنشاط المضاد للبكتيريا ضد *Staphylococcus aureus*، و *Escherichia coli*، و *Pseudomonas aeruginosa*(باستخدام الانتشار في حفر الآجار والتخفيف الدقيق في المرق)، والنشاط المضاد للفطريات ضد *Candida albicans*و *Candida tropicalis*(مقايسة تعكر المرق)، والسمية الخلوية على خلايا L-929 fibroblasts باستخدام مقايساتMTT ، وLDH ، و(neutral red uptake ، والنشاط المتعلق بنمو الشعر على خلايا human hair follicle dermal papilla cells ( المدمجةBrdU ، وإفراز IGF-1 و (VEGF إلى جانب التثبيط المختبري لإنزيم 5-α reductase. النتائج :(Results) احتوى كلا المستخلصين على السابونين، والفلافونويدات، والعفص (التانين)، والتربينويدات، والكومارين، والفيتوستيرول؛ وحدد تحليل GC–MS مركب glycidyl oleate كمكون رئيسي في الشيكاكاي، ومركب cis-vaccenic acid كمكون رئيسي في الريثا. أظهرت الشيكاكاي قيمة تركيز مثبط نصف أقصى (IC50) لـ DPPH أقل بشكل طفيف (حوالي 50 ميكروجرام/مل) مقارنة بالريثا (حوالي 60 ميكروجرام/مل)، وسعة مضادة للأكسدة كلية (TAC) أعلى بشكل طفيف (75 ± 5 مقابل 68 ± 4 مجم مكافئات حمض الأسكوربيك/جم)، ولم يصل أي من الفارقين إلى مستوى الدلالة الإحصائية (القيمة الاحتمالية .($p > 0.05$) أنتجت الريثا مناطق تثبيط أكبر وتراكيز مثبطة دنيا (MIC) أقل مقارنة بالشيكاكاي ضد السلالات البكتيرية الثلاث (على سبيل المثال، 4 مقابل 8 مجم/مل ضد (*S. aureus*، وثبطت نمو فطر *Candida* بنسبة 67–85% عند تركيز 5 مجم/مل، مقارنة بـ 15–17% للشيكاكاي. لم يقلل أي من المستخلصين حيوية خلايا L-929 إلى أقل من 80% عند تركيز 200 ميكروجرام/مل. زاد كلا المستخلصين من تكاثر خلايا dermal papilla cells مقارنة بالعينة الضابطة )الشيكاكاي حوالي 120%، الريثا حوالي 130%؛ مقارنة بـ minoxidil حوالي 150(%، وزادا من إفراز IGF-1 و VEGF، وثبطا نشاط 5 α-reductase بنسبة تصل إلى 45–50% عند تركيز 200 ميكروجرام/مل. الخلاصة :(Conclusion)تجمع مستخلصات الشيكاكاي والريثا بين النشاط المضاد للأكسدة، والمضاد للميكروبات، والمحفز لبصيلات الشعر عند تراكيز لم تُظهر سمية خلوية ملحوظة ضمن تراكيز اختبارات نمو الشعر، حيث تقود الريثا معظم التأثير المضاد للميكروبات، بينما يساهم كلا النباتين بشكل متقارب في النشاط المضاد للأكسدة، والمحفز للتكاثر، والمضاد للأندروجين في المختبر.(in vitro)

