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A green *Hibiscus cannabinus* oil emollient cream for potential topical applications

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ABSTRACT:

A green emollient cream with *Hibiscus cannabinus* seed oil and an alkyl polyglucoside surfactant has been formulated. It can serve as biological alternatives to synthetic formulations that normally incorporate chemical constituents as surfactants and stabilizers mainly to increase consumer compliance in terms of textural and visual aesthetics. FAME analysis of the oil showed the presence octanoic and decanoic acids. The cream after formulation and ultrasonication, presented a smooth and soft appearance with visual and textural appeal. It showed a mean particle size of 138 nm with a zeta potential of -59.2 mV and an electrophoretic mobility of -0.000459 cm^2/Vs. Its SEM image projected well dispersed oil globules in water. FTIR spectrum showed extensive hydrogen bonding. Accelerated stability tests under conditions of freeze thawing, heating cooling and centrifugation revealed no cracking, creaming or phase separation. Similar results were observed during the shelf life studies. It is concluded that this *Hibiscus cannabinus* cream can be utilized as an emollient base for loading cosmopharmaceutic ingredients for their topical delivery, without any toxicity concerns, as it is formulated from completely natural constituents.

KEYWORDS: *Hibiscus cannabinus*, Alkyl polyglucosides, Emollient cream, Topical applications.

INTRODUCTION:

Hibiscus spp.,is known for its applications in traditional medicine[1]. Specifically, *Hibiscus cannabinus* has been pharmacologically explored for its activities as an anti-hypertensive, anti-inflammatory, antipyretic, antidiabetic, antioxidant and antimutagenic agent[2,3]. Its constituents include limonene, phellandrene, citral, ethyl and butyl alcohol, benzene acetaldehyde, methyl furfural and phytols that contribute to its activity[4]. However, the probability of risk in its concentrated form has posed limitations due to dose dumping, raising toxicity concerns[5]. Its emulsions are used to overcome risks due to inadvertent over dosages[6].

Such emulsion formulations contain parabens, phthalates, Tweens, and Spans. These are merged with synthetic bases like polyethylene glycol and cocamide to formulate end products[7]. This enhances textural and visual aesthetics and also to increases their stability[8]. However, several of them have deleterious effects on biological systems[9,10]. Hence green formulations of *Hibiscus cannabinus* seed oil, that ensures minimal dermal toxicity upon topical applications is required to exploit its full potential.

In this study, an aqueous *Hibiscus cannabinus* seed oil based emollient cream has been formulated with an alkyl polyglucoside surfactant. The smooth and creamy product has well dispersed microsized oil droplets stabilized by surfactant molecules as evidenced from its size measurements, zeta potential, electrophoretic mobility and SEM image. Its FTIR spectrum shows extensive hydrogen bonding. It has a shelf life of a minimum period of 90 days at 37^0C and is stable to accelerated stability tests. To best of our knowledge, an emollient cream matrix, that can be used in base form as well as be employed for loading active ingredients for topical delivery, has not been reported so far.

MATERIALS AND METHODS:

Materials:

Pure *Hibiscus cannabinus* oil was purchased from a branded dealer in the local market. Alkyl polyglucoside surfactant was procured from SEPPIC, Bangalore, India. Milli Q water was used for the formulation.

FAME analysis of *Hibiscus cannabinus* seed oil:

The oil was esterified using methanol and sodium hydroxide[11] and subjected to GC-MS analysis (JOEL GCMATE II) using a HP 5Ms ultra inert column with very low bleeding characteristics. Pure helium was used as a carrier gas (1ml/minute). The GC inlet temperature was set to 220^0C, and the oven temperature was ramped from 50^0C to 250^0C at a rate of 10^0C/minute. The GC interface and ion chamber temperature were both set to 250^0C. Electron Impact ionization (70eV) mode was employed with a scan rate of 50 to 600amu. Quadruple double focusing mass analyzer and photo multiplier tube were used and NIST library was employed to identify the compounds.

