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Development and Evaluation of Phytosome -Based Herbal Soap Containing Caesalpinia sappan (Fabaceae) Extract for the Management of Vitiligo

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ABSTRACT

The present study was ai med at developing and evaluating a phytosome-based herbal soap containing Caesalpinia sappan (Fabaceae) extract for its potential application in the management of vitiligo. The study focused on improving the delivery of the bioactive constituents of the plant extract through phytosome formulation and incorporating the optimized formulation into a topical soap.

The stem bark of Caesalpinia sappan was extracted using a maceration extraction method and subjected to phytosome preparation to enhance the delivery of its phytoconstituents. The prepared phytosome were evaluated for their physicochemical characteristics and incorporated into different soap formulations (F1–F4). The formulations were evaluated for appearance, ph, foamability, spreadability, skin compatibility, and other relevant physicochemical parameters. The optimized formulation was further evaluated for its potential melanogenic activity using a tyrosinase assay

The developed phytosome-based herbal soap formulations showed acceptable physicochemical properties and were suitable for topical application. Among the formulations, F3 showed the best overall performance with satisfactory physical characteristics and stability. Cell-line studies demonstrated moderate biological activity, as evidenced by the tyrosinase assay, indicating the potential of the formulation to influence melanogenesis-related activity.

The findings suggest that phytosome-based incorporation of Caesalpinia sappan extract into a herbal soap may provide a promising topical approach for the management of vitiligo. Formulation F3 demonstrated comparatively better performance, while the moderate activity observed in cell-line tyrosinase studies supports the need for further optimization and detailed in-vitro and in-vivo investigations to establish its therapeutic potential.

Keywords: Caesalpinia sappan, phytosome topical soap, tyrosinase assay

INTRODUCTION

Medicine is a substance having nutritive, curative or preventive properties, while the word "herbal" simply refers to a botanical or plant-based preparation. Put together, "herbal medicine" stands for plant-derived substances used for their nutritive, curative or preventive value. It is really an interdisciplinary field, drawing on botany, medicinal plant research, pharmacognosy, phytochemistry, phytotherapy, Ayurveda, Siddha, Unani medicine, biotechnology and biochemistry all at once [1].

Herbal medicine (HM) forms the fulcrum of complementary and alternative medicine, and its popularity has grown considerably in recent years, gradually finding its way into mainstream healthcare systems as well. Much of this rise in patronage can be attributed to low cost, the appeal of being a "natural" product with a perceived low toxicity, reasonable efficacy in several otherwise difficult-to-treat conditions, and the sheer flexibility in how these preparations can be accessed, made and used [2].

Caesalpinia sappan L.: An under-explored candidate

Caesalpinia sappan L. (family Fabaceae/Caesalpiniaceae), commonly known as sappan wood, and referred to as Pattanga in Ayurveda, Bakam in Unani and Patangam in Siddha medicine, is a small thorny tree native to South and Southeast Asia (especially in Kerala). Its dried heartwood has been used for centuries as a traditional remedy for wounds, skin diseases, diabetes and menstrual disorders across Ayurvedic, Siddha and traditional Chinese medicine [37].

Phytochemical studies of the heartwood have turned up a fairly diverse set of secondary metabolites: homoisoflavonoids, chalcones, flavones, xanthones, coumarins and cassane-type diterpenoids among them, of which the homo isoflavonoid brazilin is by far the most extensively studied constituent [40,41]. Brazilin is generally regarded as the principal marker compound of the heartwood; on exposure to air and light it undergoes oxidation to brazilein, the reddish-orange pigment historically responsible for the wood's traditional use as a natural dye [39]. Beyond brazilin, additional constituents reported from the heartwood include protosappanins, sappanone A and B, sappanchalcone, deoxysappanone B and episappanol, several of which have distinct pharmacological profiles of their own, credited with notable antioxidant, anti-inflammatory, antimicrobial and immunomodulatory activity [40,41,39].

The pharmacological profile of C. Sappan extracts and their isolated constituents has been investigated extensively over the past decade, most consistently for antioxidant, anti-inflammatory, antimicrobial and anticancer activity, with additional reports of antidiabetic, antiplatelet, neuroprotective and wound-healing effects [38,39]. Brazilin-rich extracts have repeatedly demonstrated potent free-radical-scavenging activity in DPPH and related in vitro assays, attributed to the polyphenolic hydroxyl groups of brazilin and brazilin, along with suppression of pro-inflammatory mediators in stimulated macrophages and phagocytic immunomodulatory effects [40]. Antimicrobial screening has shown activity against a range of Gram-positive and Gram-negative bacteria, while anticancer studies, largely in vitro and in rodent models, report cytotoxic and pro-apoptotic effects of brazilin and related cassane diterpenoids against several tumour cell lines [39]. In traditional Chinese medicine, the heartwood decoction continues to be prescribed to promote blood circulation and resolve blood stasis, and has more recently been examined for antiplatelet, cardioprotective and neuroprotective activity in experimental models of stroke and Parkinsonian neurodegeneration [38]. Collectively, this body of work indicates that the therapeutic relevance of C. Sappan rests on a broadly antioxidant and immunomodulatory phytochemical fraction, which is precisely the pharmacological direction that appears most relevant to melanocyte protection in vitiligo.

