

DEVELOPMENT AND EVALUATION OF MUKIA MADERASPATANA LOADED SOLID LIPID NANOPARTICLE-BASED NANOEMULSION FOR ANTIFUNGAL SCALP THERAPY

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ABSTRACT

Mukiamaderaspatana (L.) M. Roem. is a medicinal folk creeper of the family Cucurbitaceae having antimicrobial and anti-inflammatory properties [1,2]. In the present study, we developed and evaluated a nanoemulsion of SLN loaded with M. maderaspatana hydroalcoholic extract for antifungal therapy of the scalp. Leaves were macerated in ethanol-water (70:30); screening confirmed alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids and phenolic compounds [3]. UV-Visible spectroscopy showed absorption maxima at 266 nm and 331 nm, and total flavonoid content was 21.56 mg QE/g by the aluminium chloride method [12,13]. Thin-layer chromatography gave a distinct band with an R_f value of 0.78, and FTIR confirmed groups consistent with phenols, flavonoids and terpenoids [11]. SLNs were synthesised by the solvent injection technique [9] using glyceryl monostearate as the lipid matrix, incorporated into an oil-in-water nanoemulsion using castor oil, Tween 80 and PEG 400. Three formulations (F1-F3) were evaluated; F1 performed best, with nanometre-range particle size, polydispersity index of 0.30, scalp-compatible pH of 5.3, drug content of 90% and highest entrapment efficiency of 93.1%. F1 showed satisfactory spreadability, viscosity (2.77 cP), and withstood centrifugation and heating-cooling stress without phase separation [16]. Dye, dilution and conductivity tests confirmed an oil-in-water architecture. Antifungal evaluation against *Candida albicans* [14,15] showed dose-dependent inhibition zones of 13 mm and 15 mm at 100 and 150 micrograms, versus 20 mm for fluconazole. The results showed that the nanoemulsion is a stable nanocarrier with antifungal activity and is suitable for scalp therapy.

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INTRODUCTION:

Warm and humid climatic conditions in tropical and subtropical regions favour the proliferation of microorganisms, which leads to fungal infections on the scalp and are one of the most persistent dermatological complaints. [14]

Factors that are well known to predispose to excessive sebum production, poor scalp hygiene, immunocompromised states, hormonal fluctuations, and sharing personal grooming items are all recognised.

The opportunistic dimorphic yeast *Candida albicans* colonises the skin and mucous membranes of humans harmlessly, but can be a primary cause of scalp folliculitis and seborrheic dermatitis when conditions are right for it to switch to an invasive, hyphal form. It attaches itself to the keratinised tissues of the scalp and produces hydrolytic enzymes which break down keratin and sebum, and has the ability to create protective biofilms which significantly diminish the effectiveness of conventional antifungal treatment. [14,15]

One big drawback to the antifungal shampoos, lotions and creams available now is that they can't reach deeper into the skin beyond the outer

epidermis and into the hair follicle, where *Candida* is the main hiding place. As a result, the recurrence rate has remained high, even with the use of azole and polyene antifungal agents, some of which are becoming increasingly resistant and causing skin irritation when used long-term. To overcome these limitations, topical delivery of nanotechnology-based carriers, especially Solid Lipid Nanoparticles (SLNs), has gained significant interest. Due to their sub-micron size, lipophilic matrix, increased follicular penetration, protection of labile phytoconstituents from degradation, sustained release profile at the target site, and surfactant-free manufacturing, SLNs can be used to deliver deeper penetration, protection for labile phytoconstituents, and sustained release at the target site. [7,8,9]

Mukiamaderaspatana (L.) *Musumusukkai* (M. Roem.), locally known as *Musumusukkai*, is a creeping herb with widespread occurrence in India and has been documented with various medicinal uses such as respiratory, digestive and dermatological diseases in traditional and folk medicine. The secondary metabolites present in its leaves are reported to include flavonoids, alkaloids, tannins and phenolic compounds, some of which

have been shown to have antimicrobial and antioxidant properties. In spite of this phytochemical profile, the plant has not been investigated as an active ingredient of a nanoparticulate delivery system for fungal infection of the scalp. Thus, the present study was designed to isolate and characterise the phytoconstituents present in the leaves of *M. maderaspatana*, formulate a stable nanoemulsion of the extract using the SLN solvent injection method, and analyse the in vitro antifungal activity of the formulated nanoemulsion by testing against *C. albicans* for the possible development of a stable plant-based alternative for antifungal therapy for the scalp. [1,2,3,4,5,6]