Synthesis of Emollient cream:

Ten ml of *Hibiscus cannabinus* seed oil was preheated to 50^0C and 2g of powdered surfactant was dissolved in it. 30ml of water was then added in 10ml aliquots with constant stirring. The resulting emollient cream was sonicated in an ultrasonic atomizer (Model VCX500, Sonics and Materials, Inc., USA), for 60 minutes. During sonication, 10 ml aliquots of water were added at 5 minute intervals till the total volume reached 100 ml. The resulting emollient cream was exposed to UV light for 10 minutes and stored in a brown bottle at 37^0C for 48 hours, before further characterization.

Characterization studies:

Horiba Scientific SZ 100 instrument was employed along with Windows [Z type] version 2.00software for particle size analysis, after a one in hundred dilution. Polydispersity index was also recorded. A 90^0 scattering angle and a temperature of 25^0C were employed. The percentage frequency of particles against their diameter was plotted by using the software. After suitable dilutions, the zeta potential and electrophoretic mobility were recorded using the same instrument. The SEM image was captured with Carl Zeiss EVO 18 Research instrument. FT-IR spectra were recorded for the oil and cream with an FT-IR Spectrophotometer (Bruker Optics, Germany) using ATR technique, between 4000 and 500cm^{-1}. Shelf life stability was assessed by transferring about 10g of cream into a 25ml glass beaker and maintaining it at 37^0C for a period of 90 days. After every 15 days, it was observed for signs of visual destabilization like cracking, creaming and formation of oil droplets and/or phase separation. For recording the stability, under accelerated test conditions, 10 grams of cream were transferred into 25 ml glass beakers. They were covered with aluminum foil and stored for of 48 hours, at 40 ± 0.5^0C for heating cycles, 5 ± 0.5^0C for cooling cycles and -18 ± 0.5^0C for freeze thaw cycles. After this time interval, they was brought to 37^0C and observed for any signs of destabilization as described above. All the cycles were repeated four times and the incubation temperatures were strictly monitored. 10g of the cream was also centrifuged for 20 minutes at 5000 rpm in a laboratory REMI centrifuge and observed for any signs of destabilization.

RESULTS:

A simple formulation procedure was used here. Ultrasonication was adopted as a method of choice to break down surfactant stabilized oil droplets into microsized ones. The emollient cream

was creamy with a smooth silky feel and appearance (Figure 1). It was left undisturbed for a period of 48 hours at 37^0C for attaining stability.

Figure 1: *Hibiscus cannabinus* emollient cream

FAME analysis of the oil using GC-MS showed the presence of six fatty acids namely caprylic, capric, undecylic, tridecylic, pentadecylic and oleic acids. Their lipid numbers were further identified and their relative percentages were recorded (Table I). The major fatty acids were substituted and methylated octanoic and decanoic acids. Other studies have also revealed similar compositions[12].

Table 1: Fatty acid constituents of *Hibiscus cannabinus* seed oil

Fatty acid Constituents	Lipid number	Relative percentage
Caprylic acid	8:0	16.9
Capric acid	10:0	13.4
Undecylic acid	11:0	34.2
Tridecylic acid	13:0	21.2
Pentadecylic acid	15:0	9.8
Oleic acid	18:1	4.5

Mean particle size was measured after 48 hours. It was found to be 138 nm. Polydispersity Index (PDI) was less than 0.5. Zeta potential and electrophoretic mobility was -59.2 mV and -0.000459cm^2/Vs respectively (Figures 2 and 3).

Figure 2: Particle size analysis of the emollient cream

Figure 3: Zeta potential analysis of the emollient cream

The SEM image of the cream is shown in Figure 4. It can be seen that the oil droplets are well dispersed in water and stabilized by the surfactant molecules.

