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Research Article

Study Of Regulatory Requirements In Indian Context For Formulated Nutricosmeceutical Product

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ABSTRACT:
The present study focuses on the design and evaluation of ethosomes with *Hibiscus sabdariffa* and *Murraya koenigii* extract with emphasis on enhanced antioxidant activity and transdermal delivery. Ethosomes, being recent lipid-based vesicular carriers, offer enhanced permeation and stability for bioactive compounds. Hibiscus sabdariffa being a concentrated source of anthocyanins, polyphenols, and essential nutrients contains promising antioxidant, antihypertensive, and hepatoprotective activities. The study involves the formulation of ethosomes with optimized phospholipid and ethanol concentration, followed by physicochemical characterization involving the study of vesicle size, zeta potential, etc. Furthermore, the study critically examines the regulatory environment for nutraceuticals in India, with special reference to compliance under the Food Safety and Standards Authority of India (FSSAI) regulations. The formulation of *Hibiscus sabdariffa* and *Murraya koenigii* in a stable and efficient ethosomal system, along with regulatory perspective, positions this formulation as a lead candidate for future nutraceutical development. The research offers practical recommendations aimed at addressing existing regulatory disparities while maintaining high safety standards. By advocating for increased harmonization, the study seeks to establish a more unified global framework for the herbal products sector, ensuring both safety and transparency. This work serves as an essential resource for regulators, industry participants, and consumers who wish to navigate the complexities of herbal product labelling in a dynamic market.

Keywords: Nutricosmeceuticals, Ethosomes, Regulations, FSSAI, CDSCO

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❖ INTRODUCTION

Over the past few years, the intersection of nutrition and dermatology has given rise to a new generation of products referred to as nutricosmeceuticals. They are oral products that deliver both nutritional and cosmetic benefits, acting from the inside out to enhance skin health, slow aging, and deliver

overall wellness. Nutricosmeceuticals fill the gap between cosmetics and pharmaceuticals by marrying the potency of bioactive molecules with the preventive and therapeutic potential of nutrients. (Orasan et al., 2016) Among the wide range of botanicals used in nutricosmeceuticals, *Hibiscus sabdariffa* (roselle) and *Murraya koenigii* (curry leaves) have also gained considerable attention owing to their high content of phytochemicals and high antioxidant activity. *Hibiscus sabdariffa* is valued for its high content of anthocyanins, flavonoids, and organic acids, which render it anti-inflammatory, anti-aging, and cardioprotective. Similarly, *Murraya koenigii* is a goldmine of nutrients and bioactive phytochemicals such as carbazole alkaloids, vitamins A and C, iron, and calcium, which are essential to enhance skin, hair, and overall well-being. For effective delivery of such bioactives to target locations, sophisticated drug delivery systems are the need of the hour. Ethosomes, a new generation of lipid-based vesicular carriers, are a new horizon in this regard. (A.K Johnson, 2021)Comprising phospholipids, ethanol, and water, ethosomes enhance permeability of the active principles through the stratum corneum with increased penetration of the skin and improved bioavailability. Their cosmetic and nutricosmeceutical use offers targeted and sustained delivery of natural compounds with the maximum therapeutic benefit and least side effects. This integrated approach that unites natural nutrition with innovative delivery systems is a move forward for personal care that is in harmony with the growing demand for science-based and all-inclusive beauty and wellness solutions. .(Lentjes, 2019)

Nutraceuticals are those food-based products that offer benefits to health beyond mere nutrition and are used for disease prevention or cure. They comprise dietary supplements, functional foods, and herbal preparations. Cosmeceuticals, however, are cosmetic products that incorporate biologically active ingredients thought to have therapeutic effects for the skin in terms of anti-aging, anti-inflammatory, and skin repairing properties. Nutricosmeceuticals are a mix of the two, formulated to enhance beauty from within by incorporating nutritional and cosmetic effects, for example, nourishing healthy skin, hair, and nails through oral supplementation.(Gokce et al., 2022)

In India, nutraceuticals are regulated mostly by the Food Safety and Standards Authority of India (FSSAI) through its 2022 guidelines, with regulation from the Central Drugs Standard Control Organization (CDSCO) and Advertising Standards Council of India (ASCI). For cosmeceuticals, the regulatory framework is the Drugs and Cosmetics Act of 1940 and Rules of 1945, with additional oversight by CDSCO, Bureau of Indian Standards (BIS), and ASCI for advertisement compliance. Collectively, these frameworks ensure safety of the products, proper labelling, and honesty in marketing for these new health and beauty product categories.

