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FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLES OF *CASSIA ALATA* EXTRACT FOR MANAGEMENT OF WOUND

Mohit Khare, *Vinod K Dhote, Kanika Dhote and Surendra Jain

Truba Institute of Pharmacy Bhopal-462038.

***Corresponding Author: Prof. Vinod K Dhote**
Truba Institute of Pharmacy Bhopal-462038.
Email Id:

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ABSTRACT

The objective of the present research was to investigate the wound-healing potency of solid lipid nano particles of *cassia alata*extract. Preliminary phytochemical screening revealed the presence of various active constituents in the methanolic extract, including alkaloids, glycosides, carbohydrates, flavonoids, and tannins, which are known for their pharmacological benefits. These findings justified the use of the methanolic extract for SLN formulation. The methanolic extract demonstrated a higher percentage yield and richer phytochemical profile compared to petroleum ether extract, making it more suitable for nanoparticle preparation. The extraction process using methanol and petroleum ether yielded 2.26% and 0.786% respectively, confirming methanol as a more efficient solvent for extracting bioactive compounds. Among the five formulations, SLN3 and SLN4 exhibited desirable particle size and PDI values, essential for effective drug delivery. SLN4, with the smallest particle size (97.23 nm), and SLN3, with the lowest PDI (0.212), showed excellent potential in terms of particle uniformity and bioavailability. Particle size analysis showed that SLN4 had the smallest particle size (97.23 nm), followed by SLN3 (142.51 nm), with SLN3 having the lowest polydispersity index (PDI = 0.212), indicating high uniformity and stability. Zeta potential values ranged from -8.7 mV to 19.4 mV, suggesting acceptable colloidal stability across all formulations. In conclusion, the optimized formulations, particularly SLN3 and SLN4, hold potential for further in vivo studies and future development into stable, effective herbal-based nanomedicines for treating infections, inflammation, and related conditions.

KEYWORDS: *Solid lipid nanoparticles, cassia alata*, Wound healing, Antioxidant, Zeta potential, Soxhlet extraction, Phytochemical analysis.

1. INTRODUCTION

Cassia alata is a traditional herb belonging to the Fabaceae family. Various parts of the plant have been used traditionally to treat skin diseases (including eczema, ringworm, and itching), hepatitis, jaundice, and gastrointestinal issues (including constipation, food poisoning, and dysentery) **(Chew et al., 2022).** *C. alata* has been traditionally used for treating skin diseases and has shown remarkable potential as a wound healing agent.

SLNs are submicron size carriers ranging from 50–1000 nm and are made up of lipids that are biocompatible and biodegradable. These lipids are capable of incorporating both lipophilic and hydrophilic drugs. SLNs have been perceived as excellent carriers for the delivery of several drugs like antidepressants, anticancer agents, antioxidants and wound healing**(Ghaffari *et al.*, 2018).** SLNs are particularly useful in enhancing the bioavailability of drugs that are used in treating CNS disorders **(Ravi *et al.*, 2014).**

Effective wound care and management relies on mainly on the development of novel and effective formulations. Chronic wound care management will continue to be a focused area of research **(Rajendran *et al.*, 2018).** The process of wound healing involves a series of events leading to the repair wound of injured tissues. Three phases associated with wound healing are: inflammatory phase, proliferative phase and remodelling phases. The process of wound healing may also be delayed by aerobic and anaerobic bacterial infections**(Landén *et al.*, 2016).**

This research to discuss and highlight the solid lipid nanoparticles (SLNs) of *Cassia alata* extract relevant wound healing mechanisms exhibited and its phytochemicals.

2. MATERIALS AND METHODS

2.1 Chemical

Triethanolamine was procured from Sigma-Aldrich Drugs Pvt Ltd. Glacial Acetic Acid, Nitroprusside,

Sodium Hydroxide and Ammonia was received as sample from Merck. Ethanol was acquired from Molychem. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Clorofiltind and himedia.

2.2 Plant collection

Cassia alata, a therapeutic herb (plant), was collected. Plant parts (leaves) were cleaned, then dried for three days at room temperature in the shade before being dried completely at 45°C in an oven. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant - Medicinal plant *Cassia alata* was authenticated by a plant taxonomist in order to confirm its identity and purity.

2.3 Extraction Method

Using Soxhlet apparatus and the continuous hot percolation process, plant material was extracted for the current experiment. Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Materialused}} \times 100$$

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use **(Rifkowaty and Wardanu 2021).**

2.4 Methodology of Phytochemical Screening

The following steps were taken in order to employ standard phytochemical screening methods to show which plant metabolites were present in different extracts of Cassia alata leaves (Sahaet al., 2020).