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Glyceryl monostearate, Tween 80, castor oil and polyethylene glycol 400 (PEG 400) were purchased and utilised without further refining or polishing. Ethanol was of analytical grade, and double distilled water was used throughout the study. All the reagents used in the analytical procedures were of analytical grade, as follows: Quercetin, Aluminium chloride, Sodium nitrite, Sodium hydroxide and common reagents required for qualitative phytochemical tests such as Dragendorff's, Mayer's and Wagner's reagents, Ferric chloride and Lead acetate. For the antifungal assay, Sabouraud Dextrose Agar (SDA) and fluconazole were used.

COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

The process of gathering and verifying plants. The gathering and authentication of plant material. *Mukiamaderaspatana* leaves were picked in disease-free condition from the Cuddalore District, Tamil Nadu, India and authenticated by a qualified botanist, and kept for future reference. The leaves were shade-dried and then coarsely powdered and kept in well-labelled containers in the refrigerator for future use.

PREPARATION OF THE PLANT EXTRACT

The leaf powder was macerated in the hydroalcoholic solvent, ethanol: water (70:30 v/v), for 72 hours with an orbital shaker (120 rpm) at room temperature; 30 g of the powder of the coarse leaves. The marc was re-extracted twice with the same method to ensure complete extraction, and the combined filtrates were concentrated by solvent (alcoholic + water) evaporation at 45-50 °C to form a thick hydroalcoholic extract. The percentage yield was calculated as actual/theoretical, then multiplied by 100 to get a percentage.

PRELIMINARY PHYTOCHEMICAL SCREENING

The hydroalcoholic extract was analysed for the presence of alkaloids (Dragendorff, Mayer and Wagner tests), flavonoids (Shinoda and alkaline reagent test), saponins (froth test), tannins (ferric chloride test), phenols (lead acetate test),

glycosides (Keller–Killiani and Borntrager's tests), terpenoids (Salkowski and copper acetate test), protein (Biuret test) and carbohydrate (Molisch and Benedict's tests) by performing the standard tests. [10,11]

UV–VISIBLE SPECTROSCOPIC ANALYSIS

The extract was subjected to UV-Visible scanning (in distilled water) in the 200-800 nm range and the wavelength(s) of maximum absorbance (λ_{max}).

THIN-LAYER CHROMATOGRAPHY (TLC)

The pre-coated silica gel 60 F₂₅₄ plate was used for the phytochemical profile. Overriding with standard quercetin, ethyl acetate:formic acid:acetic acid:water (100:11:11:26) as mobile phase. The developed plates were observed under UV light after spraying with 10% aluminium chloride solution, and the RF values were calculated as the ratio of the distance travelled by the spots to the distance travelled by the solvent front.

TOTAL FLAVOUR CONTENT (TFC)

The aluminium chloride colourimetric method, using quercetin as the reference standard, was used to determine TFC. A stock solution of quercetin (1 mg/mL in ethanol) was diluted to prepare standards ranging from 20 to 100 µg/mL. Two millilitres of distilled water, 0.3 mL of 5 per cent sodium nitrite, and then 0.3 mL of 10 per cent aluminium chloride were added to the extract or standard in turn, and the resulting solution was made up to 10 mL with distilled water after 5 min; then 1 mL of 1 M sodium hydroxide was added and further diluted to 10 mL with distilled water. Absorbance was measured at 415 nm, and total flavonoids were expressed as mg of quercetin equivalents (QE)/g of dry extract. [12,13]

FTIR SPECTROSCOPIC ANALYSIS

THE DRIED EXTRACT OF THE PLANT WAS GROUND AND FORMED INTO A KBR PELLET, THEN ANALYSED USING FOURIER TRANSFORM INFRARED SPECTROSCOPY OVER THE WAVELENGTH RANGE OF 4000 TO 400 CM⁻¹ TO IDENTIFY THE PRIMARY FUNCTIONAL GROUPS PRESENT.