❖ MATERIAL & METHODS

The calyx and leaves of *Hibiscus sabdariffa* and *Murraya koenigii* plant were collected from local area of Nagpur. The Mumbai-based Loba Chemicals Pvt. Ltd. Supplied us solvent methanol and ethanol. Quercetin, DPPH, ABTS, catechol, ferrozine, ferrous sulphate, guaiacol, gallic acid, sodium carbonate, ammonium molybdate, sulphuric acid, sodium phosphate, ascorbic acid, potassium ferricyanide, TCA, etc were purchased from Hi-Media, India. Hydrogen peroxide and Folin-Ciocalteu reagent were purchased from Central Drug House (P) Ltd. India. Every chemical was of the analytical grade.

1. Extraction

The coarse powdered of dried calyx of *Hibiscus sabdariffa* (100g) and leaves of *Murraya koenigii* (100g) was extracted with 650ml of ethanol by hot solvent extraction for 24hr using Soxhlet assembly. The extract was concentrated at 40℃ to obtain dark reddish colour sticky mass. The extracts dried by evaporation weighed and subjected to phytochemical screening.(Luque de Castro & Priego-Capote, 2010)

2. Quantitative estimation

Phytochemical evaluation was done by performing tests for Carbohydrates, Proteins, Flavonoids, Alkaloids, Glycosides, polyphenols, Tannins, saponins etc. Quantitative estimation was done for estimating total flavonoid content in the extract.

- **Total flavonoid content:** Stock solution of Rutin (1mg/ml) in methanol was prepared then Different aliquots of 100-1000µg/ml was prepared in a 10ml volumetric flask & 4 ml distilled water was added along with 0.3ml 5% $NaNO_2$. After 5 min add 0.3 ml f 10% $AlCl_3$ & further after 5-6 min add 2ml 1M NaOH. After the addition of NaOH, the solution turns red color. Incubate/kept at dark a place for 30 min. Absorbance was measured at 510 nm and Calibration curve was plotted as concentration v/s Absorbance.(Aryanti et al., 2021)(Salim et al., 2024)

3. DPPH(2,2-Diphenyl-1-picrylhydrazyl)Radical Scavenging Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a general assay for determining the free radical scavenging capacity of compounds, extracts, or formulations. Dissolve 3.94 mg of DPPH in methanol (100 mL).Store the solution in a dark bottle at room temperature. Stabilize for 30 minutes prior to use. Prepare the test sample (pure compound or extract)of varying concentrations in methanol (Regupathi & Chitra, 2015).Prepare ascorbic acid as standard in identical solvent and concentrations. In a test tubes, add 1 mL of 0.1 mM DPPH solution.1 mL of test sample of varying concentrations. As control, 1 mL of DPPH solution and 1 mL of methanol (no antioxidant). As blank, take 1 mL of methanol and 1 mL of sample (no DPPH), mixed well and incubate the reaction at room temperature in the dark for 30 minutes.Record absorbance at 517 nm using a UV-Vis spectrophotometer. (Alam et al., 2013, Sundara rajan wr al., 2017)

The percentage of **DPPH radical scavenging activity** is calculated using the formula:

$$\%ScavengingActivity=(Acontrol-Asample/Acontrol)\times 100$$

Where, Acontrol = Absorbance of DPPH solution (without sample). Asample = Absorbance of DPPH solution with test sample.