It is worth being upfront about one thing here: part of the pharmacological literature on C. Sappan has specifically identified isolated compounds, sappanone A being a good example, as inhibitors of tyrosinase and melanogenesis in the context of skin-lightening research, which is

Essentially the opposite of what vitiligo therapy is trying to achieve, and this needs to be acknowledged rather than quietly set aside [42,43]. That said, a separate and fairly substantial body of evidence characterises brazilin-rich C. Sappan extracts as having marked free-radical-scavenging and anti-inflammatory activity, including the suppression of pro-inflammatory mediators in stimulated macrophages and phagocytic immunomodulatory effects [40]. Given that oxidative stress and immune-mediated melanocyte destruction are now widely recognised as central drivers of vitiligo pathogenesis, and that structurally comparable antioxidant phytochemicals have already shown melanocyte-protective, regimenting effects through Nrf2-dependent pathways, it seems reasonable to hypothesise that the antioxidant fraction of C. Sappan could work in a melanocyte-protective rather than a purely tyrosinase-inhibitory direction under the specific pathophysiological conditions seen in vitiligo.

Topical drug delivery to diseased skin is inherently constrained by the barrier function of the stratum corneum, and this challenge is amplified for plant-derived polyphenolic actives, most of which are relatively large, poorly lipophilic molecules with limited passive permeability [41]. Over the past two decades, a range of nanocarrier platforms, including liposomes, niosomes, transferosomes, ectosomes, solid lipid nanoparticles and phytosomes , have been developed to improve the cutaneous delivery of such actives by modifying their partitioning behaviour, protecting them from degradation, and in several cases enabling more targeted deposition within the epidermis and upper dermis, where the therapeutic target, the melanocyte, actually resides [44].

Phytosome technology: concept, mechanism and advantages

Without any chemical interaction between the two, the phytosome complex forms a discrete molecular entity in which each phytoconstituent molecule is individually surrounded by the lipid component, giving it an amphiphilic character that improves partitioning across both aqueous and lipid-rich biological barriers [44]. This structural distinction translates into several practical advantages: improved aqueous solubility and dissolution of poorly soluble polyphenols, protection of oxidation-prone actives from degradation, enhanced

permeation across the stratum corneum for topical use, and, in oral or systemic applications, markedly improved bioavailability compared with the unformulated extract [12,44]. Phytosomes are typically prepared by solvent evaporation, thin-film hydration, antisolvent precipitation, co-solvent lyophilisation or salting-out methods, and can subsequently be incorporated into conventional dosage forms such as gels, creams, lotions or capsules without loss of the complex's integrity . Phospholipid complexation, or phytosome formulation, along with other nanocarrier-based delivery systems, has been used to work around such bioavailability limitations for herbal actives in general, and is increasingly being explored specifically for vitiligo therapeutics to improve cutaneous retention and local bioavailability [11].

Despite the therapeutic potential of plant-derived extracts, their incorporation into conventional formulations may be associated with certain limitations, including poor aqueous solubility, chemical instability, limited membrane permeability, and inadequate delivery of bioactive constituents to the target site. Such limitations may reduce the effective utilization of phytoconstituents in topical applications. Phytosomes are lipid-based phytopharmaceutical delivery systems developed to enhance the delivery characteristics of plant-derived bioactive compounds. In phytosomes, phytoconstituents are molecularly complexed with phospholipids, which may improve their interaction with biological membranes and enhance their physicochemical properties, stability, and bioavailability [45,46]. Therefore, incorporation of Caesalpinia sappan extract into a phytosomal system may provide a promising strategy for improving the delivery of its bioactive constituents in a topical formulation.

Soap is a widely used topical cleansing preparation that provides convenient application to the skin. Herbal soaps can serve as suitable vehicles for incorporating plant-derived ingredients while providing cleansing and skin-care benefits. However, the incorporation of herbal or phytosomal ingredients into soap requires careful formulation to ensure acceptable physicochemical characteristics, stability, skin compatibility, and uniform distribution of the

Active constituents. Parameters such as ph, moisture content, hardness, foamability, cleansing ability, total fatty matter, and stability are important in determining the quality and acceptability of the final formulation. Incorporation of C. Sappan extract-loaded phytosomes into a herbal soap may therefore provide a combined approach in which the potential antioxidant and anti-inflammatory properties of sappanwood constituents are coupled with the enhanced delivery characteristics of phytosomal technology.

The present study is therefore focused on the development and evaluation of sappanwood (Caesalpinia sappan L.) Extract-loaded phytosomal herbal soap. The study is designed to investigate the feasibility of incorporating sappanwood extract into a phytosomal delivery system and subsequently formulating it as a topical herbal soap. The developed formulation will be evaluated for relevant physicochemical, pharmaceutical, and stability characteristics to determine its suitability as a topical herbal formulation. This approach may provide a promising platform for further investigation of C. Sappan phytoconstituents in the context of supportive topical management of vitiligo.

Vitiligo is a common skin disorder in which the skin loses its natural colour due to the reduction or destruction of pigment-producing cells called melanocytes. It can affect people of different ages and may occur on different parts of the body. Although vitiligo is not contagious or life-threatening, it can have a major effect on a person’s confidence, emotional well-being, and quality of life.

The exact cause of vitiligo is not completely understood, but factors such as autoimmune reactions, oxidative stress, genetic factors, and environmental triggers may be involved. Currently available treatments mainly aim to restore skin pigmentation and control the progression of the disease, but the response to treatment can vary from person to person. Some treatments may also require a long treatment period and regular application. Therefore, there is a need to study vitiligo in detail and understand its causes, progression, symptoms, and available management approaches. Studying the disease can help in identifying suitable treatment strategies and developing safer and more effective herbal approaches. Hence, the present study focuses on understanding vitiligo and exploring the potential of Caesalpinia sappan as a herbal approach for its management.