PREPARATION OF MUKIA MADERASPANTANA LOADED SOLID LIPID NANOPARTICLES

The *Mukiamaderaspatana*-loaded solid lipid nanoparticles were prepared using the melt-emulsion method. To get the lipid phase, 1.5 g of glyceryl monostearate was melted to 70-75°C. The lipid melt was mixed with *Mukiamaderaspatana* extract 500 mg in 8 mL of ethanol (with constant stirring). Tween 80 (2 mL) was separately dissolved in 50 mL of distilled water, and the solution was heated to 70-75 °C to make the aqueous phase. To get a homogeneous SLN dispersion, the hot lipid phase was injected dropwise into the hot aqueous phase with constant magnetic stirring (800-1000 rpm) and stirring was

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then performed for 45–60 minutes. The dispersion was cooled to room temperature and kept in amber glass containers at 2–8 °C. [9]

PREPARATION OF THE ALN-BASED NANOEMULSION

The SLN-based nanoemulsion was prepared. The nanoemulsion was prepared using SLN. Castor oil (10 mL), Tween 80 (5 mL) and PEG 400 (5 mL) were mixed to form the oil phase. Then, a pre-nanoemulsion was prepared dropwise by adding distilled water (30 mL) under mechanical stirring (1200–1500 rpm) for 20–30 minutes. The SLN dispersion prepared above (50 mL) was then slowly dropped under continuous stirring (1200–1500 rpm for 30–45 minutes) to form a uniform SLN-based nano-emulsion, which was cooled to room temperature. In order to prepare the formulations, the following three formulations (F1–F3) were prepared by making variations in extract and excipient ratios, as shown in Table 1.

COMPONENT	F1	F2	F3
PLANT EXTRACT	500 mg	300 mg	100 mg
GLYCERYL MONOSTEARATE	5 g	5 g	5 g
CASTOR OIL	10 mL	9 mL	8 mL
TWEEN 80	7 mL	6 mL	5 mL
PEG 400	5 mL	4.5 mL	4 mL
DISTILLED WATER	Q.S. to 100 mL	Q.S. to 100 mL	Q.S. to 100 mL

The composition of *M.maderaspatana*-loaded SLN-based nanoemulsion formulations (F1–F3) is enumerated in Table 1.

EVALUATION OF THE NANOEMULSION

The formulations were evaluated for organoleptic characteristics (colour, odour, homogeneity and phase separation), particle size and polydispersity index (PDI) by dynamic light scattering technique, total drug content and entrapment efficiency (EE%) by the UV-spectrophotometric estimation at 415 nm using the calibration curve of quercetin, pH by a calibrated digital pH meter, spreadability by the compression between the glass slides under fixed load, viscosity was measured with an Ostwald viscometer at 25 ± 1 °C, determination of emulsion type by the dye test using methylene blue, by the dilution test and by electrical conductivity/TDS measurement at room temperature, and physical stability by high-speed centrifugation (10,000 rpm, 30 min) and heating-cooling cycle (2–8 °C / 24 h at room temperature, repeated 3 times). A microscope was used for evaluation of morphological uniformity. [16]

IN VITRO ANTI-FUNGAL ACTIVITY

The ability to inhibit fungal growth in vitro. Activity against fungi in vitro.

Assessment of Antifungal activity was done for Sabouraud Dextrose Agar (SDA) plates by the agar well diffusion method. *Candida albicans* was swabbed uniformly to obtain a confluent lawn, and wells of about 6 mm diameter were punched out with a sterile borer. The nano-emulsion formulation was loaded into the wells at 50, 100 and 150 µg and 100 µg of fluconazole, and an appropriate blank was used as a standard. Plates were incubated for 24–48 hours, and the diameter of the clearing zone around the well was measured in millimetres. [15]

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The qualitative phytochemical screening of hydroalcoholic leaf extract of *M. maderaspatana* revealed the presence of alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, and phenolic compounds, with no presence of proteins and carbohydrates (Table 2). [1,2,3]

The high concentration of flavonoids, phenolic acids and tannin compounds is in accordance with the previous ethnobotanical studies of the antimicrobial and antioxidant properties of this species and justifies the choice to use the species as the active phytoconstituent source in the system of nanostructures. [1,2,3,4]

