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Formulation and In Vitro Anticandidal Evaluation of a Herbal Face Serum Containing Combined Aqueous Leaf Extracts of *Artocarpus Heterophyllus* Lam. and *Terminalia Catappa* L.

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ABSTRACT

Background: Superficial fungal infections are increasingly difficult to treat because of azole resistance and drug-related adverse effects, which has renewed interest in plant-derived topical antifungals. The leaves of Artocarpus heterophyllus (jackfruit) and Terminalia catappa (Indian almond) are rich in phenolics, flavonoids and tannins with reported antimicrobial activity.

Objective: To authenticate and characterise both leaves, to prepare a face serum containing their combined aqueous extracts, and to evaluate its physicochemical properties and in vitro anticandidal activity.

Methods: Leaves were authenticated, studied macroscopically and microscopically, and extracted by aqueous maceration. Ash values, loss on drying, moisture content and preliminary phytochemical screening were determined. A xanthan gum-based serum containing 2% w/w of each extract was evaluated for appearance, pH, viscosity, spreadability, homogeneity, washability, absorption time and short-term stability. Antifungal activity against Candida albicans MTCC 183 was assessed by agar well diffusion, with fluconazole (1 mg/mL) as positive and placebo serum as negative control.

Results: Extractive yields were 13.8% w/w (T. catappa) and 10.24% w/w (A. heterophyllus). Both extracts contained alkaloids, flavonoids, tannins/phenolics, steroids, reducing sugars and amino acids. The serum was homogeneous and easily spreadable, with a pH of 5.12 and a viscosity of 1300 cP, and was visually stable for 10 days under light and dark storage. The test serum produced a 12 mm zone of inhibition against Candida albicans (fluconazole, 20 mm; placebo, none).

Conclusion: The combined-extract serum is a stable, skin-compatible formulation with measurable anticandidal activity. Because individual extracts were not tested in parallel and dermatophytes were not included, synergy and dermatophyte activity remain to be established.

Keywords: Artocarpus heterophyllus; Terminalia catappa; herbal antifungal serum; Candida albicans; well diffusion assay; topical formulation; phytochemical screening

1. Introduction

Fungal infections of the skin, hair and nails are among the most common infectious diseases worldwide, and their burden is greatest in patients with diabetes, HIV infection or prolonged immunosuppression. Species of *Candida* are the leading cause of superficial and invasive mycoses, while dermatophytes (*Trichophyton, Microsporum* and *Epidermophyton*) cause tinea infections. Treatment relies on azoles, polyenes, allylamines and echinocandins, but prolonged use has been followed by azole-resistant *Candida* strains, local irritation and systemic toxicity [9,13]. Plant secondary metabolites such as phenolic acids, flavonoids and hydrolysable tannins offer a reservoir of structurally diverse antimicrobial leads, and herbal topical products are generally well accepted by patients.

Terminalia catappa L. (Combretaceae; Indian or tropical almond) and *Artocarpus heterophyllus* Lam. (Moraceae; jackfruit) are widely distributed in India. Their leaves are used in folk medicine for skin diseases, wounds and infections, and contain flavonoids, tannins, saponins and phenolic acids with antioxidant, anti-inflammatory and antimicrobial properties [1,8]. A serum is a light, fast-absorbing, easily spreadable dosage form suited to delivering water-soluble plant extracts to the skin, yet few studies have formulated leaf extracts of these two species into a topical product and tested it. The present work therefore aimed to (i) authenticate and characterise both leaves, (ii) prepare aqueous extracts and screen their phytoconstituents, (iii) formulate a face serum containing the combined extracts, and (iv) evaluate its physicochemical properties and in vitro anticandidal activity.

2. Review of Literature

Jackfruit leaf extracts obtained by conventional and emerging techniques were shown to inhibit phytopathogenic fungi, and high hydrostatic pressure extraction gave the highest metabolite content and the strongest antifungal effect, with phenolic acids and flavonoids implicated [2,5]. A recent microcalorimetric study also confirmed antimicrobial activity of jackfruit leaf extract [7]. Phytochemical and nutritional reviews describe phenolics, flavonoids, terpenoids, saponins and tannins in *A. heterophyllus* with antioxidant and antimicrobial effects [1,3,4,6].