AIM AND OBJECTIVES

The primary aim of this research is to develop, formulate, and comprehensively evaluate a novel phytosomal herbal soap loaded with Caesalpinia sappan (Sappanwood) extract for the effective management and adjunctive treatment of vitiligo. Vitiligo is a chronic depigmenting disorder characterized by progressive loss of functional melanocytes, primarily driven by oxidative stress, autoimmune mechanisms, and chronic inflammation. Sappanwood contains bioactive phenolic compounds, notably brazilin and sappanone A, which possess potent antioxidant, anti-inflammatory, and melanocyte-protective properties. However, these compounds exhibit poor skin permeation and limited bioavailability when delivered via conventional topical

formulations. By employing phytosomal technology—a phospholipid-based nanocarrier system—this study seeks to enhance the dermal delivery, stability, and therapeutic efficacy of Caesalpinia sappan extract. The formulated phytosomal herbal soap will be systematically evaluated for its physicochemical characteristics, antioxidant capacity, safety profile, and potential to promote repigmentation, thereby offering a safe, patient-compliant, and scientifically validated herbal intervention for individuals affected by vitiligo.

The specific objectives of this study are multifaceted and designed to ensure the successful development and comprehensive evaluation of the Sappanwood extract-loaded phytosomal herbal soap.Third, to assess the in vitro studies using tyrosinase assay.

MATERIALS AND METHODS

Caesalpinia sappan was selected for the study on the basis of ethnobotanical information, traditional uses, literature survey, availability and economy of medicinal plants. The wood Caesalpinia sappan was collected from Kerala and authenticated from Siddha Central Research Institute, Chennai, India. The plant specimen number was given as B13032602S.

The Organoleptic evaluation such as color, odour, taste was recorded by sensory characters [47,48]

Fresh wood material was used for anatomical studies. Transverse sections of the selected wood was prepared using a sharp blade/microtome to obtain thin sections. The sections were cleared and stained with appropriate histological stains to differentiate the various tissue components. The stained sections were mounted on clean glass slides and examined under an Olympus microscope at suitable magnifications. The anatomical characteristics, including epidermis, cortex, vascular bundles, xylem, phloem, parenchymatous tissues, and other distinctive cellular structures, were observed. Representative sections were photographed using a Nikon digital camera attached to the microscope. The obtained photomicrographs were used for identification and documentation of the diagnostic anatomical characteristics of the plant material.

The dried wood material was separately powdered and passed through a suitable sieve to obtain a uniform powder. A small quantity of the powder was placed on a clean glass slide and mounted in glycerin. For the detection of specific cellular structures and diagnostic characters,

Suitable reagents such as phloroglucinol–hydrochloric acid staining reagents was used as required. The prepared slides were examined under an Eclipse E200 microscope (Nikon, Japan) using appropriate magnifications. The characteristic microscopical features, including epidermal cells, trichomes, vessels, fibres, starch grains, crystals, and other diagnostic structures, were observed and recorded. Photomicrographs were captured using an axiocam erc5s digital camera attached to the microscope.

About 2 gm of air-dried powdered drug was accurately weighed and taken in a tared silica crucible. Incinerated at a temperature not exceeding 450□C until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air-dried drug. [49,50]

The ash obtained as directed under total ash was boiled with 25 ml of 2M Hcl for 5 min. The insoluble matter was collected on an ashless filter paper, washed with hot water, ignited and weighed. Then calculated the percentage of acid insoluble ash with reference to the air-dried crude drug. [49,50]

The total ash obtained was boiled with 25 ml of water for 5 min. The insoluble matter was collected on an ashless filter paper, washed with hot water and ignited for 15min at a temperature not exceeding 450□C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried crude drug. [49,50]

The previously ignited crucible was cooled in a desiccator and weighed (W_1). About 1–2 g of the sample was added to the crucible and weighed again (W_2), then gently heated until completely charred. After cooling, a few drops of concentrated sulfuric acid were added. The crucible was ignited in a muffle furnace at 600 ± 25°C until a constant white residue was obtained. It was cooled, weighed (W_3). And calculated. [49,50]

About 1.5 gm of the powdered drug was accurately weighted and transferred into a weighed flat and thin porcelain dish. Dried in an oven at 100□C, cooled and weighed again. The loss in weight is usually recorded as moisture.

The powdered drug was dissolved in cold water and hot water and the solubility was observed.

The presence or absence of phytochemical compounds can be determined by performing certain qualitative tests[55].

Few drops of dragendroffs's reagent were added to each 1ml of plant extract, and it resulted in the formation of orange brown-colored precipitate which indicates the presence of alkaloids

Few drops of lead acetate were added to the plant extract (wood, stem, root). It shows yellow colour precipitate indicates the presence of flavonoids.

1ml of plant extract and treated with a few drops of α- naphthol alcohol and 2ml of concentrated H2SO4 along the walls of the tube. If brown ring appears it indicates the presence of carbohydrates.

Few drops of plant extract and diluted with distilled water to up to 25 ml and mixed thoroughly for 10 minutes after that it shows a layer of foam which indicates the presence of saponins in the extract.

In 2 ml of plant extract add a few drops of fecl3 and its showed blue black colour which indicated the presence of phenols.

In 2 ml of plant extract add few drops of fecl3 which showed blue or green colour precipitate indicated the presence of tannin.

Add a few drops of Biuret reagent (copper sulfate and sodium hydroxide) to the sample. A purple or violet color indicates the presence of proteins.

Add concentrated nitric acid; a yellow color indicates the presence of aromatic amino acids (like tyrosine and tryptophan).

Add Millon's reagent (mercuric nitrate in nitric acid); a red color indicates the presence of tyrosine.

Add Ninhydrin reagent and heat; a blue or purple color indicates the presence of free amino acids or proteins.