**الكلمات المفتاحية:**

الشيكاكاي

الريثا

الفحص الكيميائي النباتي

النشاط المضاد للأكسدة

خلايا الأديمة

## Introduction

Rising concern over antimicrobial resistance and the side effects associated with several synthetic hair-loss therapies has renewed interest in plant-derived alternatives for both antimicrobial and cosmeceutical use, and plants remain a major reservoir of structurally diverse antimicrobial secondary metabolites that continue to be screened for this purpose [1]. Reetha (Sapindus mukorossi, also known as Ritha or Indian soapnut) and Shikakai (Acacia concinna) are two plants long used in Ayurvedic and South Asian traditional medicine as natural hair cleansers and tonics: S. mukorossi is valued for its high saponin content and is reported to possess antimicrobial and anti-inflammatory properties [2], while A. concinna, whose Hindi name translates to "fruit for hair," is documented to have antioxidant and broader medicinal activity in addition to its traditional cleansing use [3,4]. Each plant has separately been examined for isolated properties — antibacterial activity of Shikakai pod extracts [5], antifungal activity favoring Sapindus over Acacia [6], and hair-follicle stimulation by Shikakai extract in animal skin [7] — yet the two plants are almost always combined in traditional formulations, and side-by-side data spanning their phytochemistry, antioxidant capacity, antimicrobial spectrum, in vitro cytocompatibility profile, and direct effects on human hair follicle cells remain limited. The present study therefore evaluates Shikakai and Ritha extracts under matched experimental conditions, characterizing their phytochemical and GC–MS profiles, antioxidant activity, antibacterial and antifungal spectra against common skin and scalp pathogens, in vitro cytocompatibility profile in cultured mammalian cells, and direct effects on human dermal papilla cell proliferation, growth-factor secretion, and 5α-reductase activity, in order to build a comparative pharmacological basis for the long-standing joint use of these two plants in hair and scalp care.

## Materials and Methods

### *Plant material and authentication*

Dried pods of Acacia concinna (Shikakai) and dried fruit pericarps of Sapindus mukorossi (Ritha) were procured from a certified local herbal market and authenticated by a botanist at the Department of Pharmacognosy, Faculty of Pharmacy, Sebha University (voucher specimens AC-2025 and SM-2025, respectively). Samples were inspected for adulterants and adequate drying before processing.

### *Preparation of extracts*

Each plant material was cleaned and pulverized to a fine powder using a mechanical grinder. For biological assays, aqueous extracts were prepared by mixing 50 g of powder with 500 mL of distilled water and heating at 60–70°C for 2 hours; an autoclave-assisted variant (5 g of powder in 50 mL distilled water, autoclaved at 121°C/15 psi for 30 minutes) was used where a sterile extract was required. Extracts were filtered through muslin cloth followed by Whatman No. 1 filter paper (Cytiva, Marlborough, MA, USA) and stored at 4°C. For phytochemical profiling and GC–MS, 25 g of each plant material was extracted with 250 mL of 95% ethanol (Sigma-Aldrich, St. Louis, MO, USA) by cold maceration for 72 hours, filtered, and concentrated

under reduced pressure at ≤40°C on a rotary evaporator (Buchi Rotavapor R-300, Büchi Labortechnik, Flawil, Switzerland); the resulting crude ethanolic extracts were stored in amber vials at 4°C. For the biological assays, the aqueous extracts were diluted directly into the corresponding broth or cell-culture medium; matching vehicle-control wells contained the same assay medium without plant extract. The percentage extraction yield was not determined gravimetrically in the original study.

***Phytochemical screening and GC–MS profiling***

Standard qualitative tests were performed on the aqueous extracts following established pharmacognostic protocols [8,9]: the froth test for saponins, Shinoda's test for flavonoids, ferric chloride test for tannins/polyphenols, Salkowski's test for terpenoids and sterols, ultraviolet fluorescence for coumarins, and Dragendorff's and Mayer's tests for alkaloids. All reagents (magnesium turnings, hydrochloric acid, ferric chloride, Dragendorff's and Mayer's reagents) were of analytical grade (Sigma-Aldrich). GC–MS analysis of the ethanolic extracts was performed on a gas chromatograph coupled to a mass-selective detector (Agilent 7890B GC/5977A MSD, Agilent Technologies, Santa Clara, CA, USA); compounds were tentatively identified by comparison of mass spectra with the NIST library.

***Antioxidant assays***

DPPH radical-scavenging activity was measured following the method of Blois [10], using 2,2-diphenyl-1-picrylhydrazyl reagent (Sigma-Aldrich) across a concentration series of each extract, with ascorbic acid (Sigma-Aldrich) as the reference standard; absorbance was read at 517 nm and IC50 values were derived from the dose–response curves. Total antioxidant capacity was determined by the phosphomolybdenum method of Prieto et al. [11], using ammonium molybdate, sodium phosphate, and sulfuric acid (Sigma-Aldrich), with results expressed as milligrams of ascorbic acid equivalents (AAE) per gram of extract.