2.4.1 Total phenols content

Amount of *Cassia alata*extract was dissolved in the distilled water. Add to it a few drops of dilute solution of ferric chloride. Formation of dark blue colour indicated the presence of tannins. amount of *Cassia alata*extract was dissolved in the distilled water.2ml of 1% gelatin solution containing 10% sodium chloride were added. Presence of phenolic compounds indicated by the development of white precipitate. Few amount of *Cassia alata*extract was dissolved in a test tube along with distilled water; to it few drops of lead acetate solution were added. Formation of white precipitate indicates presence of phenolic compounds.

2.4.2 Total flavonoid content

The *Cassia alata*extract was dissolved in 5ml of alcohol (95%) and treated with few drops of con. HCl and 0.5 gm of magnesium metal. Development of a pink colour within a minute indicated the presence of flavonoids.

2.5 Formulation of solid lipid Nanoparticles

Solid lipid Nanoparticlesformulation was made using the solvent evaporation technique.In brief, 20 milliliters of chloroform was used to dissolve the required amount of extract and compritol. It was added into 200 ml of sodium lauryl sulphate aqueous solution while homogenizing at 600 rpm for 10 min and at the same time, stirred by mechanical mixer at 300 rpm to prevent any dead zone of unhomogenized area in the mixing beaker. After that, it was stirred mechanically at for 2 h during that period all the solvent will evaporate leaving solid residue of Nanoparticles. The Nanoparticles was retrieved by centrifugation for 1 h and washed twice with deionized water to remove surfactant. Before centrifugation, 1 ml sample was withdrawn for size distribution measurements. The obtained freeze-dried products were kept in refrigerator until further analysis **(Rahman *et al.*, 2010)**.

Table 1: Ingredients used in Solid lipid Nanoparticles formulation.

Ingredients	SLN 1	SLN2	SLN3	SLN4	SLN5
Extract (mg)	200	**200**	200	200	200
Compritol (Lipid) (mg)	100	**150**	200	250	300
Sodium lauryl sulphate (Surfactant) (%)	0.2	**0.2**	0.2	0.2	0.2
Stirring time (Min.)	10	**15**	20	25	30
chloroform (ml)(Solvent)	20	**20**	20	20	20
Water	q.s	**q.s**	q.s	q.s	q.s

2.6 Evaluation parameter of Solid lipid Nanoparticles formulation

2.6.1 Particle size

The Malvern Zeta sizer (Malvern Instruments) was used to measure the size and zeta potential of the nanoparticles.(**Balla *et al.*, 2020).**

2.6.2 Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the optimized extract loaded solid lipid Nanoparticles were coated with a thin layer (2–20 nm) of metal (s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pre-treated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons.

From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography **(Sarangi *et al.*, 2018).**

2.7 Stability study determination

The extract loaded solid lipid nanoparticles formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing ($25^0C \pm 2^0C$ and 60 ± 5% RH) and ($40^0C \pm 2^0C$ and 70 ±5% RH) for 3 months. The formulation was checked for evaluation parameter particle size and zeta potential studies at the interval of 30, 45, 60, 90 days (3 month) months. The formulation was tested for stability under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines. Formulation was analyzed for the change in evaluation parameter like particle size and zeta potential **(Narayan and Choudhary 2017).**

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Cassia alata*is shown in Table: 2.

Table 2: Percentage Yield of crude extracts of *Cassia alata*extract.

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Cassia alata*	Pet ether	300	2.36	0.786%
2		Methanol	279	6.10	2.26%

3.2 Preliminary Phytochemical study

Table 3: Phytochemical testing of extract.

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Present	Present
2.2	Legal's test	Present	Present
2.3	Killer-Killiani test	Present	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Present	Absent
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Present	Absent
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Absent
8.2	Libbermann-Burchard's test	Present	Absent

3.3 Characterization of solid lipid nanoparticles

3.3.1 Particle size determination

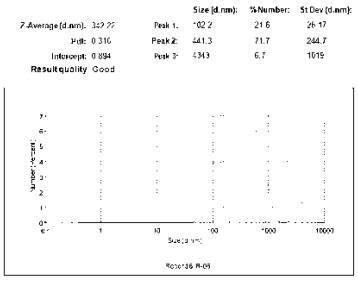

Figure 1: Particle size (SLN 1)

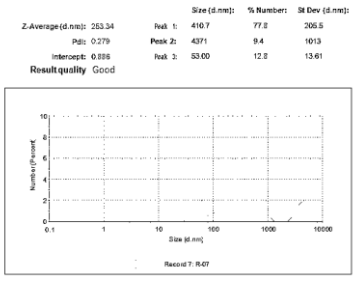

Figure 2: Particle size (SLN 2)

