EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 1
Year: 2025

FORMULATION AND EVALUATION OF NANO SUSPENSION FROM THE ETHANOLIC EXTRACT OF *GARCINIA INDICA*

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Article Received on 04/11/2024 **Article Revised on 24/11/2024** **Article Accepted on 14/12/2024**

ABSTRACT
The nano-suspension formulation of herbal extracts from *Garcinia indica* shows significant potential for improved bioavailability and therapeutic efficacy. This is attributed to enhanced solubility, stability, and dissolution properties, with reduced particle size increasing surface area for better absorption and faster onset of action. However, further research is required to evaluate the long-term stability, effects, and scalability of the nano-suspension for its pharmaceutical and therapeutic applications.

KEYWORD: Nano-suspension, *Garcinia Indica.*

1. INTRODUCTION

The word 'Pharmaceutics' is used in the pharmacy and pharmaceutical science to encompass many subject areas that are all associated with the steps to which a drug is subjected towards the end of its development, i.e. it is the stags that follow the discovery or synthesis or the drug, its isolation and purification, and testing for advantageous pharmacological effects and absence of serious toxicological problems. Put at its simplest – pharmaceutics converts a drug into a medicine.

A drug is defined as an agent intended for use in the diagnosis, mitigation, treatment, cure or prevention of disease in humans or in other animals (Food, Drug, and Cosmetic Act, 1938). One of the most astounding qualities of drugs is the diversity of their actions and effects on the body. This quality enables their selective use in the treatment of a range of common and rare conditions involving virtually everybody organ, tissue and cell.

Drugs are rarely delivered as pure chemical entities but are approximately usually provided as prepared formulations i.e. dosage form. After converting them into an appropriate dose formulation, they are delivered in several dosage forms. To create an alternative dosage form, non-medicinal chemicals (also known as pharmaceutical ingredients or excipients) are added. By adding pharmaceutical ingredients that solubilize or suspend or thicken or dilute or emulsify or stabilize or preserve them, drug dosage forms can be made more effective and appealing.

Liquid dosage forms are the aqueous solutions of medicaments that are used to induce therapeutic effects, internally and externally. These forms are less stable than solids. Different routes of administration are used for liquid dosage forms, such as oral, intravenous, intramuscular, subcutaneous, subcutaneous, etc. A pharmaceutical suspension may be defined as a coarse dispersion containing finely divided insoluble material suspended in a liquid medium. The physical chemist defines the word "suspension" as two-phase system consisting of an un-dissolved or immiscible material dispersed in a vehicle (solid, liquid, or gas).

Nanoparticles are sub ionized colloidal structure composed of synthetic or semi-synthetic polymers. Size ranges 10-1000 nm. The drug is dissolved encapsulated or attached to a nano particle matrix. The materials which are used for the preparation of nano-particles should be non-toxic, biodegradable, sterilizable etc.

1.1 Nano Suspension

Nano suspension is submicron colloidal dispersions of non-ionisized drug particles stabilized by surfactants. Nano suspension consists of poorly water soluble drug without any matrix materials suspended in dispersion. These can be used to enhance the solubility of drugs that are poorly soluble in water as well as lipid media.

A nano-suspension is a colloidal dispersion of drug particles or active ingredients, typically in the size range of 1 to 1000 nanometers, suspended in a liquid medium. This formulation technique is commonly used in pharmaceuticals, especially for drugs that have poor solubility or bioavailability.

1.2 Here are some key points about nano-suspensions

- **Improved Solubility and Bioavailability**: Nano-suspensions increase the surface area of the drug, improving its dissolution rate in the body. This leads to better absorption and enhanced therapeutic effects, especially for poorly water-soluble drugs.
- **Stability**: The small particle size reduces the tendency of the drug to crystallize, which enhances stability and shelf-life.
- **Faster Onset of Action**: The small size of the particles allows for quicker absorption into the bloodstream, leading to faster therapeutic effects.
- **Delivery Methods**: Nano-suspensions can be delivered via oral, intravenous, or topical routes, depending on the formulation. They are often used for drugs that need to be delivered in small doses or for controlled-release therapies.
- **Applications**: Nano-suspensions are particularly useful in delivering herbal extracts, poorly soluble synthetic drugs, and bioactive compounds, making them a promising strategy for pharmaceutical and therapeutic applications.