***Antibacterial activity testing***

Antibacterial activity was assessed against Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, and Pseudomonas aeruginosa ATCC 27853 by the agar well diffusion method on Mueller–Hinton agar (Oxoid, Basingstoke, UK), with gentamicin discs (10 µg, Oxoid) as the positive control and sterile distilled water as the negative control; zones of inhibition were measured after 24 hours of incubation at 37°C. Minimum inhibitory concentrations were determined by broth microdilution in Mueller–Hinton broth (Oxoid) across a two-fold dilution series (2–32 mg/mL), with the MIC defined as the lowest concentration showing no visible growth after 24 hours.

***Antifungal activity testing***

Antifungal activity was evaluated against clinical isolates of Candida albicans (skin and nail) and a reference Candida tropicalis strain in Sabouraud dextrose broth (Oxoid) in a 96-well format, with extract concentrations of 1.25–10 mg/mL. Growth was quantified by optical density at 530 nm after 24 hours at 28°C, with percentage inhibition calculated relative to untreated growth controls; fluconazole and itraconazole (10 µg/mL, Sigma-Aldrich) were included on parallel plates as reference antifungal agents, and sterility controls (broth and extract without inoculum) confirmed that the extracts themselves did not contribute to turbidity.

***Cytotoxicity assays (MTT, LDH, and neutral red uptake)***

Cytotoxicity was assessed on L-929 mouse fibroblasts (ATCC CCL-1) cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1% penicillin–streptomycin (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). For the MTT assay, following Mosmann [12], cells were treated with extract concentrations from 25 to 1000 µg/mL for 24 hours, followed by MTT reagent (Sigma-Aldrich) and solubilization in dimethyl sulfoxide, with absorbance read at 570 nm (reference 630 nm). LDH release was measured in parallel using a commercial LDH cytotoxicity assay kit (Pierce LDH Cytotoxicity Assay Kit, Thermo Fisher Scientific), with percentage cytotoxicity calculated relative to Triton X-100-lysed cells as the maximum-release control. Neutral red uptake was assessed using neutral red dye (Sigma-Aldrich) after 3 hours of incubation, with absorbance of the eluted dye read at 540 nm. All assays were performed in at least triplicate wells across three independent experiments; data were analyzed by one-way ANOVA with Dunnett's test or unpaired Student's t-test as appropriate, with $p < 0.05$ considered significant.

***Hair growth–promoting assays***

Human hair follicle dermal papilla cells (HFDPC, adult scalp origin; ScienCell Research Laboratories, Carlsbad, CA, USA) were cultured in RPMI-1640 medium with 10% fetal bovine serum (Gibco) and used at passages 3–5. For the BrdU proliferation assay, cells were treated with Shikakai extract (50 µg/mL), Ritha extract (50 µg/mL), minoxidil sulfate (5 µM, Sigma-Aldrich) as a positive control, or vehicle for 48 hours, followed by 4 hours of incubation with 10 µM BrdU; incorporation was measured colorimetrically at 450 nm using a Cell Proliferation ELISA, BrdU kit (Roche Diagnostics, Basel, Switzerland) and expressed relative to untreated control (100%). In parallel wells, conditioned medium was collected after 48 hours of treatment and assayed for IGF-1 and VEGF using human Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA), read at 450 nm with a 570 nm reference. 5α-Reductase inhibition was measured in a cell-free assay using rat liver microsomes as the enzyme source, incubated with testosterone substrate (100 µM), NADPH cofactor (1 mM; both Sigma-Aldrich), and either extract (50 or 200 µg/mL) or finasteride (1 µM, Sigma-Aldrich) as the positive control; dihydrotestosterone produced was quantified with a competitive DHT ELISA kit (Abcam, Cambridge, UK), and percentage inhibition was calculated relative to the no-inhibitor control. All hair-growth assays were performed in triplicate or greater; BrdU proliferation was evaluated at a single screening concentration (50 µg/mL), whereas 5α-reductase inhibition was evaluated at 50 and 200 µg/mL.

***Statistical analysis***

Data were analyzed using GraphPad Prism 9 (GraphPad Software, Boston, MA, USA) and are presented as mean ± standard deviation when replicate-level values were available. One-way ANOVA with Dunnett's multiple-comparison test was used for experiments with more than two groups, and unpaired Student's t-test was used for two-group comparisons, with $p < 0.05$ considered significant. Normality and homogeneity-of-variance assumptions were checked, with log-transformation applied where required. MIC values and antifungal inhibition percentages were interpreted descriptively because the archived analysis dataset retained these as summary endpoints rather than complete replicate-level observations. Effect magnitude is therefore also reported as absolute differences, percentage-point differences, or fold changes where appropriate. Exact p values are reported when preserved in the original analysis output; threshold-only outputs (for example, $p > 0.1$) are retained as such rather than retrospectively reconstructed. Replication is stated only where documented in the source record: cytotoxicity assays comprised at least triplicate wells across three independent experiments, and hair-growth assays were performed in triplicate or greater.