For *T. catappa*, ethanolic and aqueous leaf extracts inhibited *Candida* species, and active fractions were rich in punicalin, punicalagin, gallic acid and flavonoids [8,9]. Methanol and aqueous extracts showed antimicrobial and antioxidant activity [10]; leaf extracts also acted against selected dermatophytes in vitro [13] and potentiated antibiotics against resistant bacteria [18]. Combinations of *T. catappa* with other nutraceutical plants have been reported to enhance antimicrobial effects [12]. To our knowledge, a serum combining the leaf extracts of both species has not been evaluated, and the contribution of each species to the combined activity is unknown.

3. Materials and Methods

3.1 Plant material and authentication

Mature leaves of *Terminalia catappa* and *Artocarpus heterophyllus* were collected from trees growing in Mysuru, Karnataka, and authenticated by the Head, Department of Botany, Sarada Vilas College, Mysuru (certificate SVC/Bot/Auth/07/2026-27, dated 01.07.2026). The leaves were washed under running water, shade-dried, powdered and stored in airtight containers at room temperature.

3.2 Macroscopic and microscopic studies

Colour, odour, touch, shape, arrangement and size of fresh and dried leaves were recorded. Transverse sections were cleared with chloral hydrate and stained with phloroglucinol-HCl. Leaf powders were examined with phloroglucinol-HCl and iodine, with and without stain, and photomicrographs were taken. Stomatal number, stomatal index, vein-islet number and vein-termination number were determined per mm^2.

3.3 Extraction, physicochemical constants and phytochemical screening

Powdered leaf (25 g of each species) was macerated in 250 mL distilled water (1:10 w/v) in a closed flask at room temperature for 24-72 h with occasional shaking. The filtrates were passed through Whatman No. 1 paper and concentrated on a water bath (40-50 °C). Percentage yield = (weight of dried extract / weight of powder) × 100. Total ash, acid-insoluble ash, water-soluble ash, loss on drying and moisture content were determined by standard pharmacopoeial procedures [14,15]. Extracts were screened for alkaloids (Mayer, Wagner, Dragendorff), flavonoids (Shinoda, alkaline reagent), tannins and phenolics (ferric chloride, lead acetate, gelatin), saponins (froth), steroids and triterpenes (Liebermann-Burchard, Salkowski), cardiac glycosides (Keller-Killiani), anthraquinones (Borntrager), carbohydrates (Molisch, Fehling/Benedict) and proteins/amino acids (Biuret, ninhydrin) using standard methods [14,16].

3.4 Formulation of the serum

The serum (100 g) contained *Terminalia catappa* extract 2% w/w, *Artocarpus heterophyllus* extract 2% w/w, xanthan gum 0.5 g, glycerin 10 mL, sodium benzoate 0.2 g and purified water to 100. Xanthan gum was sprinkled into 70-80 mL of water under continuous stirring and hydrated for 30-60 min; glycerin was then added. Sodium benzoate dissolved in a little warm water was added with stirring, followed by the combined extracts, and the volume was made up to 100 mL. The serum was packed in clean amber glass bottles. A placebo serum without extract was prepared in the same way.

3.5 Evaluation of the serum

Appearance, colour, odour and texture were assessed visually and by touch. pH was measured with a calibrated digital pH meter after dispersing 1 mL of serum in 50 mL of water. Viscosity was measured with a Brookfield viscometer (spindle no. 62, 50 and 100 rpm). Spreadability was evaluated by the filter-paper method, homogeneity visually and by microscopy, absorption time on skin by timing, and washability by rinsing with water. For stability, samples were stored for 10 days in direct sunlight and protected from light and checked for changes in physical properties.

3.6 Antifungal assay

Candida albicans MTCC 183 was grown in potato dextrose broth for 24-48 h at 30 °C. Potato dextrose agar pre-inoculated with the organism (1%) was poured into sterile Petri plates and 5 mm wells were bored with a sterile cork borer. The test sample was prepared by mixing 120 mg of extract with 1 mL serum, and 100 µL (equivalent to 12 mg extract) was added per well; placebo serum served as negative control and fluconazole (1 mg/mL) as positive control. Plates were held at 4 °C for 30 min for diffusion, incubated at 30 °C for 24-48 h, and the zone of inhibition (ZOI) was measured in mm.