2. DISEASE PROFILE

2.1 Ulcer

Peptic ulcer occurs in that part of the gastrointestinal tract which is exposed to gastric acid and pepsin the stomach and duodenum. It results probably due to an imbalance between the aggressive (acid, pepsin, bile and *H. Pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, nitric oxide, high mucosal blood flow, innate resistance of the mucosal cells) factors. A variety of psychosomatic, humoral and vascular derangements have been implicated and the important of Helicobacter pylori infection and recurrence has been recognized.

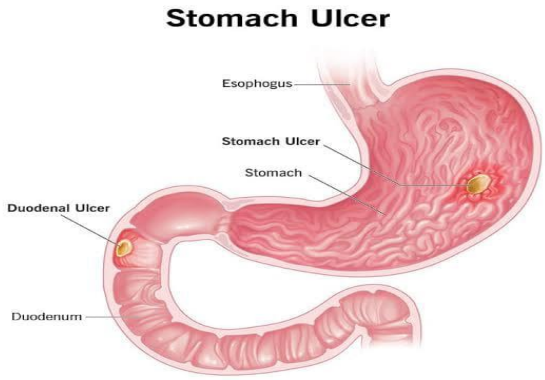


Fig. 1: Ulcer.

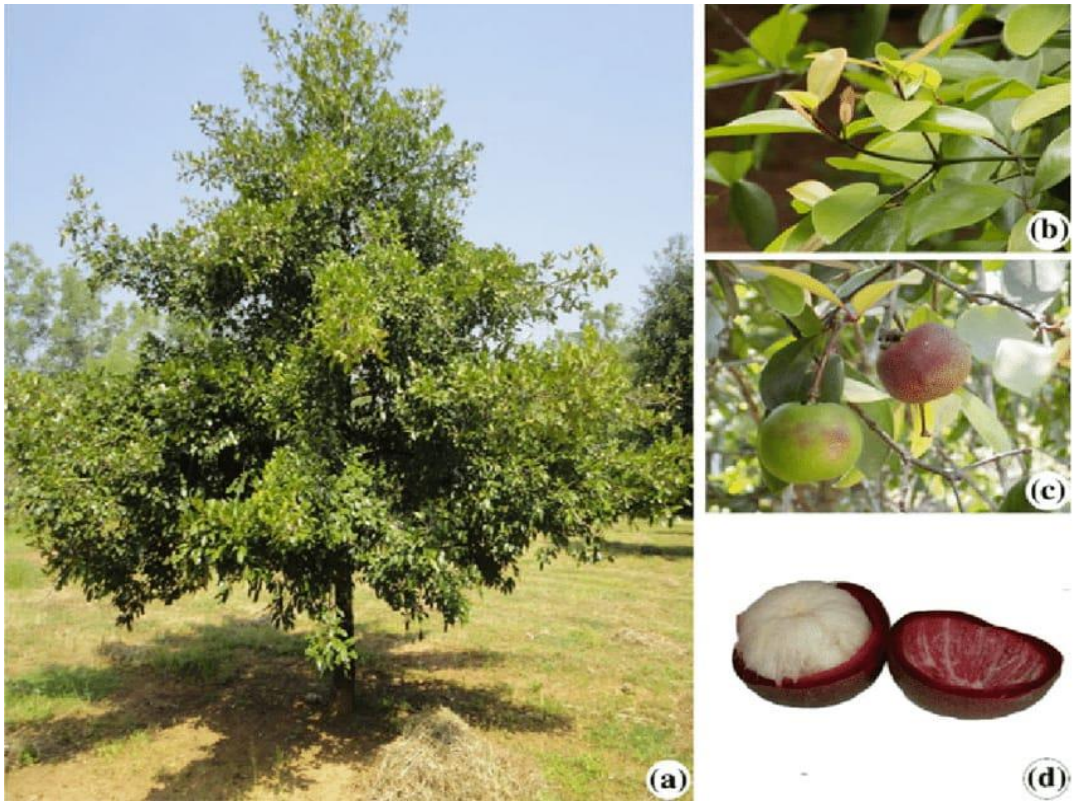
3. PLANT PROFILE

Kokum botanically *Garcinia indica* Choisy (Thouars), is a commercially under-utilized perennial tree species, found widespread as native species in Goa. *Garcinia* contains 200 species out of which over 20 are found in India. Kokum is an ever green, perennial, monopodial and all growing tree found in the West coast of India, in Northern Kerala, Coastal Karnataka, Goa and Konkan belt of Maharashtra. *G. indica* is commonly known as *Brindonia Tallo* tree or Kokum Butter tree in English. The tree grows up to 10-18 meters with drooping branches. It flowers from November to February with fruits ripening from April to May. After 15 years, a properly acred single plant yields about 30 to 50 kg of fruit.

The other vernacular names are kokum, birand, amsol (Konkani and Marathi), brind on (Portuguesein Goa), murugalu (Kannada) and punarpuli (Malayalam). Ayurveda, Unani and allopathy which *Garcinia indica* is one of the traditional medicinally imported deciduous plants available all over India. The fruit has been reported to be rich in polysaccharide. Unani system of drug medicine uses plant as antibacterial, antiviral and antitumor. Poly herbal formulations are extensively used by the masses in India for the treatment of common Functional dyspepsia (Indigestion).anti-tumor activity, anti-cancer, anti- inflammatory, anti-microbial & anti-ulcer activity.

Table 1: Taxonomical Profile.

PROFILE	TAXONOMICAL PROFILE
DOMAIN	Eukaryota