Add lead acetate; a black precipitate indicates sulfur-containing amino acids (like cysteine)

About 20-50 mg of sample was taken into the Teflon microwave digestion vessel and 1 ml of ultrapure nitric acid was added to digest about 45 minutes using Anton Paar microwave digestion unit. After that the sample is made up to a 50 ml in standard measuring flask. The calibration standard solution was prepared from 2 µg/ml to 10 µg/ml by using ultrapure nitric acid and blank also. Agilent ICP-OES 5100 VDV instrument was used with the following operation conditions: a RF power 1.2 kw, a plasma gas flow rate 12 L min-1, and a nebulizer gas flow rate 0.70 L min-1. The samples are introduced into the plasma using nebulizer and spray chamber for the analysis [54].

Wood of Caesalpinia sappan were taken and dried completely. The wood was pulverized into fine powder. The fine powder was subjected to maceration using alcohol (90%) as solvent. The extract was concentrated and evaporated and the yield was found to be 10% w/w.

Thin Layer Chromatography (TLC) is a simple, quick, and effective technique used to separate and analyze compounds present in a mixture. It is widely used in pharmaceutical, biochemical, and forensic analysis [55]

TLC works on the principle of differential adsorption. The components of a mixture migrate at different rates on a stationary phase due to their varying affinities for the mobile phase (solvent) and stationary phase (adsorbent).

The TLC plate was prepared by coating it with silica gel or another adsorbent. The sample solution was applied as small spots near the plate's base. The plate was placed in the development chamber containing the mobile phase. The solvent was allowed to move up by capillary action until it reached a certain height. The plate was removed, the solvent front was marked, and it was allowed to dry. The spots were detected using UV light or chemical reagents.

The HPTLC analysis of the phytosomal formulation was performed using a Merck HPTLC silica gel 60 F_{254} plate (100 × 100 mm) as the stationary phase. The sample solution prepared in methanol was applied onto the plate using a Linomat 5 applicator with a dosage speed of 150 nl/s and a pre-dosage volume of 0.20 µl. Four tracks of the phytosomal formulation were applied in volumes of 2.0 µl, 4.0 µl, 6.0 µl, and 8.0 µl, with the first track positioned at 15 mm from the edge and a track distance of 19.4 mm, while the application position was maintained at 8 mm from the bottom of the plate. The plate was then placed in a TTC 20 × 10 development chamber previously saturated with the selected mobile phase for 20 minutes using a saturation pad. The chromatogram was developed up to a solvent front distance of 70 mm, after which the plate was removed and dried at room temperature for 5 minutes. The developed plate was visualized and documented using a TLC Visualizer under white light, UV 254 nm, and UV 366 nm, and the images were recorded for further analysis

The specific amount of wood extract of Caesalpinia sappan and soya lecithin were taken into a 100 ml round bottom flask and refluxed with 30 ml of ethanol at a temperature 40 – 50°C for 4 h. The mixture was concentrated to 5-10 ml to obtain the sticky precipitate which was lyophilized to obtain the phytosomes. The dried phytosomes complex was placed in amber colored glass bottle and stored in refrigerator[56].

Accurately weighed 10 mg of the phytosomal formulation was transferred into a 10 ml volumetric flask. It was dissolved in methanol and the volume was made up to 10 ml to obtain a stock solution of 1000 µg/ml. From the stock solution, appropriate dilution was made to obtain a 10 µg/ml solution. The solution was scanned in the range of 200–400 nm using methanol as the blank. The wavelength corresponding to the maximum absorbance was recorded as λmax. [57,58]

The phytosomal formulation was dried in a desiccator to remove moisture prior to analysis. Approximately 1–2 mg of the sample was accurately weighed. The sample was triturated with dry potassium bromide (kbr) powder in a ratio of about 1:100. The mixture was compressed using a hydraulic press to form a transparent kbr pellet (or placed directly on the ATR crystal if ATR mode was used). The prepared sample was carefully placed in the FTIR sample holder. The spectrum was recorded using an FTIR spectrophotometer over the range of 4000–400 cm^{-1}. A suitable background scan with pure kbr was performed prior to sample scanning. The interferogram obtained was converted into an IR spectrum by Fourier transformation using the instrument software. Characteristic absorption peaks were identified and assigned to the functional groups of the phytosomal formulation. The spectrum was compared with the spectra of the pure drug/extract and phospholipid to confirm the formation of the phytosome complex. [58,63]

The X-ray diffraction (XRD) analysis of the phytosomal formulation was carried out using a BRUKER diffractometer operating in continuous scan mode with a locked-coupled scan type. The powdered sample was mounted on the goniometer stage with a specimen length of 10 mm and measured at a temperature of 25 °C. The diffraction pattern was recorded over a 2θ range of 10°–80° with a step size of 0.040° and a scan step time of 13.44 s under pre-set time scanning conditions. A Cu anode X-ray source with wavelengths Kα1 = 1.54060 Å, Kα2 = 1.54443 Å, and Kβ = 1.39225 Å (Kα2/Kα1 ratio = 0.5) was used, and the generator was operated at 40 kv and 30 ma. A fixed divergence slit and a receiving slit size of 0.100 mm were employed during the measurement. The instrument operated in a Theta/Theta diffractometer configuration with a goniometer radius of 240 mm and a focus–divergence slit distance of 91 mm. No incident beam monochromator was used, and the sample was not spun during the measurement. The diffraction data were collected along the goniometer scan axis and stored in BRUKER-binary V4 (.RAW) format for further analysis. The obtained diffractogram was used to evaluate the crystalline nature of the phytosomal formulation and to confirm complex formation. [59,64]

The phytosomal formulation was appropriately diluted with distilled water to obtain a suitable scattering concentration. The diluted sample was gently sonicated for 2–5 minutes to ensure uniform dispersion and removal of aggregates. The sample was visually inspected to confirm the absence of air bubbles and large particles. A clean, dry disposable folded capillary cell (zeta cell) was selected for the analysis. The prepared sample was carefully injected into the zeta cell using a syringe without introducing bubbles. The filled cell was placed into the zeta potential analyzer based on electrophoretic light scattering. Measurements were carried out at 25 ± 1 °C under automatic voltage conditions. The electrophoretic mobility obtained was converted into zeta potential using the Smoluchowski equation by the instrument software. Each sample was measured in triplicate, and the mean zeta potential (mv) with standard deviation was recorded. [46,52]