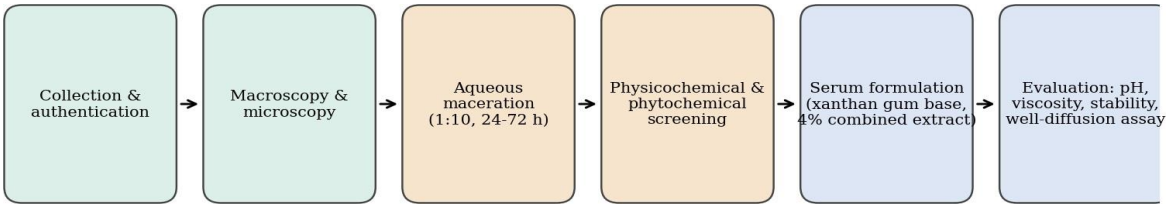


Fig. 1. Workflow of the study, from leaf authentication to formulation and evaluation of the serum.

4. Results

4.1 Macroscopic characters

Both leaves were simple, alternate, leathery and glossy dark green above and paler beneath, with an entire margin and a mild, non-pungent herbal odour; on drying they turned greenish-brown to brown and brittle (Table 1). *T. catappa* leaves were broadly obovate and clustered at the branch tips with short petioles and were considerably larger (15-30 × 8-15 cm) than the elliptic to obovate *A. heterophyllus* leaves (7-23 × 3-13 cm), which were spirally arranged and petiolate.

Table 1. Macroscopic characters of the leaves

Feature	*T. catappa*	*A. heterophyllus*
Shape	Broadly obovate to oval, entire margin, rounded/blunt apex, prominent pinnate venation	Elliptic to obovate or oblong, entire margin in mature leaves, blunt to obtuse apex, prominent midrib
Arrangement	Alternate, clustered at branch ends, short petiole (0.5-2 cm)	Alternate, spirally arranged, petiolate
Size (cm)	Length 15-30; width 8-15	Length 7-23; width 3-13
Colour (fresh / dry)	Dark green glossy above, pale green below / greenish-brown to brown	Dark green glossy above, pale green below / greenish-brown to brown

4.2 Microscopic characters

Transverse sections of both leaves showed cuticle, upper and lower epidermis, palisade and spongy mesophyll, collenchyma and a central vascular bundle with xylem and phloem. Powder microscopy revealed epidermal cells, stomata with guard and subsidiary cells, vein fragments, trichomes and parenchyma. The leaves differed markedly in quantitative microscopy (Table 2, Fig. 2): *T. catappa* had roughly two to three times the stomatal number and stomatal index, and several-fold higher vein-islet and vein-termination numbers, than *A. heterophyllus*. These values can serve as diagnostic parameters for identifying the two leaf drugs.

Table 2. Quantitative microscopy of the leaves (range of observations)

Parameter	*T. catappa*	*A. heterophyllus*
Stomatal number (per mm²)	20-25	7-9
Stomatal index (%)	19-25	13.8-15.6
Vein-islet number (per mm²)	18-26	4-5
Vein-termination number (per mm²)	16-24	2-4