KINGDOM	Plantae
PHYLUM	Spermatophyte
SUBPHYLUM	Angiospermae
CLASS	Dicotyledons
FAMILY	Clusiaceae
GENUS	Garcinia
SPECIES	Indica



a) Tree b) Leaves c) Fruit d) Pulp and Rind
Fig. 2: *Garcinia Indica.*

4. MATERIALS AND METHODS

4.1 Preparation Of Ethanolic Extract Of Garcinia Indica Fruit Rind

The fruits rinds were cut into pieces and shade dried at room temperature. The dried fruits were subjected to size reduction to a coarse powder by using mixer grinder. This powder was defatted with petroleum ether then filtered. The residue was allowed to dry at room temperature. This residue was extracted with ethanol (95%) into soxhlet apparatus. The extract was dried at room temperature till semi solid mass obtained. The sweet scented, chocolate coloured semi solid residue formed after the complete dryness was kept in air tight and water proof container, which is stored in a refrigerator.

4.2 Determination Of Hydroxy Citric Acid Content

Separation and estimation of HCA was carried using C_{18} reverse phase column (250x 4.6mm) with 8Mm sulfuric acid as a mobile phase using UV detector at 210nm. The flow rate was 1.0 mL/min, and the sample injection volume for HPLC (shimadzu Nexera X2 ultra high performance liquid chromatography) was 20µl. Quantitative estimation of HCA was done against HCA calibration standard.

4.3 Formulation of Nano Suspension From Ethanolic Extract of *Garcinia Indica*

Nano precipitation (bottom – up approach) was used for the formulation of the nano suspension, with some modification. The plant extract was completely dissolved in ethanol, and the organic phase was slowly injected (1 ml / mins) with syringe connected to the thin Teflon tube into a aqueous phase containing the stabilizer (HPMC **)** with continue stirring at 6000 rpm for 6 hrs at room temperature.

4.4 Characterization Of Nano Suspension

4.4.1 Total Drug Content

Analiquot (0.5) was evaporated to dryness. The residue was then dissolved in acetone and filtered with 0.45µm filter paper. The samples were analyzed Total drug content (TDC) and %TDC were calculated. Using UV spectrophotometer at max of 253 nm.