The phytosomal formulation was appropriately diluted with distilled water to obtain suitable scattering intensity. The diluted sample was gently sonicated for 2–5 minutes to ensure uniform dispersion and to remove aggregates. The sample was visually inspected to confirm the absence of air bubbles and visible particulates. For particle size analysis, the sample was filled into a

The phytosomal formulation was properly dried under vacuum or in a desiccator to remove residual moisture. A small quantity of the dried sample was uniformly sprinkled onto a clean aluminium SEM stub using double-sided carbon adhesive tape. Excess particles were gently removed by tapping or mild air blowing to obtain a thin layer. The mounted sample was subjected to sputter coating with a thin layer of gold or gold–palladium to improve conductivity. The coated stub was placed into the SEM specimen chamber. The chamber was evacuated to obtain the required high vacuum. The sample was scanned using SEM at an accelerating voltage typically between 5–20 kv. Micrographs were captured at different magnifications to observe the surface morphology and particle shape of the phytosomal formulation. The obtained images were analyzed for particle size, surface texture, and morphological [61,62,63]

To determine encapsulation efficiency (% EE), a phytosome sample is dissolved in methanol/ethanol to disrupt the lipid complex and measure total drug absorbance (Abstotal) at 287 nm and 303 nm using UV spectrophotometry, while another equal sample is centrifuged at 10,000–12,000 RPM for 30–45 minutes to separate the unencapsulated wood extract supernatant and record free drug absorbance (Absfree) at the same wavelengths. The concentration of both fractions is quantified against a standard calibration curve, and encapsulation efficiency is calculated using the formula

CELL LINE STUDIES-ENZYMATIC ASSAY OF TYROSINASE

50Mm potassium phosphate buffer (ph: 6.5) at 25ºc, 1Mm L-Tyrosine solution, tyrosinase enzyme solution.

Reaction cocktail 290 µl were added 1.5 ml fresh tube and sample (R38) added in five concentration 10 µl and blank(OD mean- 0.123). Immediately mix by inversion and record the increase in 280nm for approximately 10 min.

SOAP FORMULATION:

To prepare the soap base, 100 ml of virgin coconut oil was placed in a 500-ml glass beaker. This was then put in a water bath with a temperature between 40°C to 45°C and the mixture was constantly stirred until a strong consistency formed. Then 18.2g naoh was dissolved in

41.4 ml distilled water in another 500-ml glass beaker. This solution was slowly incorporated in the coconut oil mixture. This mixture was heated to 40-45°C until base consistency was achieved and this was used as the soap base.

The Soap base was taken in a beaker and was melted in a hot water bath.

Table 1. Composition used for phytosome preparation

S. No	Ingredients	Quantity	Uses
1.	Soya lecithin	0.2g	Polymer
2.	Ethanol	30ml	Solvent
3.	Caesalpinia sappan	1ml	Extract

Table 2. Composition of soap base

S.NO	INGREDIENTS	REQUIRED QUANTITY
1.	Coconut Oil	100ml
2.	Potassium Hydroxide	18.2 g
3.	Distilled Water	41.4ml

Table 3. Soap formulations

INGREDIENTS	F1	F2	F3	F4
Extract(ml)	0.5	1	1.5	2
Soap base(gram)	30	30	30	30
Rose oil	Qs	Qs	Qs	Qs

RESULTS AND DISCUSSION

The Organoleptic characters such as color, odor and taste were evaluated

Caesalpinia sappan L. (Sappanwood) is a well-known medicinal plant that can be identified using its organoleptic characteristics. The crude drug consists of the dried heartwood, which is reddish-brown colour because of natural pigments such as brazilin and brazilein. This distinctive colour is one of the key features used to identify good-quality plant material. The heartwood has a faint, pleasant woody odour that becomes more noticeable when it is cut or powdered. It also has a slightly astringent taste. Mainly due to the presence of phenolic compounds and brazilin. These sensory characteristics are simple but reliable indicators for identifying the crude drug and assessing its quality. Therefore, organoleptic evaluation remains an important first step in the pharmacognostic studies of the wood.

Anatomy of Caesalpinia sappan wood

Microscopic examination of Caesalpinia sappan wood revealed characteristic xylem features useful for pharmacognostic identification. The transverse section showed numerous large and small circular to oval vessels surrounded by parenchyma cells and thick-walled fibres. The lignified fibres provide strength and hardness, while vessels aid in water and mineral transport. Distinct growth rings were visible, with larger vessels and less compact tissue in early wood and smaller, closely arranged cells in late wood. The smaller cells surrounding them are tracheid, fibers, and parenchyma cells, which provide support and storage. The thick, reddish-brown cell walls indicate lignification, giving the tissue strength. Radial and longitudinal cell arrangements were also observed. These anatomical features provide important diagnostic characteristics for identifying and evaluating Sappanwood.

Powder microscopy revealed several characteristic anatomical features useful for the

The physicochemical analysis of Caesalpinia sappan powder showed a moisture content of 7.0% and total ash of 0.65%. Water-soluble ash, acid-insoluble ash, and sulphated ash were found to be 0.41%, 0.50%, and 1.2%, respectively. The drug was freely soluble in hot water and soluble in cold water. The water-soluble and alcohol-soluble extractive values were 10.89% and 16.38%, indicating the presence of appreciable water- and alcohol-soluble constituents.

Where, (+) indicates presence and (-) indicates absence.