**Results**

Qualitative phytochemical screening of the aqueous extracts detected saponins, flavonoids, tannins/polyphenols, terpenoids, coumarins, and phytosterols in both Shikakai and Ritha extracts (Table 1). Alkaloids were not detected in either extract using Dragendorff's or Mayer's reagents.

**Table 1.** Preliminary phytochemical screening of Shikakai and Ritha aqueous extracts.

| Phytochemical class | Shikakai (A. concinna) | Ritha (S. mukorossi) |
|---|---|---|
| Saponins | + | + |
| Flavonoids | + | + |
| Tannins / polyphenols | + | + |
| Terpenoids | + | + |
| Coumarins | + | + |
| Sterols (phytosterols) | + | + |
| Alkaloids | – | – |

GC–MS analysis of the ethanolic extracts identified fatty-acid derivatives as the predominant semi-volatile constituents in both plants (Table 2). The Shikakai extract showed major peaks corresponding to glycidyl oleate, methyl palmitate, and methyl stearate, whereas the Ritha extract was dominated by cis-vaccenic acid; methyl linoleate was detected as a minor component in both

extracts. No peaks corresponding to synthetic contaminants were observed in either chromatogram.

**Table 2.** Major compounds tentatively identified by GC–MS in Shikakai and Ritha ethanolic extracts.

| Compound (tentative ID) | Compound class | Detected in |
|---|---|---|
| Glycidyl oleate | Fatty-acid epoxy ester | Shikakai |
| Methyl palmitate | Saturated C16 fatty-acid ester | Shikakai |
| Methyl stearate | Saturated C18 fatty-acid ester | Shikakai |
| cis-Vaccenic acid | Unsaturated C18 fatty acid | Ritha |
| Methyl linoleate | Polyunsaturated C18:2 ester | Both (minor) |

In the DPPH radical-scavenging assay, the Shikakai extract had an IC50 of approximately 50 µg/mL and the Ritha extract an IC50 of approximately 60 µg/mL, compared with 20 µg/mL for ascorbic acid (Table 3); the difference between the two extracts was not statistically significant ($p > 0.05$). In the phosphomolybdenum total antioxidant capacity assay, the Shikakai extract measured 75 ± 5 mg AAE/g and the Ritha extract 68 ± 4 mg AAE/g (Table 3), also not significantly different ($p > 0.05$).

**Table 3.** Antioxidant activity of Shikakai and Ritha extracts (DPPH and total antioxidant capacity).

| Sample | DPPH IC50 (µg/mL) | TAC (mg AAE/g) |
|---|---|---|
| Shikakai extract | ~50 | 75 ± 5 |
| Ritha extract | ~60 | 68 ± 4 |
| Ascorbic acid (standard) | ~20 | — |

*AAE, ascorbic acid equivalents. Lower DPPH IC50 indicates stronger scavenging activity.*

Both extracts produced measurable zones of inhibition against S. aureus, E. coli, and P. aeruginosa in the agar well diffusion assay and detectable MICs in broth microdilution (Table 4). The Ritha extract produced larger inhibition zones and lower MICs than the Shikakai extract against every strain tested: for S. aureus, zones measured 18 ± 1.5 mm (Ritha) versus 12 ± 1.0 mm (Shikakai), with MICs of 4 and 8 mg/mL, respectively; for E. coli, zones were 15 ± 1.0 mm versus 10 ± 0.5 mm, with MICs of 8 and 16 mg/mL; and for P. aeruginosa, zones were 11 ± 0.5 mm versus 8 ± 0.0 mm, with MICs of 16 and 32 mg/mL. No inhibition zone was observed around the water control, and gentamicin (10 µg) produced zones of 22–26 mm across the three strains. Expressed as absolute effect magnitudes, Ritha increased the mean inhibition-zone diameter by 6 mm for S. aureus, 5 mm for E. coli, and 3 mm for P. aeruginosa relative to Shikakai, while reducing the MIC two-fold for each organism.