Figure 4: SEM image of the emollient cream showing surfactant stabilized oil droplets

The FTIR spectra of the oil and cream are depicted in Figure 5 and Figure 6 respectively. The *Hibiscus cannabinus* seed oil spectrum showed characteristic bands in the frequency range of 1110-1230cm^{-1} for its triglyceride moiety. The -CH_2 bending and rocking frequencies are shown at 1462cm^{-1} and 720-725cm^{-1} respectively. The latter frequency is also used to determine total unsaturation present in vegetable oil in addition to the frequency range of 2852-2954cm^{-1}. The band at 1741cm^{-1} characterizes carbonyl frequency of the saturated fatty acids. For the surfactant, IR finger prints have been obtained for -OH, -CH, -CH_2, -CH_2OR groups from alkyl polyglucoside and saturated fatty alcohols. Its spectrum shows bands at 719.45cm^{-1}, 1060.8cm^{-1}, 1462.04cm^{-1}, 1739cm^{-1}, and 2846-2954cm^{-1}, that correspond to C-H rocking, C-O stretching, -C-H bending, -C=O stretching and C-H stretching with an additional band at 3275.1cm^{-1}. This indicates O-H stretching of alcohols. Extensive hydrogen bonding is seen in alkyl polyglucoside surfactant[13] during and after the formation of the cream. This is evidenced by a broad band in the range 3200-3600cm^{-1}. Further, the absorption band at 1641cm^{-1} is a finger print for O-H bending vibration of water[14].

Figure 5: FTIR spectrum of *Hibiscus cannabinus* seed oil

Figure 6: FTIR spectrum of the emollient cream

The accelerated stability tests showed no observable change in its color and texture even after a period of six months when stored at 37^0C. An extended shelf life is thus validated for this formulation. Further, there was no creaming/sedimentation, cracking, flocculation or phase separation during the heating-cooling, freezing-thawing and centrifugation studies. It can thus be inferred that the emollient cream is stable to accelerated stability tests also.

DISCUSSION:

Plants that belong to the *Hibiscus* genus are classified under the *Malvaceae* family[15]. Their flowers, canes, roots and barks are incorporated in products related to food, traditional medicine and horticulture. Their seeds are rich sources of phospholipids, sterols, triglycerides, proteins and other natural products[16]. But in spite of this, they have not been exploited to a large extent. Seed oils are extracted from cultivated hybrid and natural varieties of the *Hibiscus* species. Their oil content ranges between 8.9 and 29.8 weight percent. It has been suggested that *Hibiscus* oil extracts, with this biochemical profile, could find potential applications in cosmetic formulations[17]. Wound healing properties have also been attributed to *Hibiscus sabdariffa* extract. It shows a

synergistic effect with gentamycin when applied to wounds[18]. The seed oil obtained from this variety is also a rich source of gamma tocopherols[19]. Hence it has been suggested as an alternative natural antioxidant to synthetic ones like butylated hydroxyl toluene and anisole as the latter pose intense health hazards[20]. Tocopherol being the vitamin of choice today in anti-ageing creams and formulations, it has been envisaged that *Hibiscus* extracts would have tremendous potential as a cosmetic base. It was therefore selected for the formulation of the emollient cream in this study.

An arachidyl glucoside surfactant, with arachidyl and behenyl alcohol groups, has been employed to emulsify the *Hibiscus cannabinus* seed oil. The surfactant is non-ionic, biocompatible, biodegradable and is of 100% vegetable origin. It is derived from corn or manioc[21]and incorporates no solvents or preservatives. It aids emulsification of oils at low concentrations between 1 to 2% and across a wide range of pH. It promotes growth of liquid crystals and retains the skin moisturization for hours after the initial application. It can be used on oily as well as on sensitive skin. It also provides an evanescence effect. It stabilizes the formulations by obstructing the creaming and phase separations. By varying its concentrations, emollients of optimum viscosity can be obtained. It is an ideal choice for emulsifying *Hibiscus cannabinus* seed oil. Ultrasonication was adopted as a method of choice to homogenize the emollient cream as it aids in the break down of surfactant stabilized oil droplets into microsized ones. This increases the surface area for effective end applications[22]. The excess surfactant molecules present in the system facilitates further droplet stabilization by charge and steric based mechanisms.