The ethanolic extract of Caesalpinia sappan tests positive for alkaloids, phenols, tannins (including hydrolysable types), flavonoids, terpenoids, and saponins—key plant compounds pulled out well by alcohol, glycosides, cardiac glycosides, amino acids, proteins, or carbohydrates, as ethanol favors semi-polar

chemicals over watery ones. This pattern fits standard lab tests for Caesalpinia sappan extract confirming the extract's quality and consistency.

The lead content of Caesalpinia sappan wood powder was found to be 0.88 mg/kg using the AOAC method. This result indicates that the lead level in the crude drug was low. Thus, the sample showed acceptable purity with respect to lead content.

Stationary Phase: Silica gel adsorbent Mobile Phase: Chloroform: Methanol (5:1)

The TLC analysis of Sappanwood showed the presence of brazilin, an important phytochemical constituent of Caesalpinia sappan L. The standard brazilin showed an Rf value of 0.54, while the sample showed an Rf value of 0.50. The solvent travelled 6.5 cm, and the sample spot travelled 3.3 cm. The close Rf values of the standard and sample indicate the possible presence of brazilin concentration, solvent composition, or experimental conditions. Thus, the TLC result supports the identification of brazilin in Caesalpinia sappan extract.

Table 11: HPTLC of ethanolic extract of Caesalpinia sappan wood

HPTLC fingerprinting of Caesalpinia sappan extract showed several distinct bands at Rf 0.19, 0.23, 0.30, 0.48 and 0.92 under white light, 254 nm and 366 nm, indicating the presence of different phytoconstituents. The lower Rf bands may represent polar to moderately polar phenolic constituents, while the band at Rf 0.48 may indicate an intermediate-migrating phenolic constituent. The band at Rf 0.92 represents a highly mobile constituent. The variation in band colour and intensity at different wavelengths confirms the presence of multiple constituents and provides a characteristic HPTLC fingerprint of the extract.

Ultra-violet analysis of Caesalpinia sappan wood phytosomes

UV-Visible analysis of sappanwood phytosome showed absorption around 300–305 nm. This indicates the presence of UV-absorbing polyphenolic compounds.The absorption may be mainly due to brazilin and other phenolic constituents. Absorption in the 340–400 nm region may indicate conjugated compounds. Overall, the results suggest that the important constituents of sappanwood were retained in the phytosome.

Fourier Transform Infrared:

Discussion:

X-ray diffraction

The X-ray diffraction (XRD) pattern of sample measured using a Copper radiation source over a of 10° to 80° demonstrates a predominantly amorphous structural nature. The diffraction profile shows a broad humped feature centered roughly between accompanied by significant baseline noise and low overall signal intensity (peaking below 150 counts). The absence of sharp, well-defined Bragg reflections indicates a lack of long-range periodic order in the atomic lattice. This behavior is typically characteristic of non-crystalline materials, amorphous glass or polymer matrices, synthesized bio-nanomaterials prior to thermal treatment, or extremely small crystallite sizes causing extreme peak broadening. Higher intensity at low angles suggests low-angle diffuse scattering, and calcination at elevated temperatures would be required if a crystalline phase transformation is intended

Table 13: Zeta potential and Electrophoretic mobility of Caesalpinia sappan wood phytosomes

The Zeta potential of the Caesalpinia sappan-loaded phytosomes was found to be approximately −70 mv with a single, sharp peak. This strong negative surface charge creates high electrostatic repulsion between particles, preventing them from clumping together. The sharp peak indicates a uniform particle size, confirming that the prepared phytosomal formulation is physically stable and well-dispersed.

Intensity Distribution ACF

Particle size analysis of the formulation using a Zeta sizer based on Dynamic Light Scattering (DLS) revealed an average hydrodynamic diameter (z-average) of 109.3 nm with a primary distribution peak centered around 200.6 nm, confirming the successful generation of nanoscale phytosomal structures. The Zeta sizer operates by detecting fluctuations in scattered light intensity caused by the Brownian motion of particles suspended in a liquid medium, where smaller particles move more rapidly than larger ones, allowing the software to calculate the hydrodynamic size and Polydispersity Index (PDI). The presence of the dominant peak at 200.6 nm along with a lower overall mean diameter indicates that while the bulk of the particle population resides around 200 nm, a significant fraction of smaller nanostructures shifts the average to 109.3 nm. Overall, these DLS results establish that the Caesalpinia sappan-loaded phytosomes fall well within the desirable sub-micron nanometer range, which facilitates enhanced biological membrane permeability, improved solubility, and optimal colloidal dispersion quality.

SEM (Scanning Electron Microscopy)

Scanning Electron Microscopy (SEM) confirmed the successful formation of Caesalpinia sappan-loaded phytosomes, revealing small, well-distributed particles with an irregular, slightly rough surface morphology. The distinct surface texture and close association of the particles stem from the effective complexation between the herbal extract and the phospholipid matrix during preparation. Crucially, the absence of large aggregates demonstrates good particle dispersion, which enhances both the physical stability and effective delivery of the phytosomal formulation

Table 14: Encapsulation efficacy values of Caesalpinia sappan wood phytosomes

The Encapsulation efficiency of the C. Sappan-loaded phytosomes improved significantly as the formulation level was raised from MO11-0.5 to MO11-20, jumping from 17.374% to 77.710%. This means that adding more of the formulation allows the phospholipid matrix to trap and hold a much higher amount of the active herbal extract. While MO11-20 achieved the highest success rate among all tested batches, the efficiency was still going up at this level. Therefore, MO11-20 is currently the best option, but testing even higher concentrations might unlock an even better result.