PHYTOCONSTITUENT	TEST PERFORMED	RESULT
Alkaloids	Dragendorff's, Mayer's and Wagner's tests	Present
Flavonoids	Shinoda test, Alkaline reagent test	Present
Saponins	Sugar stearate Froth (foam) TEST	Present
Tannins	Ferric chloride test	Present
Phenols	Lead acetate test	Present
Glycosides	Keller–Killiani test, Borntrager's test	Present
Terpenoids	Salkowski test, Copper acetate test	Present
Proteins	Biuret test	Present
Carbohydrates	Molisch test, Benedict's test	Absent

Table 2 presents the preliminary phytochemical screening of the extract of *M. maderaspatana* leaf.

UV-VISIBLE SPECTRAL CHARACTERISTICS

The extract showed two significant absorption maxima at 266.00 nm (absorbance 0.7046) and 331.00 nm (absorbance 0.5971) when optically scanned using a UV-Visible spectrometer. The shift at the former wavelength value is attributed to $\pi \rightarrow \pi^*$ a single-electronic transition that is typical of phenolics and aromatic ring systems, and this shift at the latter is assigned to an $n \rightarrow \pi^*$ a single-electronic transition that is characteristic of flavonoids and conjugated chromophores, thus confirming the qualitative screening results.

Total flavonoid content

When the total flavonoids in the extract were determined with the Aluminium chloride method, 21.56 ± 0.48 mg QE/g was observed, indicating an appreciable amount of flavonoids in the extract, which corresponds to its reported antioxidant and antimicrobial activity from the literature. [12,13]

THIN-LAYER CHROMATOGRAPHIC PROFILE

The TLC was carried out on the extract developed in ethyl acetate:formic acid:acetic acid:water (100:11:11:26), which showed a well-resolved and distinct spot with a solvent front travel of 8.3 cm and a spot travel of 6.47 cm, representing an R_f value of 0.78. The profile exhibits the presence of different characteristic peaks indicative of a specific flavonolic group of bioactive compounds and could be used as a fingerprint for future standardisation of the extract.

FTIR SPECTRAL ANALYSIS

FTIR spectroscopy of the extract exhibited characteristic absorption bands at 3386, 2926, 2118, 1629, 1413, 1254, 1052, 924 and 839 cm^{-1} (Table 3). The O–H stretching of the phenolic and flavonoid hydroxyl groups was assigned to the broad band at 3386 cm^{-1} , that of aliphatic C–H stretching to the band at 2926 cm^{-1} and that of the aromatic C=C stretching of a flavonoid or an alkaloid ring system to the band at 1629 cm^{-1} . Both the bands at 1254 and 1052 cm^{-1} were assigned as C–O for phenolics and C–O–C for glycosidic functional groups, which validated the phenolic, flavonoid and glycosidic presence in the extract.

Peak (cm ⁻¹)	Bond/vibration	Probable functional class
3386	O–H stretch (broad)	Phenols, flavonoids, tannins
2926	C–H stretch (asym./sym.)	Aliphatic CH ₂ /CH ₃ (terpenoids, fatty acids)
2118	C≡C stretch (weak)	Acetylenic/secondary metabolites
1629	C=C aromatic / N–H Bending	Flavonoids, alkaloids

1413	C–H bending / O–H deformation	Phenolic
1254	C–O stretch (aryl ether)	Phenolic / flavonoid
1052	C–O–C stretch	glycosidic linkages
924	C–H out-of-plane bending	Aromatic fingerprint region

Table 3 shows the peak assignments observed for the FTIR spectra of the extract from *M. maderaspatana* leaves.

NANOEMULSIFICATION CAN BE ASSESSED BY A PHYSICOCHEMICAL ANALYSIS.

The pH obtained from the physicochemical evaluation of the nanoemulsion was between 5.3 (F1) and 6.0 (F3) (Table 4). They match the mildly acidic pH of the human scalp and should help to decrease topical irritation. In Dynamic Light Scattering mode, it was confirmed that the nanocarrier was successfully formed in the nano-size region with a Z-average particle size of approximately 10 nm and a monomodal particle size distribution. Moreover, the low polydispersity index value (PDI = 0.30) suggested the presence of a narrow and uniform population of droplets, providing physical stability and regular drug release.