TDC = Vol. total / Vol. Aliquot drug x amount in aliquot x 100

%TDC = TDC / TAX100

4.4.2 Particle Charge (zeta potential)

Zeta potential of formulation was measured using micro trac analyser. The samples were diluted 10 times with solvent before analysis. Physically stable nano suspensions stablilized by electrostatic repulsion, a zeta potential of ±30 mV is required as a minimum. Combined with the steric stabilization, the absolute value of zeta potential about ±20 mV is sufficient to fully stabilize the nano suspenstions system.

4.4.3 Scanning Electron Microscopy

The samples were viewed under scanning electron microscope. The morphological features of *Garcinia*

indica nanosuspension are observed by scanning electron microscope at different Magnifications.

4.4.4 In-vitro Drug Release Studies

The invitro release of various nanosuspension formulations were performed by dialysis bag diffusion technique. Dialysis tubing will act as dialysis sac. (Sigma dialysis membrane MW 12000 Da). Length of dialysis tube is 4 -5 cm. The sac was hermetically sealed and filed with 0.1N HCl and examined for the leaks. The sac was then emptied and 1ml of the formulated liquid nano suspension was accurately transferred into the sac, which served as the donor compartment. the sac was once again examined for leak and then suspended in the stoppered vessel containing 100ml 0.1N HCl, which behave has the receptor compartment. The Media temperature should be 37º±0.5º at 500 rpm speed. At predetermined time interval, 3ml of sample was withdraw from the receptor compartment and analyzed for the quantity of drug released. Fresh buffer was used to replenish the receptor compartment at each time point. The sample were withdrawn at 5, 10,15,20,25 and 30 min. The diffusion studies and sample analysis was carried out for all the developed formulations. Collected samples were suitably diluted with 0.1N HCl and analysed at 253 nm using 0.1N HCL as blank by using a UV spectrophotometer. The cumulative percentage drug release was calculated and graphs were plotted against time Vs % cumulative drug release.

5. RESULTS AND DISCUSSION

5.1 Physical Evaluation

- **Color:** Red to dark red
- **Odour:** Mildly sweet aroma
- **Taste:** Slightly sour
- **Viscosity:** 2.150 cp
- **pH:** 4.5 – 5.0
- **Solubility:** Soluble in water and other organic solvents.

5.2 Formulation Of Nano Suspension From Ethanolic Extract Of *Garcinia Indica*

Fig. 3: Soxhlet In Heating Mantle.

Fig. 4: Soxhlet Extraction Process.

Fig. 5: Sonication Under Process.

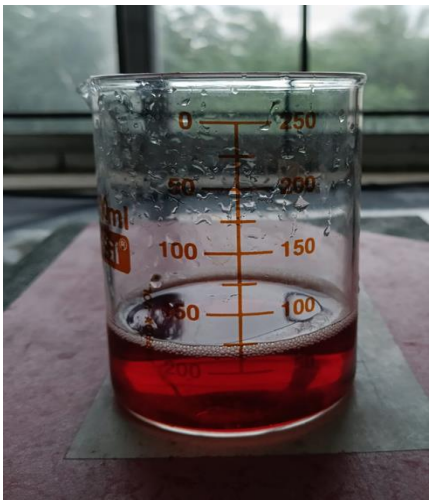


Fig. 6: Formulated Nano Suspension.

5.3 Determination Of Particle Size

			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm):	171.7	Peak 1:	165.4	99.2	73.96
PdI:	0.349	Peak 2:	24.70	0.8	4.258
Intercept:	0.873	Peak 3:	0.000	0.0	0.000
Result quality :	Good				

