ABSTRACT

FORMULATION AND DEVELOPMENT OF A NOVEL HERBAL DRUG DELIVERY SYSTEM WITH SPECIAL EMPHASIZE ON PHYTOSOME INCORPORATING BIOACTIVE COMPOUNDS OF FLOWERING BUDS OF *MESUA FERREA* LINN. AND EVALUATION OF ITS THERAPEUTIC POTENTIAL ON ATOPIC DERMATITIS

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BACKGROUND

Atopic dermatitis (AD) is a critical chronic inflammatory skin disease with symptoms of itching, scarring, rough skin, debris, swelling, behavioural problems, enhanced risk of depression, and mood disorders, and the reasons for AD are multifactorial and complex. AD carries a substantial disease burden and influences the quality of life that varies with its severity. The flowering buds of the *Mesua ferrea* Linn plant (FBMF) are globular, with two millimeters diagonally and scales in the axillary buds. To assess the potential of topical standardized n-hexane extract of flowering buds of *Mesua ferrea* Linn. (SMf) loaded phytosome gel towards AD.

OBJECTIVES

To formulate and optimize the topical application SMf-loaded phytosome-gel and to characterize its physicochemical, pharmaceutical properties, and skin diffusion study, skin irritation study, and also to evaluate the efficacy of the SMf-loaded phytosome-gel by monitoring the therapeutic potential in DNCB-induced mice AD-like models.

METHODOLOGY

The study has scientifically explored the FBMF powder samples and extracts based on pharmacognostic and phytochemical parameters. The Botanical verification and the Universal DNA barcoding technique were used to identify this medicinal plant. The FBMF powder's characteristics were measured by the micromeritics properties such as tapped density, bulk density, Carr's index, Hausner ratio, optical microscopy method, and angle of repose. The physicochemical assessments were accomplished with the ash values, extractive values, loss on drying, foaming index, and swelling index. The glycoside tests, alkaloids, carbohydrates, steroids, tannins, and flavonoids performed the FBMF extract's phytochemical screening. The presence of phytoconstituent (β-sitosterol) of each low-polar to high-polar solvent extract of FBMF was identified by the qualitative HPTLC study compared to the reference β-sitosterol R_f value of 0.83 at the wavelength of 254 nm and the solvent system of [toluene: ethyl acetate: acetic acid, 6:2:0.1 (v/v)]. A well diffusion method was used for anti-microbial study and, also measured MIC (Minimum inhibitory concentration) and compared to the marketed API (Active pharmaceutical ingredient).

The prepared mobile phase was used in a qualitative HPTLC study of each extract of FBMF and also studied simultaneous quantification of β-sitosterol in samples. The calibration curve for β-sitosterol was created, and the peak areas were measured by graphing practical concentrations of β-sitosterol *vs.* peak areas. The methods were validated by the limit of quantification, limit of detection, precision, accuracy, recovery studies, and specificity.

SMf-loaded phytosomes were prepared by the different ratio of soya lecithin, and a small amount of cholesterol was characterized by the zeta potential, PDI (Polydispersity

index), particle size, loading capacity, percentage of entrapment efficiency, and HR-TEM (High-resolution transmission electron microscopy) was used to perceive the morphology of the phytosome preparation. After that, the best optimized phytosome formulation was incorporated in gel and evaluated for viscosity, pH and homogeneity, organoleptic properties, extrudability, spreadability, drug content, and syneresis. The kp (Permeability coefficient), flux (Drug permeation rate) in Jss (Steady state), Er (Enhancement ratio), stability study, and FTIR (Fourier transform infrared spectroscopy). The phytosome gel was selected according to the evaluation parameters, and the test samples were applied topically to the DNCB-induced AD-like mice model. The therapeutic potential includes spleen weight, ear thickness, serum test (IL4, IFN-γ, TNF-α), and blood tests like neutrophils, WBC, eosinophils, monocytes, and lymphocytes were observed in the DNCB-induced AD-like mice model.

RESULTS

The Universal DNA barcoding of this plant has shown a high percentage of identity. The physicochemical and micrometrics properties of the powder sample of FBMF were evaluated within an acceptable range. All extracts contain steroids and carbohydrates, and flavonoids were found in ethyl acetate and ethanol extract according to the preliminary phytochemical screening. The HPTLC study shows that all extracts contain β-sitosterol and n-hexane extract, demonstrating anti-microbial activity with a MIC value of 0.062 mg/ml.

The HPTLC technique was found to be accurate, and in intra-day analysis, the RSD (Relative standard deviation) values of β-sitosterol ranged from 1.16 % to 1.60 %, and inter-day analyses 0.68 % to 1.77 %. The recovery study for the standard chemicals

was resolute at 99.98 %. This TLC-densitometric method was detected to be accurate, sensitive, specific, particular, and accessible. It can be active in regular quality control to analyzed β-sitosterol from FBMF.

Phytosome formulation (P3) was optimized based on the highest percentage of entrapment efficiency, lowest PDI, less drug loading, highest yield, high zeta potential, and less particle size. The phytosome gel G-1.0 formulation shows better results compared to the other gel formulations. The drug permeation rate (flux) in steady state (Jss) is 0.30± 0.001 μg. $h^{-1}.cm^{-2}$, permeability coefficient (Kp) 1.90± 0.006 h^{-1}. Cm^{-2} and an enhancement ratio (Er) of 1.317 was best for the skin. Compared with extract-loaded plain gel, the G-1.0 showed good pharmaceutical properties with better activity in the DNCB-induced AD-like mice model. The therapeutic activity of the standard drug was improved with G-1.0.

CONCLUSION

Our research demonstrated that the plant *Mesua ferrea* Linn. has been successfully authenticated with the DNA barcoding technique, and every extract of FBMF contains β-sitosterol. The n-hexane extracts have shown the highest anti-microbial effects compared to other extracts.

The HPTLC-validated method was used for the sample's solvent extracts with β-sitosterol, support precision, specificity assessment, the limit of quantification, accuracy, the limit of detection, and recovery according to ICH guidelines. The percentage of RSD of intra-day and inter-day is within the acceptable range (2 %) of the method. This approach for the concurrent measurement of *Mesua ferrea* Linn's blooming bud extracts was found to be accurate, precise, and specific and also gave acceptable

results according to the statistical data, parameters, and experimental results. This research proposed that SMf-loaded phytosome gel with a DNCB-induced AD-like mouse model may be a novel topical drug delivery system for possible AD intervention.

Keywords *Mesua ferrea* Linn, DNA barcoding, HPTLC study, anti-microbial activity, Phytosome, DNCB induced Atopic dermatitis-like mice model

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Date: 22/04/2024