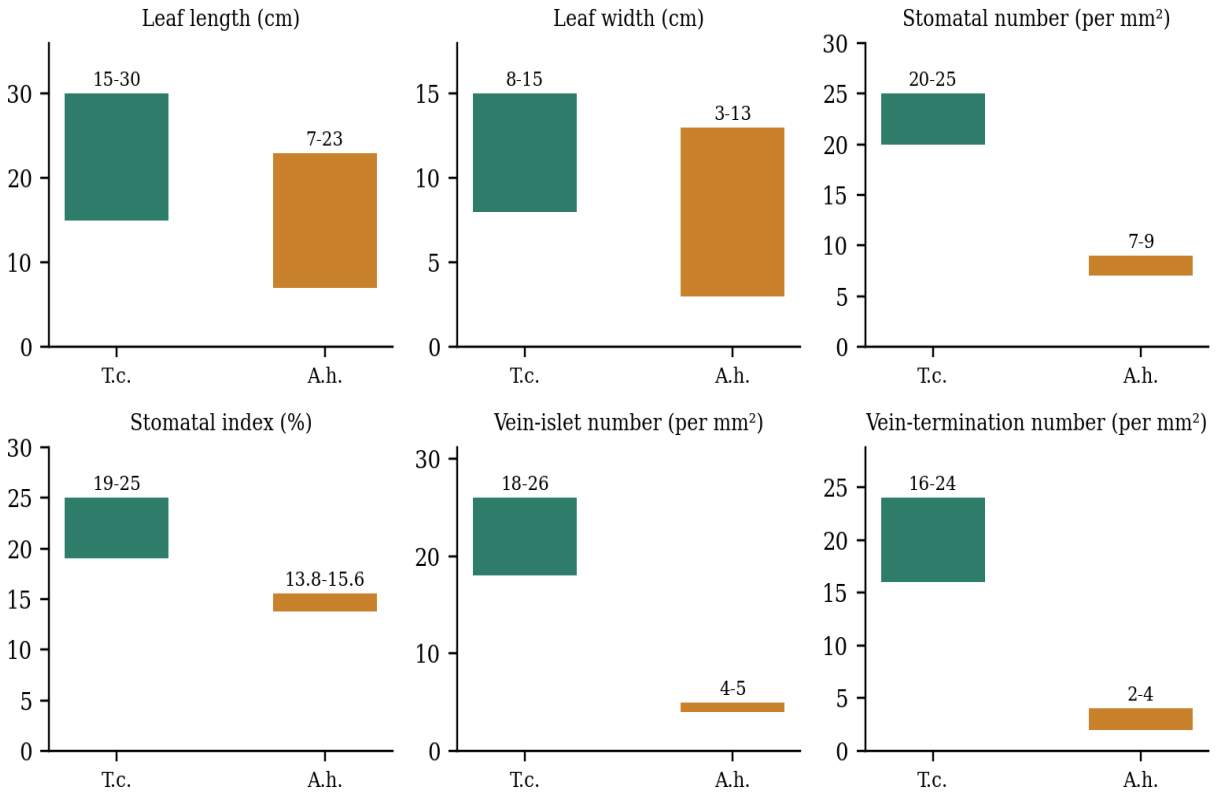


Fig. 2. Comparison of leaf dimensions and quantitative microscopic constants of *T. catappa* (T.c., green) and *A. heterophyllus* (A.h., orange). Bars span the observed range; labels give the range.

4.3 Extractive yield and physicochemical constants

Aqueous maceration of 25 g of powder gave 3.45 g of dried extract from *T. catappa* (13.8% w/w) and 2.56 g from *A. heterophyllus* (10.24% w/w) (Fig. 3a). Loss on drying was nearly identical (9.4% and 9.3% w/w), whereas total ash (22.6% vs 25.6%), acid-insoluble ash (7.6% vs 15.2%), water-soluble ash (2.2% vs 3.2%) and moisture content (7.04% vs 8.02%) were higher in jackfruit leaf (Table 3, Fig. 3b). The acid-insoluble ash of jackfruit leaf was twice that of Indian almond leaf, indicating more adhering siliceous or earthy matter.

Table 3. Physicochemical constants and extractive yield of the leaf powders

Parameter	***T. catappa***	***A. heterophyllus***
Loss on drying (% w/w)	9.4	9.3
Total ash (% w/w)	22.6	25.6
Acid-insoluble ash (% w/v)	7.6	15.2
Water-soluble ash (% w/v)	2.2	3.2
Moisture content (%)	7.04	8.02
Extractive yield, aqueous maceration (% w/w)	13.8	10.24