**Table 4.** Antibacterial activity of Shikakai and Ritha extracts (agar well diffusion, 100 mg/mL) and minimum inhibitory concentrations (broth microdilution).

| Bacterial strain | Shikakai zone (mm) | Ritha zone (mm) | Gentamicin zone (mm) | Shikakai MIC (mg/mL) | Ritha MIC (mg/mL) |
|---|---|---|---|---|---|
| S. aureus | 12 ± 1.0 | 18 ± 1.5 | 26 ± 1.2 | 8 | 4 |
| E. coli | 10 ± 0.5 | 15 ± 1.0 | 24 ± 1.0 | 16 | 8 |
| P. aeruginosa | 8 ± 0.0 | 11 ± 0.5 | 22 ± 2.0 | 32 | 16 |

Zone values are mean ± SD; no zone was observed for the water control. MIC values are descriptive broth-microdilution endpoints.

Against Candida yeasts in the broth turbidity assay, the Ritha extract produced substantially greater growth inhibition than the Shikakai extract at 5 mg/mL (Table 5): 67% versus 17% for a C. albicans skin isolate, 85% versus 15% for a second C. albicans (nail) isolate, and 30% versus 5% for C. tropicalis. The reference antifungal agents fluconazole and itraconazole achieved near-complete inhibition in parallel wells, and no turbidity was observed in the sterility controls. The corresponding Ritha–Shikakai differences were approximately 50, 25, and 70 percentage points for the skin C. albicans isolate, C. tropicalis, and the nail C. albicans isolate, respectively; these normalized antifungal endpoints were interpreted descriptively.

**Table 5.** Antifungal activity of Shikakai and Ritha extracts against Candida species at 5 mg/mL (24 h); values are normalized growth-inhibition percentages.

| Candida strain | Shikakai (% inhibition) | Ritha (% inhibition) |
|---|---|---|
| C. albicans (skin isolate) | ~17% | ~67% |
| C. tropicalis (reference) | ~5% | ~30% |
| C. albicans (nail isolate) | ~15% | ~85% |

In the MTT assay, L-929 fibroblast viability remained above 80% for both extracts at concentrations up to 200 µg/mL, with Shikakai-treated cells at approximately 88% and Ritha-treated cells at approximately 80% viability at this concentration (Table 6); at 50 µg/mL, viability for both extracts was 95–98%. The concentration causing 50% loss of viability was not reached for either extract up to 1000 µg/mL. LDH release remained at 10–15% at 200 µg/mL for both extracts, close to the 5% background release in untreated cells, and neutral red uptake showed 85–95% retention over the same concentration range.

**Table 6.** Viability of L-929 fibroblasts after 24 h treatment with Shikakai or Ritha extract (MTT assay; three independent experiments with at least triplicate wells per experiment).

| Extract | Viability at 50 µg/mL (%) | Viability at 200 µg/mL (%) |
|---|---|---|
| Shikakai extract | ~98% | ~88% |
| Ritha extract | ~95% | ~80% |

Treatment of human dermal papilla cells with Shikakai or Ritha extract (50 µg/mL) for 48 hours increased BrdU incorporation relative to untreated control cells (set at 100%): Shikakai treatment reached approximately 120% of control ($p < 0.05$) and Ritha treatment approximately 130% of control ($p < 0.01$), compared with approximately 150% for minoxidil (5 µM) (Table 7); the 10-percentage-point difference between Shikakai and Ritha was not statistically significant in the original analysis (threshold output $p > 0.1$; an exact value was not retained). In parallel cultures, on the normalized scale used for Table 7, IGF-1 secretion was approximately 112% and 125% of control after Shikakai and Ritha treatment, respectively, while VEGF was approximately 130% and 140% of control; these growth-factor endpoints were retained as descriptive normalized values in the archived dataset.

**Table 7.** Dermal papilla cell proliferation (BrdU) and growth-factor secretion after 48 h treatment (triplicate or greater; values normalized to untreated control).

| Treatment | BrdU proliferation (% of control) | IGF-1 (% of control) | VEGF (% of control) |
|---|---|---|---|
| Control | 100 (baseline) | 100 (baseline) | 100 (baseline) |
| Shikakai extract (50 µg/mL) | ~120% | ~112% | ~130% |
| Ritha extract (50 µg/mL) | ~130% | ~125% | ~140% |
| Minoxidil (5 µM) | ~150% | — | — |

Both extracts inhibited 5α-reductase activity in the cell-free enzyme assay in a dose-dependent manner (Table 8): at 50 µg/mL, inhibition was approximately 20% for Shikakai and 25% for Ritha, rising to approximately 45% and 50%, respectively, at 200 µg/mL. Finasteride

(1 µM), the positive control, inhibited approximately 95% of enzyme activity.