4. Preparation of Ethosomes

a. Place the required amount of lecithin (1-4% w/v) into a small round bottom flask.
b. Add the ethanol (10-50% v/v) containing the drug to the flask.
c. Start the magnetic stirrer to solubilize the lecithin and the drug in the ethanol. Ensure thorough mixing.
d. Cover the round bottom flask with its lid or an appropriate cover to prevent ethanol evaporation during stirring.
e. Slowly add distilled water to the mixture while maintaining continuous stirring. This step is crucial to obtain the colloidal suspension.
f. Continue stirring for a sufficient time until the nanoparticle are formed. This usually takes around 30 minutes at room temperature.
g. After 30 minutes, stop the stirring and let the final nanoparticle suspension sit at room temperature.

5. Evaluation of Ethosomes

- **Particle size and zeta potential**

Particle size and zeta potential of ethosomes are determined by dynamic light scattering (DLS) or electrophoretic light scattering (ELS) techniques. Light scattering data are measured at a suitable angle (typically 90°c or 173°c for backscattering), and hydrodynamic diameter is calculated using the Stokes-Einstein equation (O'brien et al., 1995).

The zeta potential is calculated by software from the Smoluchowski equation, and it is a measure of surface charge and colloidal stability of the ethosomes. A rough estimate of zeta potential value of >±30 mV is a measure of good colloidal stability, andlow values are measures of the potential for aggregation(Marsalek, 2014)(Shah et al., 2014)

- **SEM (Scanning Electron Microscopy)**

The scanning electron microscopy (SEM) is done to observe the microscopy of particles in high mangnification. In this the sample is fixed, dehydrated, and covered with a conductive coating such as gold or platinum if non-conductive. The sample is mounted on an aluminum stub and placed in the SEM vacuum chamber. The sample is scanned with an electron beam, and secondary and backscattered electrons are produced due to interactions, and high-resolution images are created. Images are analyzed to examine surface morphology, particle size, and structural features.(Datye & DeLaRiva, 2023)(Abdullah & Mohammed, 2019)(Alam et al., 2013)

❖ RESULT AND DISCUSSION

1. Phytochemical evaluation

During phytochemical evaluation *Hibiscus sabdariffa* shows presence of Carbohydrates, Proteins, Flavonoids, Phenols, alkaloids and Tannins while *Murraya koenigii* shows presence of Proteins, Flavonoids, Phenols, and Tannins.

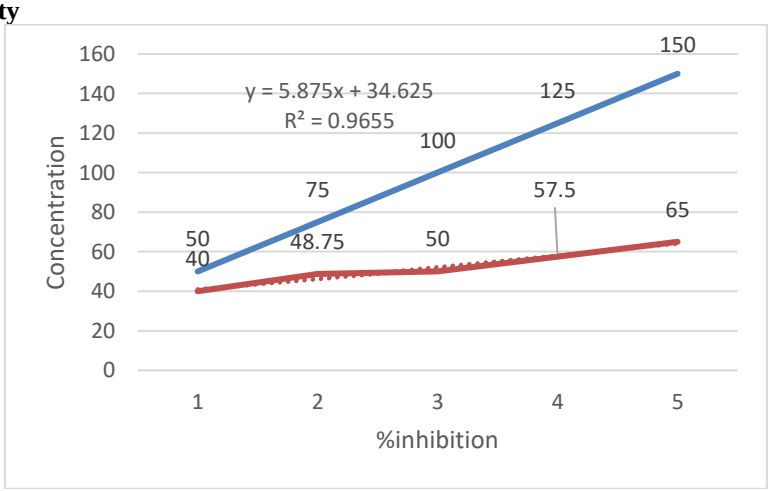
Table 1: Phytochemical estimation of extract

List of Phytochemicals	Result of Ethanolicextractof *Hibiscussabdariffa*	Result of Ethanolicextractof *Murrayakoenigii*
Carbohydrates	+	-
Proteins	+	+
Flavonoids	+	+
Phenols	+	+
Alkaloids	+	-
Tannins	+	+
Glycosides	-	-

2. Total Flavonoid content

Flavonoid content in Hibiscus and Murraya extract was found to be 17.50±0.52 mg / g in ethanolic extract and 20 ±0.52 mg/g in ethanolic extract.