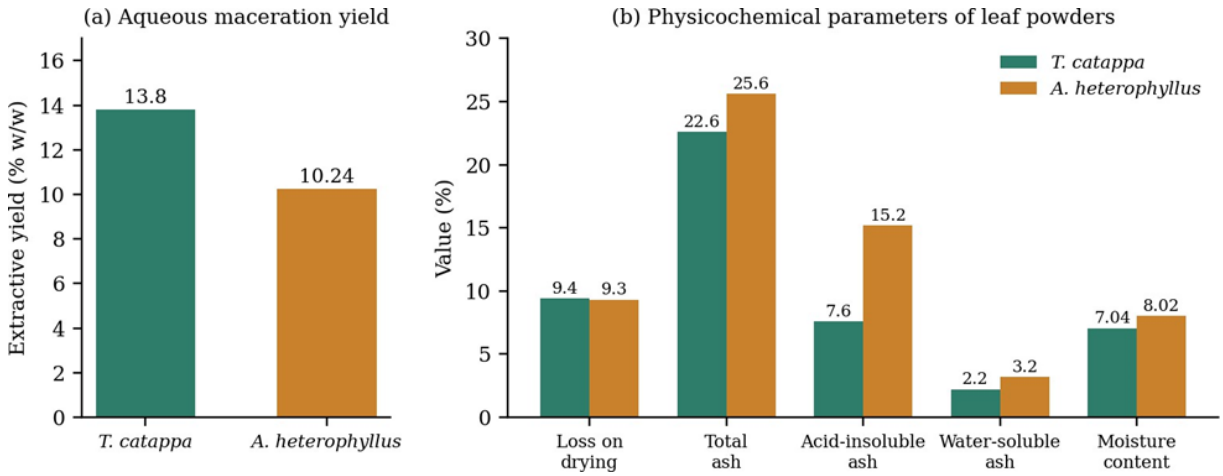


Fig. 3. (a) Extractive yield of the aqueous extracts and (b) physicochemical constants of the leaf powders of *T. catappa* and *A. heterophyllus*.

4.4 Preliminary phytochemical screening

Both extracts gave the same screening pattern (Fig. 4). Alkaloids (Mayer and Wagner positive; Dragendorff negative), flavonoids (Shinoda and alkaline reagent), tannins/phenolics (lead acetate and gelatin positive; ferric chloride negative), steroids (Liebermann-Burchard), reducing sugars (Fehling/Benedict) and amino acids (ninhydrin) were detected. Saponins, triterpenes (Salkowski), cardiac glycosides, anthraquinone glycosides, Molisch-positive carbohydrates and Biuret-positive proteins were not detected.

	T. catappa	A. heterophyllus
Mayer (alkaloids)	+	+
Wagner (alkaloids)	+	+
Dragendorff (alkaloids)	−	−
Shinoda (flavonoids)	+	+
Alkaline reagent (flavonoids)	+	+
Ferric chloride (phenolics)	−	−
Lead acetate (tannins/phenolics)	+	+
Gelatin (tannins)	+	+
Froth (saponins)	−	−
Liebermann-Burchard (steroids)	+	+
Salkowski (triterpenes)	−	−
Keller-Killiani (cardiac glycosides)	−	−
Borntrager (anthraquinones)	−	−
Molisch (carbohydrates)	−	−
Fehling/Benedict (reducing sugars)	+	+
Biuret (proteins)	−	−
Ninhydrin (amino acids)	+	+

Fig. 4. Phytochemical screening of the aqueous leaf extracts (+, present; □, absent).

4.5 Characteristics of the serum

The serum was a smooth, slightly viscous, homogeneous, non-gritty amber (brownish-yellow) liquid with a mild earthy odour. Its pH was 5.12. Viscosity measured 1300 cP, and the product spread easily, was absorbed in 1-2 min, washed off readily with water and showed no visible change in colour, consistency or homogeneity after 10 days in either light or dark storage (Table 4).

Table 4. Physical evaluation of the herbal serum

Parameter	Observation
Colour / appearance	Amber (brownish-yellow); smooth, slightly viscous liquid
Odour	Mild, characteristic, slightly earthy
Homogeneity	Homogeneous; no particulate matter
pH	5.12
Viscosity (Brookfield, spindle 62)	1300 cP
Spreadability	Very spreadable
Absorption time	1-2 min
Washability	Easily washable
Stability (10 days, light and dark)	Stable

4.6 Anticandidal activity

The placebo serum produced no zone of inhibition, confirming that the base was inactive. The test serum gave a 12 mm zone of inhibition against *Candida albicans* MTCC 183, compared with 20 mm for fluconazole (1 mg/mL), i.e. 60% of the zone of the reference drug (Fig. 5).