**Table 8.** Inhibition of 5α-reductase activity by Shikakai and Ritha extracts (triplicate or greater).

| Treatment (concentration) | 5α-Reductase inhibition (%) |
|---|---|
| Shikakai extract (50 µg/mL) | ~20% |
| Ritha extract (50 µg/mL) | ~25% |
| Shikakai extract (200 µg/mL) | ~45% |
| Ritha extract (200 µg/mL) | ~50% |
| Finasteride (1 µM, positive control) | ~95% |

**Discussion**

This study set out to characterize Shikakai and Ritha extracts under matched conditions, spanning phytochemistry, antioxidant capacity, antimicrobial spectrum, in vitro cytocompatibility profile, and direct effects on human hair follicle cells, in order to build a comparative pharmacological basis for the traditional joint use of these two plants in hair and scalp care. Read together, the results support that aim: both plants carry a broad phytochemical base capable of several simultaneous actions, and the pattern of activity across assays is internally consistent rather than incidental.

The phytochemical screening and GC–MS data show that both plants are multi-component systems rather than single-compound sources. The concurrent presence of saponins, flavonoids, tannins, terpenoids, and phytosterols in both extracts is consistent with earlier reports that Shikakai pods contain concinnoside-type saponins alongside polyphenols, while Ritha fruits are dominated by sapindoside-type saponins together with fatty acids and glycosides [2]. The GC–MS finding of glycidyl oleate as a major Shikakai constituent and cis-vaccenic acid as a major Ritha constituent adds a lipophilic dimension to this picture; a related long-chain fatty-acid signature in Sapindus fruit material has also been reported in an unrelated analytical context [13], which is consistent with fatty-acid derivatives being a genuine and recurring feature of Ritha extracts rather than an extraction artifact. No synthetic contaminants were detected in either profile, supporting the chemical quality of the preparations used in the subsequent bioassays.

The antioxidant results place both extracts in a relatively high-activity range for crude plant preparations: DPPH IC50 values of approximately 50–60 µg/mL are similar to the ~60–70 µg/mL range reported for Acacia and Sapindus fruit extracts under comparable assay conditions [4], and the marginal edge shown here by Shikakai is compatible with the antioxidant activity separately documented for A. concinna leaf material [3]. Because the difference between the two extracts did not reach statistical significance in either the DPPH or the total antioxidant capacity assay, the most defensible reading of the antioxidant data is that Shikakai and Ritha are comparably effective radical scavengers rather than that one plant is meaningfully superior to the other on this axis. Accordingly, the small numerical differences should not be interpreted as evidence of antioxidant superiority by either extract.

The most consistent and largest-magnitude finding in this study is the antimicrobial advantage of Ritha over Shikakai, evident in the agar-diffusion zones and the two-fold lower MIC values against all three bacterial strains, and even more pronounced in the descriptive Candida inhibition data. This pattern agrees with prior comparative work showing Sapindus extracts outperforming Acacia extracts against fungal pathogens [6] and with reports that Shikakai pod extracts, while active against Gram-positive organisms such as S. aureus, are comparatively modest broad-spectrum antibacterial agents [5]. A saponin-related contribution is chemically plausible because saponins can act as biosurfactants and disturb microbial membrane and cell-wall integrity; more recent experimental work has directly documented leakage of cellular constituents and structural membrane damage after exposure to plant-derived saponin fractions [14], consistent with the broader mechanistic literature [15]. However, the present study did not fractionate the extracts or quantify individual saponins, so the antimicrobial differences cannot be assigned conclusively to a specific compound or mechanism.

The L-929 experiments provide an in vitro cytocompatibility signal rather than a complete safety assessment. Fibroblast viability remained above 80% at 200 µg/mL and LDH/neutral-red readouts were broadly concordant, indicating that the concentrations used in the hair-related assays were not markedly cytotoxic under these conditions. These findings should not be extrapolated to topical or long-term safety: crude saponin-rich preparations can interact with biological membranes, and repeated scalp exposure may produce irritation or sensitization in susceptible users. Accordingly, dedicated reconstructed-skin irritation, sensitization, and repeated-exposure studies are required before either extract can be described as dermally safe.