Formulation code	pH
F1	5.3
F2	5.7
F3	6.0

The pH of the formulated nanoemulsions presented in Table 4.

Efficient incorporation of the extract during the preparation of the nanoemulsion resulted in a total drug content of 90%. The LC/N under the different conditions of extraction decreased, and it was observed that with increasing ratio of extract to lipid, the extract load per LC decreased, which resulted in a reduction in entrapment efficiency (drug remaining after centrifugation). F1 had the highest entrapment efficiency of 93.1%, while F2 and F3 had entrapment efficiencies of 88.3% and 66.2%, respectively, showing that the ratio LC/F1 of 5:1, which corresponds to a higher extract, favours more complete encapsulation of the extract in the SLN matrix. F

formulation	Extract loaded (mg)	Entrapment efficiency (%)
F1	500 mg	93.1
F2	300 mg	88.3

F3	100 mg	66.2
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Table 5 shows the entrapment efficiency of formulations (F1-F3).

Spreadability, measured under fixed-load conditions, demonstrated that all the formulations had a smooth and non-gritty feel on application to the scalp, such as F2 (66.6 g·cm/s), F1 (28.5 g·cm/s), and F3 (16.6 g·cm/s). The viscosity of the optimised formulation (F1), determined using an Ostwald viscometer, was 2.77 cP, suitable for uniform spreading without being too runny. The water is thus the continuous external phase, and the formulation was found to be an oil-in-water (O/W) type nano-emulsion after the water content was confirmed by dye test, follow-on test, and electrical conductivity /TDS measurement. The optimised formulation was found to be robust and highly stable to high-speed centrifugation at 10,000 rpm for 30 min, and to three cycles of heating-cooling stress, which did not cause any visible creaming, phase separation, change in colour, pH or odour. The narrow PDI, derived from dynamic light scattering, supported drug release from the consistent size of the globules, as evidenced by the homogeneous field seen under the microscope with a consistent distribution of globule sizes.

IN VITRO ANTIFUNGAL ACTIVITY

The antifungal activity of the *M. maderaspatana*-loaded nanoemulsion against *Candida albicans* was clearly observed with zones of inhibition of 13 mm and 15 mm for 100 µg and 150 µg doses, respectively, while no zone of inhibition was observed at the lowest concentration of the nanoemulsion tested (50 µg) (Table 6). Considering the optimal standard dose (100 µg) of the standard antifungal agent fluconazole that produced the largest zone of inhibition (20 mm), as expected of a pure synthetic drug, the zone of inhibition produced by the phytoconstituent-loaded nanocarrier registered a high value at a comparable and higher dose range – indicating the contribution of encapsulated flavonoids and phenolic-rich extract to the antifungal activity, as expected from the phytochemical and spectral evidence obtained from this study. [4,5]

Test organism	50 µg	100 µg	150 µg	Fluconazole (100 µg)
<i>Candida albicans</i>	-	13 mm	15 mm	20 mm

CONCLUSION

In the present study, a successful solid-liquid nanoparticle-based nano-emulsion comprising the hydroalcoholic leaf extract of *Mukiamaderaspatana* was created to be used for Mass Antifungal treatment of the scalp. The results obtained from the preliminary phytochemical screening, UV-Visible spectroscopy, TLC and FTIR analysis altogether established a rich phytoconstituent profile of flavonoids, phenolics, alkaloids and

tannins. The optimised solvent injection (Injection 1) formulation displayed nanoemulsion characteristics, with particle size in the nanometres range, a close size distribution, pH not far from the scalp, high drug content and entrapment efficiency, good spreadability and viscosity. The formulation exhibited strong physical stability in the accelerated stress test and an in vitro dose-dependent clear zone of inhibition for *Candida albicans*. As a whole, these results validate the *M. maderaspatana*-loaded SLN nano-emulsion as a stable, phytochemically active and promising topical nanocarrier to treat fungal scalp infections and support further evaluation through long-term stability and dermal safety, along with in vivo efficacy testing.

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