CELL LINE STUDIES

Tyrosinase is a key enzyme involved in the melanin biosynthesis pathway and catalyzes the oxidation of L-tyrosine and L-DOPA to form L-DOPA-quinone. The tyrosinase enzyme assay is used to evaluate the inhibitory activity of a test sample against tyrosinase. In the present study, the enzymatic activity was measured spectrophotometrically at 280 nm using L-tyrosine as the substrate. Different concentrations of the test sample were evaluated, and the inhibitory activity was expressed in terms of IC_{50} value

RESULT

Discussion:

EVALUATION OF SOAP:

Discussion:

The organoleptic evaluation showed that all four formulations (F1–F4) had a deep red colour, pleasant odour, and smooth texture. The deep red colour may be due to the natural colour of the Caesalpinia sappan extract as phytosome is incorporated into the soap. The pleasant odour indicates that the formulations were acceptable in terms of smell. All formulations also showed a smooth texture, suggesting good mixing and uniform distribution of the ingredients. Overall, F1–F4 showed similar and acceptable organoleptic characteristics, indicating that the prepared herbal soaps were suitable for further evaluation.

Discussion:

The ph values for formulations F1, F2, F3, and F4 were 9.0, 10.0, 7.0, and 10.5, respectively, driven by variations in the soap base, alkaline ingredients, and extract levels. F4 was the most alkaline (10.5), while F3 maintained a skin-friendly, neutral ph of 7.0. Consequently, F3 is the most suitable formulation for topical application.

Discussion:

The foam height values for formulations F1, F2, and F3 were measured at 6.5 cm, 4.5 cm, and

Discussions:

The moisture content values for formulations F1, F2, F3, and F4 were 3.79%, 4.74%, 3.42%, and 6.05%, respectively. F3 recorded the lowest moisture content (3.42%), which helps extend shelf life and preserve solid soap bar integrity, whereas F4 showed the highest moisture level (6.05%), making it more susceptible to melting or softening.

Discussion:

All formulations (F1–F4) exhibited identical foam retention of 4 minutes, indicating consistent lather stability across all batches. Consequently, all four soap formulations provide equally effective and reliable cleansing performance for the consumer.

Discussion:

Formulation F3 recorded the highest Total Fatty Matter (75.62%), classifying it as a premium Grade 1 soap. Higher TFM values indicate superior cleansing, better skin moisturizing, and enhanced soap bar durability.

Discussion:

The saponification values increased steadily from F1 (183.24) to F4 (191.02), with F3 showing the highest value. Higher saponification values indicate lower average molecular weight fatty acids, which enhance soap solubility and lather formation. Overall, all formulations fall within the optimal range for effective cleansing and quality soap production.

Discussions:

CONCLUSION

The present study comprehensively evaluated the pharmacognostic properties, phytochemical profile, formulation characteristics, and depigmenting potential of Caesalpinia sappan heartwood extract and its phytosome -loaded herbal soap intended for the management of vitiligo. Organoleptic evaluation of the crude heartwood confirmed the characteristic reddish-brown color, mild aromatic odor, and astringent taste, which are typical features associated with the presence of brazilin, brazilein, and other homo isoflavanones responsible for the plant's pharmacological activity. Proximate analysis revealed acceptable physicochemical parameters, including low moisture content and total ash and acid-insoluble ash values within acceptable limits, indicating good quality and minimal extraneous matter in the crude drug. Preliminary phytochemical screening of the ethanolic extract demonstrated the presence of important secondary metabolites such as phenols, flavonoids, tannins, terpenoids, and saponins, which collectively contribute to antioxidant, anti-inflammatory, and tyrosinase-modulating activities relevant to vitiligo management.

Chromatographic and spectroscopic studies further confirmed the presence and enrichment of key marker compounds in the extract. Thin-layer chromatography showed comparable Rf values between standard brazilin/brazilein and the sample.

Phytosome technology enhanced the lipophilic character and potential skin permeation of the hydrophilic sappanwood polyphenols. UV–Visible spectroscopy of the phytosomal complex indicated successful interaction between the polyphenolic extract and phospholipid, with shifts in λmax suggestive of complex formation. FT-IR and comparative phytochemical analysis of the phytosomal complex supported the formation of a phospholipid–polyphenol assembly, which is expected to improve stability and delivery of active constituents into the epidermis. FT-IR analysis revealed characteristic functional groups corresponding to hydroxyl, aromatic, and conjugated carbonyl systems typical of sappanwood constituents.

In vitro tyrosinase inhibition studies using the sappanwood extract and/or phytosomal complex demonstrated dose-dependent inhibition of tyrosinase activity, consistent with reported mechanisms of brazilin, brazilein, and sappanone A in suppressing melanogenesis through direct enzyme inhibition. Although vitiligo is characterized by melanocyte loss rather than hyperpigmentation, the inclusion of a tyrosinase-modulating, antioxidant-rich phytosomal system is rationalized by the need to regulate residual melanocyte activity, reduce oxidative

Stress, and create a favorable micro environment for repigmentation when used in combination with standard therapies.

Physicochemical characterization of the developed herbal soap demonstrated desirable quality attributes. The soap exhibited uniform shape and size, smooth texture, and a light pink to reddish hue indicative of incorporated sappanwood extract. Ph values were within the skin-compatible range (approximately 9–10 for traditional soap), while moisture content, total fatty matter, and alcohol-insoluble matter complied with standard specifications for herbal soaps. Foam height and foam retention were satisfactory, suggesting good cleansing and user acceptability. Among the four formulations, F3 was found to be the best formulation based on its overall evaluation results and desirable characteristics.

Taken together, the developed Caesalpinia sappan extract–loaded phytosomal herbal soap exhibited satisfactory organoleptic and physicochemical properties, evidence of successful phytosome formation, and promising in vitro tyrosinase-modulatory and antioxidant potential. These findings support its potential as an adjunctive, natural, topical formulation for the management of vitiligo, warranting further in vivo evaluation and clinical studies to establish efficacy, safety, and optimal therapeutic regimens.