The hair-growth-related assays add mechanistic leads to the traditional reputation of both plants. The 20–30% increase in dermal papilla cell proliferation produced by Shikakai and Ritha, alongside directional increases in IGF-1 and VEGF secretion, is consistent with earlier observations that Shikakai extract can accelerate hair-follicle proliferation in animal skin [7]; the present data extend that observation to a human dermal-papilla-cell model and to Ritha. The VEGF increase is directionally consistent with the established observation that minoxidil can upregulate VEGF in human dermal papilla cells [16], but the present experiment measured secretion only and therefore does not establish a vascular or angiogenic mechanism in intact follicles. The 5α-reductase findings are also biologically plausible in light of more recent natural-product studies: physcion has been shown experimentally to inhibit 5α-reductase and promote hair growth in vitro and in vivo [17], while rice-derived phytochemical preparations have demonstrated suppression of steroid 5α-reductase pathways in vitro [18]. Nevertheless, the effects observed here were more modest than the positive controls (approximately 120–130% proliferation versus ~150% for minoxidil, and 45–50% 5α-reductase inhibition versus ~95% for finasteride), supporting interpretation of Shikakai and Ritha as experimental botanical candidates rather than substitutes for established pharmacological treatments.

These conclusions should be read with several limitations in mind. All bioactivity data were generated in vitro using cultured cells, yeast/bacterial assays, or a cell-free enzyme preparation, so translation to intact human scalp remains unconfirmed. The extracts were crude preparations and the active constituents responsible for individual effects were not isolated or quantified. The original study did not record gravimetric extraction yield or evaluate chemical stability during storage, which limits inter-study standardization. The BrdU proliferation screen used only one extract concentration (50 µg/mL), so a concentration–response relationship for dermal-papilla proliferation cannot be defined. In addition, several archived endpoints (antifungal inhibition, IGF-1, VEGF, and 5α-reductase) were retained as normalized summary percentages rather than complete raw replicate values; therefore, missing standard deviations, exact p values, and standardized effect sizes were not reconstructed retrospectively. Finally, L-929 cytocompatibility does not address skin irritation, sensitization, barrier effects, or chronic exposure. Future work should quantify extraction yield and marker compounds, conduct stability testing, fractionate the extracts to identify active constituents, generate concentration–response data for the hair-cell endpoints, retain complete replicate-level datasets for inferential statistics, and confirm efficacy and dermal safety in ex vivo follicle/skin systems before any clinical or cosmeceutical claim is made.

**Conclusion**

This comparative evaluation indicates that Shikakai (Acacia concinna) and Ritha (Sapindus mukorossi) extracts share a broad phytochemical base associated with measurable antioxidant, antimicrobial, and hair-follicle-related activity. Ritha consistently showed stronger antibacterial and antifungal effects, whereas the two extracts had statistically comparable antioxidant activity and similar moderate effects on dermal papilla cell proliferation and 5α-reductase inhibition. The L-929 results support cytocompatibility at the tested hair-assay concentrations but do not establish topical or long-term dermal safety. Overall, these in vitro findings provide a comparative pharmacological basis for further standardized, dose–response, ex vivo, and clinical investigation rather than evidence of clinical efficacy or product safety.

**Declarations**

**Author contributions:** Conceptualization and supervision, S.S.S.;

methodology, investigation, formal analysis, and writing – original draft, H.A.Y.A. and Z.G.M.E.; writing – review and editing, S.S.S. All authors read and approved the final manuscript.

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**Conflict of interest:** The authors declare no conflict of interest.

**Data availability:** The data supporting the findings of this study are available from the corresponding author upon reasonable request.

**Ethics statement:** This study did not involve human participants or living animal experimentation; commercially sourced cell lines and standard tissue preparations were used in accordance with institutional laboratory practice.

**Generative AI disclosure:** Generative AI assistance was used to restructure, tighten, and copy-edit this manuscript from the authors' original graduation research project; all experimental data, scientific claims, and interpretations remain the responsibility of, and were reviewed and approved by, the authors.

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