3. DPPH Scavenging Activity



GRAPH 1:- Standard Ascorbic acid

1. Standard Ascorbic Acid

Table 1: Standard ascorbic acid

Sr no.	Concentration (mg/ml)	% Inhibition
1	50	40
2	75	48.75
3	100	50
4	125	57.5
5	150	65

Table 3: IC50 of standard Ascorbic acid, *Hibiscus sabdariffa* and *Murraya koenigii*

Particulars	IC50 Value
Ascorbic acid	90.42
Hibiscus sabdariffa	86.61
Murraya koenigii	57.86

4. Particle Size Analysis: Particle size analysis was done using Litesizer 500.

Table 4: Particle size of Murraya koenigii

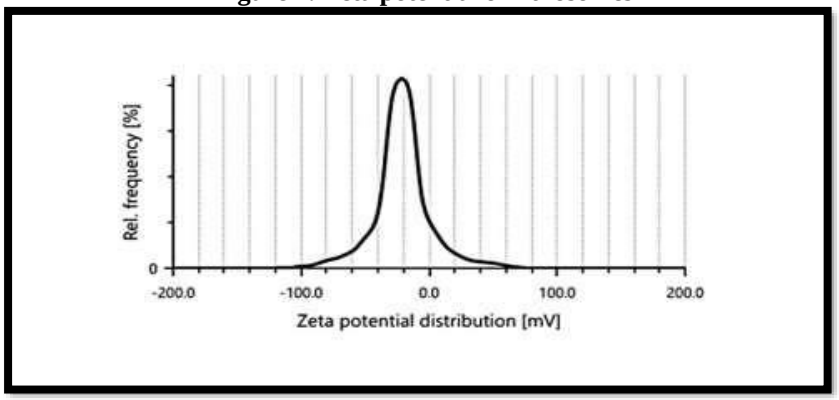
Batches	Soya lecithin	Ethanol	Drug	Water	Particle Size
1	7 ml	50 ml	10 g	20 ml	1018.6 nm
2	6.6 ml	50ml	10 g	20 ml	291.1 nm
3	1 ml	15 ml	1 g	10 ml	224.4 nm

Table 5: Particle size of Hibiscus sabdariffa

Batches	Soya lecithin	Ethanol	Drug	Water	Particle Size
1	1 ml	15 ml	1 g	10 ml	491.6 nm
2	6.6 ml	50ml	10 g	20 ml	328.6 nm
3	1 ml	15 ml	1 g	10 ml	252.9 nm