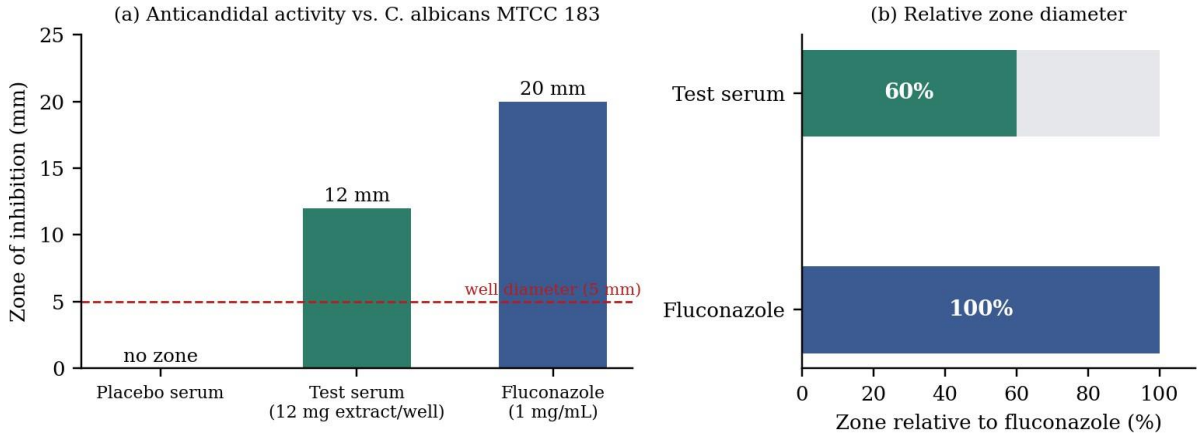


Fig. 5. Anticandidal activity of the herbal serum against *Candida albicans* by agar well diffusion: (a) zone of inhibition and (b) zone relative to fluconazole. Single determination.

5. Discussion

5.1 Pharmacognostic standards

Authentication and the macroscopic and microscopic profile establish the identity of the two leaf drugs and give diagnostic features for future quality control. The large differences in stomatal number, stomatal index and vein-islet and vein-termination numbers (Table 2) are especially useful because these constants are relatively stable within a species and are used to distinguish leaf drugs and detect adulteration [14]. The ash values were high for both leaves, and the acid-insoluble ash of *A. heterophyllus* (15.2%) in particular points to adhering soil or silica. Raw material for a topical product should be washed more thoroughly and limits for acid-insoluble ash should be set before scale-up. The moisture contents (7.04% and 8.02%) and loss on drying (about 9%) are acceptable for air-dried leaf, but are close to the level at which microbial growth becomes a risk during storage, so airtight storage is advisable.

5.2 Extraction and phytoconstituents

Water was chosen as a safe, inexpensive solvent compatible with a water-based serum. It extracted both polar phenolics and tannins efficiently, giving a higher yield from *T. catappa* (13.8%) than from *A. heterophyllus* (10.24%), in keeping with the high tannin content of Indian almond leaf. The detection of flavonoids, tannins/phenolics and alkaloids agrees with earlier reports on both species [1,3,8,10]. These classes are widely linked to antifungal action through disruption of cell-membrane integrity, inhibition of spore germination and interference with fungal enzymes and ergosterol-dependent processes [2,9]. In *T. catappa* the hydrolysable tannins punicalagin and punicalin, together with gallic acid and flavonoids, have been associated with activity against *Candida* [9]. Two points in the screening data require caution. First, the two extracts gave identical qualitative profiles, which is plausible for

broad class tests that cannot discriminate between species but means the screening has no diagnostic value; chromatographic fingerprinting (HPLC, GC-MS) is needed to show how the extracts actually differ. Second, the ferric chloride test was negative although lead acetate and gelatin tests for tannins were positive; this may reflect low concentration or the dilute aqueous extract and should be confirmed by quantifying total phenolic and tannin content.