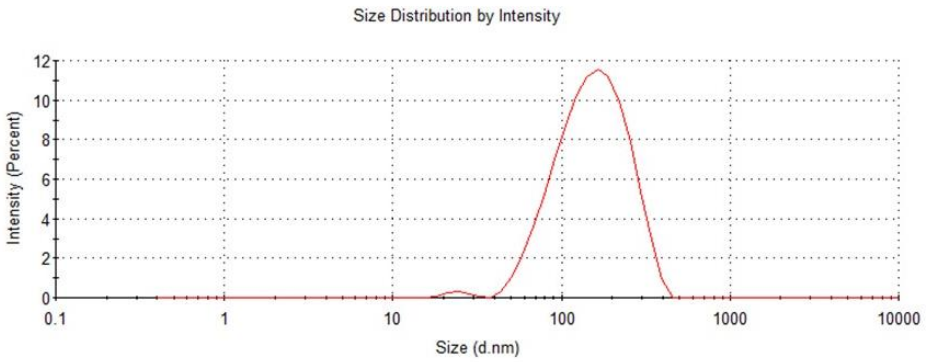


Fig. 7: Determination Of Particle Size.

5.4 Determination of Zeta Potential

			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-30.4	Peak 1:	-30.4	100.0	7.53
Zeta Deviation (mV):	7.53	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.0112	Peak 3:	0.00	0.0	0.00
Result quality :	Good				

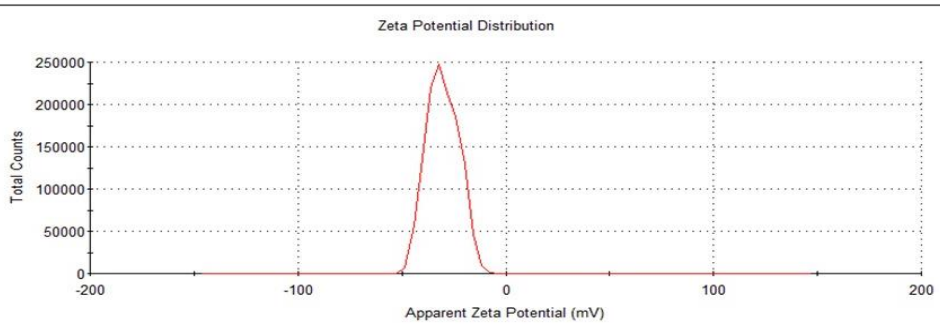


Fig. 8: Determination of Zeta Potential.

5.5 SEM Analysis of Formulated Nanosuspension

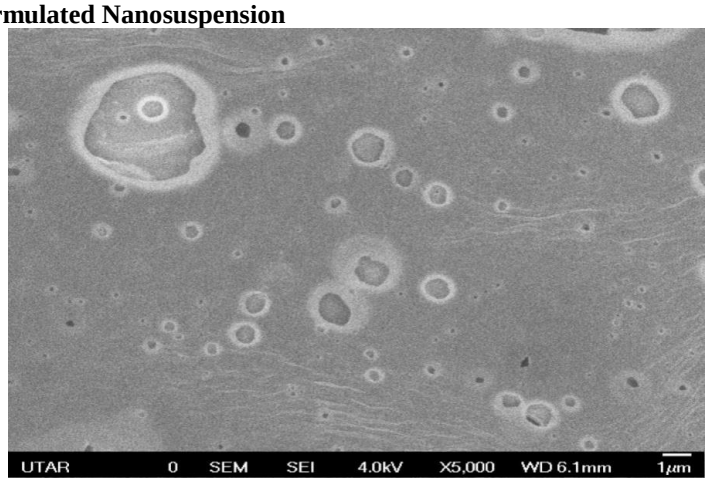


Fig. 9: SEM Analysis of Formulated Nanosuspension.

5.6 In-Vitro Drug Release Study

In-vitro drug release study was performed using dialysis bag and the results has shown 95 % of drug release within 1 Hour. By considering this parameter, the formulation has proved that it has adequate drug release when it is administered in the form of nanosuspension.

6. CONCLUSION

We concluded the nano-suspension formulation of herbal extracts from *Garcinia indica* holds great promising potential due to its enhanced bioavailability and therapeutic efficacy due to its improved solubility, increased stability and dissolution properties. The reduced particle size achieved through Nano sizing increases the surface area, leading to better absorption and potentially faster onset of action. However, further research is needed to assess stability, long-term effects and scalability of the Nano suspension, ensuring its viability for pharmaceutical and therapeutic application.

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