Further studies can be carried out to optimize the Caesalpinia sappan phytosomal herbal soap for better stability and skin delivery. Long-term stability studies can be performed to evaluate its physical and chemical stability during storage. In vivo studies may be conducted to assess the effectiveness and safety of the developed formulation. Further research can investigate its antioxidant and tyrosinase-modulating activities using suitable biological models. Skin irritation and sensitization studies can also be performed to confirm its safety for topical use. The formulation may be compared with standard and other herbal topical treatments for vitiligo. Clinical studies are required to establish its efficacy, safety, and suitable therapeutic use. Further development may help establish Caesalpinia sappan phytosomal herbal soap as a supportive natural topical formulation for vitiligo management.

Table 4. Organoleptic evaluation of crude drug

S.No	Characteristics	Caesalpinia sappan
1	Colour	Reddish brown
2	Odor	Pleasant woody odour

3	Taste	Slightly astringent

Table 5. Proximate analysis of Caesalpinia sappan wood

S.No	Proximate analysis	Caesalpinia sappan wood (%w/w)
1.	Moisture content	7.0
2.	Total ash	0.65
3.	Water soluble ash	0.41
4.	Acid insoluble ash	0.50
5.	Sulphated ash value	1.2
6.	Hot water solubility	Freely soluble
7.	Cold water solubility	Soluble
8.	Water soluble extractive value	10.89
9.	Alcohol soluble extractive value	16.38

Table 6. Phytochemical screening

S.No	Bioactive constituents	Ethanolic extract
1.	Alkaloids	+
2.	Glycosides	+
3.	Cardiac glycosides	+
4.	Phenols	+
5.	Tannins	+
6.	Hydrolysable tannins	+
7.	Flavonoids	+
8.	Terpenoids	+
9.	Saponins	+
10.	Amino acids and Protein	+
11.	Carbohydrate	+

Table 7. Heavy-metal estimation

S.No	Test parameters	Test protocol	Unit	Residue Content
1.	Lead as Pb	AOAC (22nd Edition)	Mg/kg	0.88

Table 8. TLC analysis

Sample	Distance travelled by solvent	Distance travelled by sample	Rf value
Ethanolic Extract of Caesalpinia sappan wood	6.5cm	3.3cm	0.50

Table 9. Zeta potential

Peak no	Zeta potential	Electrophoretic mobility
1.	-70.7mv	-0.00057 cm2/Vs
2.	Mv	Cm2/Vs
3.	Mv	Cm2/Vs

Table 10. Encapsulation efficacy

Sample name	287.0nm	303.0nm	Abs(eff)	%
0.5 µg/ml	0.1499	0.3235	0.1737	17.374
1 µg/ml	0.2134	0.475	0.2616	26.161
5 µg/ml	0.87	1.5608	0.538	53.825
10 µg/ml	0.907	1.561	0.654	65.42
20 µg/ml	1.515	3.287	0.777	77.71

Table 11. Tyrosinase assay: OD values (decreasing concentration)

S. No	Name of the tested sample concentration (µg/ml)	OD Value at 280 nm (in triplicates)	OD Value at 280 nm (in triplicates)	OD Value at 280 nm (in triplicates)
1.	500 µg/ml	0.169	0.185	0.179
2.	250 µg/ml	0.188	0.198	0.200
3.	100 µg/ml	0.209	0.203	0.223
4.	50 µg/ml	0.232	0.258	0.281
5	10 µg/ml	0.309	0.360	0.328
6	Control	0.956	0.972	0.988

Table 12. Tyrosinase assay: OD values (increasing concentration)

S. No	Name of the tested sample concentration (µg/ml)	OD Value at 280 nm (in triplicates)	OD Value at 280 nm (in triplicates)	OD Value at 280 nm (in triplicates)
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5	10 µg/ml	0.169	0.185	0.179
6.	Control	0.956	0.972	0.988

Table 13. Enzymatic activity

S. No	Tested sample concentration (µg/ml)	Tyrosinase U/ml	Tyrosinase U/ml	Tyrosinase U/ml	Mean value (%)
1.	500 µg/ml	0.0186	0.0237	0.0205	0.02093333
2.	250 µg/ml	0.0109	0.0135	0.0158	0.0134
3.	100 µg/ml	0.0086	0.008	0.01	0.00886667
4.	50 µg/ml	0.0065	0.0075	0.0077	0.00723333
5.	10 µg/ml	0.0046	0.0062	0.0056	0.00546667

Table 14. Evaluation of soap formulations

Parameter	F1	F2	F3	F4
pH	9.0	10.0	7.0	10.5
Foam height	6.5	4.5	6.5	3.0
Moisture content (%)	3.791	4.74	3.42	6.05
Foam retention	4 mins	4 mins	4 mins	4 mins
Total fatty matter (%)	73	71.24	75.62	74.94
Saponification value	183.24	186.57	191.02	189.03
Ethanol insoluble value (%)	3.80	3.10	2.90	3.98

CONCLUSION

The present study comprehensively evaluated the pharmacognostic properties, phytochemical profile, formulation characteristics, and depigmenting potential of Caesalpinia sappan heartwood extract and its phytosome -loaded herbal soap intended for the management of vitiligo. Organoleptic evaluation of the crude heartwood confirmed the characteristic reddish-brown color, mild aromatic odor, and astringent taste, which are typical features associated with the presence of brazilin, brazilein, and other homo isoflavanones responsible for the plant's pharmacological activity. Proximate analysis revealed acceptable physicochemical parameters, including low moisture content and total ash and acid-insoluble ash values within acceptable limits, indicating good quality and minimal extraneous matter in the crude drug. Preliminary phytochemical

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