5. Zeta potential:

Figure 1: Zeta potential of Ethosomes



Zeta potential of optimized batch of Ethosomes was found to be -21.5mV

6. Scanning Electron Microscopy:

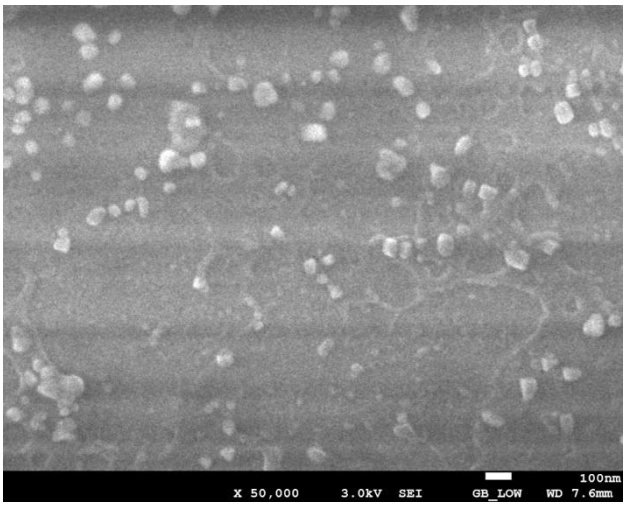


Figure 2: SEM Analysis

- **FSSAI Nutraceutical Guidelines – India**

Category	**Guideline**
Regulation Name	Food Safety and Standards (Health Supplements, Nutraceuticals, etc.) Regulations, 2016 (Amended till 2021)
Effective From	Compliance mandatory by 1st January 2018
Applicable Products	Health Supplements, Nutraceuticals, Food for Special Dietary Use/Medical Purpose, Functional Food, Novel Food
Labeling Requirements	- Mandatory declaration of nutraceutical content - Display of RDA % (if applicable) - Warnings: "NOT FOR MEDICINAL USE", side effects, etc. - Storage: “Keep out of reach of children”
Permitted Forms	Granules, Powder, Tablet, Capsule, Liquid, Jelly, Gel, Semi-solids (excluding parenteral forms)
Approved Ingredients	From Schedule I, II, IV, VI, VII, VIII. Schedule VI ingredients must have marker compound standardization
Ingredient Sources	Extracted/purified from food or non-food sources; e.g., bacterial fermentation
Nutrient Limits	Not to exceed RDA by ICMR or Codex; must retain scientific documentation if RDA is unspecified
Claims Restriction	Cannot claim to prevent/treat/cure diseases
Permissible Additives	Only as per Schedule VA, VE, VF
Approval Requirement	New ingredients must have 15 yrs (India) / 30 yrs (foreign) usage history and Food Authority approval
Sportsperson Clause	"Sportsperson" added as defined by Sports Authority of India (effective 6 Sept 2021, enforced from 1 April 2022)

- **CDSCO Cosmetic Labelling Guidelines – India**

Aspect	**Guideline**
Sale Restriction	Only licensed, properly labelled and packed Indian-manufactured cosmetics can be sold.
Export Exemption	Labels can be adapted to consignee needs; manufacturer name may be replaced with a code approved by the licensing authority.
Inner & Outer Label Must Contain	- Name of the cosmetic - Manufacturer’s name & address (or principal place with pin code if very small pack) - Net contents (except ≤60 ml liquid or ≤30 g solid)
Inner Label (Hazards)	- Directions for safe use - Warnings or precautions - Hazardous ingredient names & quantities
Batch Number	Indicated with "B", not required for ≤10g (solid) or ≤25ml (liquid). For soaps: month & year suffice.
Licence Number	Must be shown as "M [number]".
Single Label Packs	Must contain all info required for both inner & outer labels.

Ingredient List	- Ingredients >1% listed in descending order - ≤1% in any order - Prefixed by "INGREDIENTS" - Not required for packs <60 ml / <30 g
BIS Standards	Must comply with Bureau of Indian Standards (BIS) if applicable under Schedule S.
Tampering Rule	Alteration of manufacturer's label marks is prohibited unless authorized.
False Claims	No cosmetic may carry false or misleading claims.
Hair Dyes Warning	Must include caution about skin irritation & blindness risk if used on eyelashes/brows; include a sensitivity test procedure.
Fluoride Toothpaste	- Max 1000 ppm fluoride - Must declare ppm on tube & carton - Expiry date required on both
Test Report Format	Must follow Form 34 for test analysis results.
Cosmetic Standards	Must comply with standards in Schedule S.

❖ CONCLUSION

The formulation and evaluation of Nutricosmeceuticals represent a significant advancement at the intersection of nutrition and cosmetics, offering consumers holistic solutions for health and beauty from within. This study successfully demonstrated the critical steps involved in developing stable, effective, and consumer-friendly ethosomes, emphasizing the importance of ingredient selection, compatibility, and targeted benefits. Moreover, the exploration ofregulatory aspects highlighted the need for stringent compliance with national and international guidelines to ensure product safety, efficacy, and transparency. Proper regulations on product not only supports informed consumer choices but also strengthens market credibility and minimizes legal risks for manufacturers. As the nutricosmeceutical industry continues to expand, maintaining a balance between innovative product development and regulatory adherence will be key to sustainable growth and consumer trust.

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