5.3 Formulation attributes

The xanthan gum-glycerin base produced a smooth, homogeneous gel-like serum. Xanthan gum is a shear-thinning, electrolyte-tolerant hydrocolloid, which explains the combination of moderate viscosity (1300 cP) at rest and easy spreading on application; glycerin acts as a humectant and sodium benzoate, which is most effective in mildly acidic media, is well suited to a product with a pH of 5.12. This pH lies within the acidic-to-neutral range reported for skin (approximately 4.1-7.0), so irritation due to pH is unlikely, and it matches the skin's acid mantle, which helps maintain the barrier and resident flora. The rapid absorption (1-2 min) and washability are desirable for an antifungal serum that must be applied repeatedly. The amber colour reflects extract pigments and may stain clothing slightly; encapsulation or a colour-masking approach could be considered. The 10-day light and dark stability study showed no visible change, but it is a screening test only and does not replace ICH-guided accelerated testing (40 °C / 75% RH for 3-6 months) with periodic assay of marker compounds, pH, viscosity and microbial load [17].

5.4 Antifungal activity

The 12 mm zone against *Candida albicans*, with no zone from the placebo, shows that the activity is due to the extracts and not to the base. The finding is consistent with reports that *T. catappa* leaf extracts inhibit *Candida* spp. and dermatophytes [9,13] and that jackfruit leaf extracts have antifungal activity [2,5]. The serum was about 60% as effective as fluconazole by zone diameter, which is encouraging for a crude, unfractionated extract compared with a purified drug. This comparison should not be over-interpreted: zone size in agar diffusion depends on diffusion rate as well as potency, large polyphenols diffuse slowly through agar, and the extract and fluconazole were tested at different, non-equivalent concentrations. The extract is also deeply coloured, which can make the zone edge harder to read, so the result should be confirmed by broth microdilution to obtain minimum inhibitory and fungicidal concentrations.

5.5 Synergy and dermatophytes: what the data do and do not show

The combination was chosen to exploit the complementary phytochemistry of the two leaves, but the experiments performed here cannot demonstrate synergy. Synergy requires that the combination be tested against each extract alone at equivalent doses and that the interaction be quantified, for example by a checkerboard assay and fractional inhibitory concentration index (FICI of 0.5 or less indicating synergy). Only the combined serum was tested, so the present results should be read as evidence of anticandidal activity of the combination, not of synergistic action. Likewise, *Candida albicans* is a yeast that causes cutaneous candidiasis, and activity against it cannot be extrapolated to dermatophytes. Because the clinically important agents of tinea are filamentous fungi, *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum* and *Epidermophyton* should be tested next, and the earlier reports of leaf-extract activity against dermatophytes [13] make this a promising direction.

5.6 Limitations

The study has several limitations: (i) a single organism and a single determination, with no replicates or statistical analysis; (ii) no arms for the individual extracts; (iii) no MIC, MBC or time-kill data; (iv) a 10-day stability study;

(v) no skin irritation or sensitisation study, although these plants are used traditionally; (vi) a spreadability result described qualitatively; and (vii) the extract load in the assay sample (12 mg per well, prepared by adding 120 mg extract to 1 mL serum) which is higher than the nominal formulation strength of 4% w/w, so the activity of the final 4% serum needs to be confirmed.

6. Conclusion

A herbal face serum containing 2% w/w each of aqueous *A. heterophyllus* and *T. catappa* leaf extracts was formulated with a xanthan gum base. The authenticated leaves were characterised pharmacognostically, and the extracts contained flavonoids, tannins/phenolics and alkaloids. The serum was homogeneous, easily spreadable, absorbed within 1-2 min, had a skin-compatible pH of 5.12 and a viscosity of 1300 cP, and was stable for 10 days. It inhibited *Candida albicans* with a 12 mm zone (60% of fluconazole) while the placebo was inactive. These findings support further development of the serum as a natural topical antifungal. Testing against dermatophytes, comparison with the individual extracts and checkerboard analysis of interaction, MIC determination, phytochemical standardisation by HPLC or GC-MS, ICH-compliant stability studies and safety evaluation are the next steps.

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Conflict of interest, funding and ethics

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