The FAME analysis for the oil revealed the presence of mainly octanoic and decanoic acids and their methyl esters. Undecanoic acid also known as undecylic acid is anti seborrheic in nature and is a vital constituent in treating skin infections due to *Candida albicans*[23]. The acid very dynamically binds with the lipid bi-layers of the cell membrane and leads to a rise in the cell fluidity of the fungus. This eventually causes structural differences in the membrane proteins and cytoplasm and thus leads to leakage of the intracellular components and cell death. Among the potent antifungal fatty acids that target the plasmalemma and amino acid synthesis are octanoates. This 8C fatty acid causes an alteration of biological function of various organelles, including the cell nucleus, mitochondria, peroxisome, endoplasmic reticulum, golgi apparatus, lysosome, endosome, and related structures resulting in rapid exo-osmosis with cell senescence[24]. Oleic acid is an unsaturated fatty acid used for medications of dermatomycosis caused by *Trichophyton rubrum* and *Epidermophyton inguinale.* The fatty acid represses the formation and elongation of hyphae in *Candida albicans* and prevents its infection in the host by disturbing acyl-CoA synthesis during lipid oxidation. Methylated fatty acids, acquired from plant extracts, pose low environmental threats and manifest a high degree of specificity that is likely due to the hindrance of β-oxidation, thus increasing the half-lives of the fatty acids. This would allow them to be inserted more efficiently into the membrane and cause methoxylation[25,26] . It has been observed that these fatty acids present in hibiscus oil will confer it with antiarthritic, antipyretic anti-spermicidal, diuretic, antimalarial, antibacterial, anti-inflammatory, immunomodulatory and antifungal properties.

The characterization of the emollient cream in terms of mean size, zeta potential and electrophoretic mobility depicted a mean particle size of 138 nm with Polydispersity Index (PDI) of less than 0.5. An enhancement in visual appeal as well as in textural feel is a consequence of this low particle size. The polydispersity index ensures long term stability and protects it against mechanisms leading to its destabilization[27]. Zeta potential is a measure of the extent of charge distribution in the diffuse layer around the constituent particles. A value of either above +30 or below -30 is considered as a reasonable cut off for attaining stability. It was found to be -59.2 mV and this value is excellent for retaining the particles in the dispersed state. Zeta potential acts as an important determinant for delivery systems and can be applied in favorable context in this study. Electrophoretic mobility depicts the movement of the oil droplets under the influence of an electric field. In this study, the electrophoretic mobility was recorded to be -0.000459cm^2/Vs .The low value is probably due to two reasons. Surfactant induced charge stabilization of the oil droplets is the most important reason. Tight steric packing by the individual surfactant molecules in aqueous phase may also restrict the movement of particles. Hence, destabilization mechanisms that are dependent on migrations of constituent particles in standing systems, do not function effectively here. Long term stability of the emollient cream is thus ensured. Its SEM image depicted effectively distributed water molecules around the oil molecules with glucosides acting as a link between the two thus conferring stability to the oil particles against aggregation.

IR spectroscopy plays an important role in the identification and analysis of plant based oils[28].The -CH_2 bending and rocking frequencies determines total unsaturation present in vegetable oil. The IR finger prints from alkyl polyglucoside correspond to C-H rocking, C-O stretching, -C-H bending, -C=O stretching and C-H stretching at 719.45cm^{-1}, 1060.8cm^{-1}, 1462.04cm^{-1}, 1739cm^{-1}, and 2846-2954cm^{-1} respectively. Due to the higher cloud point of the alkyl polyglucosides, extensive hydrogen bonding is seen when compared to classical polyethoxylated surfactants[13]during and after the formation of the emollient cream (Figure 6).

The cream was stable to creaming, aggregation, flocculation, coalescence and phase separation. It had a shelf life for a minimum period of 90 days at 37^0C validating the fact that that it can used for this extended period of time without destabilization.

CONCLUSIONS:

A green emollient cream, formulated from *Hibiscus cannabinus* seed oil and alkyl polyglucoside surfactant, is proposed as a topical matrix in this study. The formulation protocol is simple, and the small sized oil globules are well stabilized by the surfactant molecules as evidenced from its zeta potential values, electrophoretic mobility studies and SEM image. It is stable for a minimum period of 90 days and it withstands accelerated stability tests also. It can hence be employed as such or as a base matrix for loading active pharmaceutical and cosmeceutical ingredients for their delivery by topical application.

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