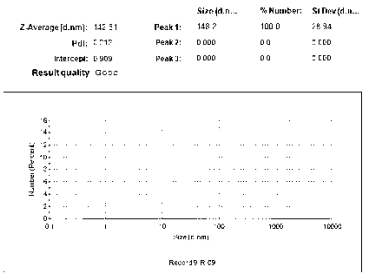

Figure 3: Particle size (SLN 3)

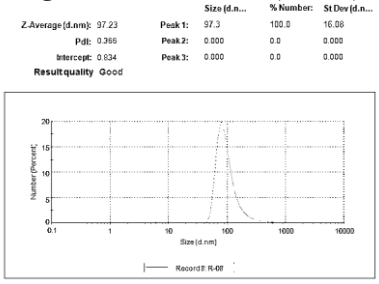

Figure 4: Particle size (SLN 4)

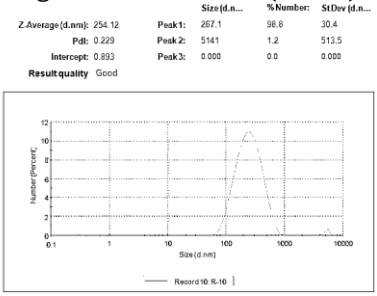

Figure 5: Particle size (SLN 5)

Table 4: Result of Particle size of all formulations.

S. No	Formulations	Particle size (nm)	PDI Value
1.	SLN 1	342.22 nm	0.316
2.	SLN2	253.34 nm	0.279
3.	SLN3	142.51 nm	0.212
4.	SLN**4**	**97.23 nm**	**0.366**
5.	SLN5	254.12 nm	0.229

DISCUSSION

The particle size and PDI results of the SLN formulations are critical indicators of their stability and drug delivery efficiency. Among the five formulations, SLN4 showed the smallest particle size (97.23 nm), ideal for enhanced cellular uptake and bioavailability, though its PDI (0.366) indicated moderate uniformity.

3.3.2 Zeta potential determination

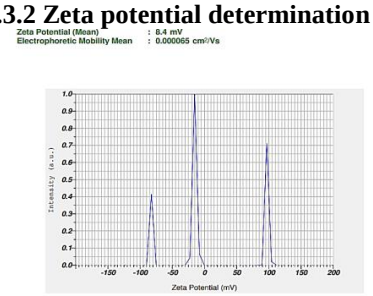

Figure 6: Zeta potential (SLN 1)

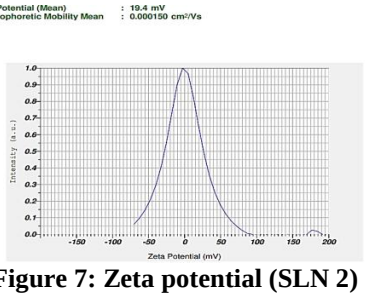

Figure 7: Zeta potential (SLN 2)

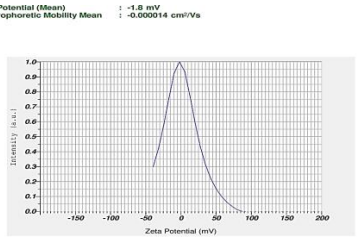

Figure 8: Zeta potential (SLN 3)

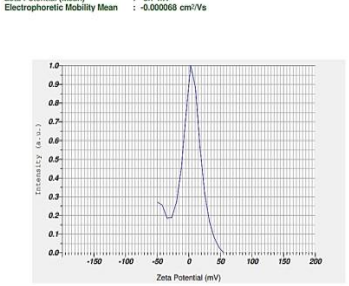

Figure 9: Zeta potential (SLN 4)

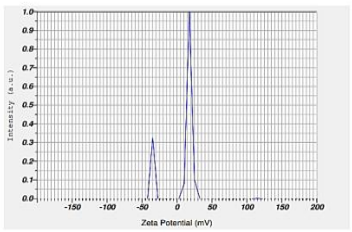

Figure 10: Zeta potential (SLN 5)

Table 5: Result of Zeta potential of all formulations.

S.No	Formulation	Zeta potential
1	SLN 1	8.4 mV
2	SLN 2	19.4 mV
3	SLN 3	-1.8 mV
4	**SLN 4**	**-8.7 mV**
5	SLN 5	10.6 mV

4. DISCUSSION

Zeta potential was found to be all formulation range -1.8 to 10.6 mV with peak area of 100% intensity. Results show in above table 5.

3.3.3 SEM analysis

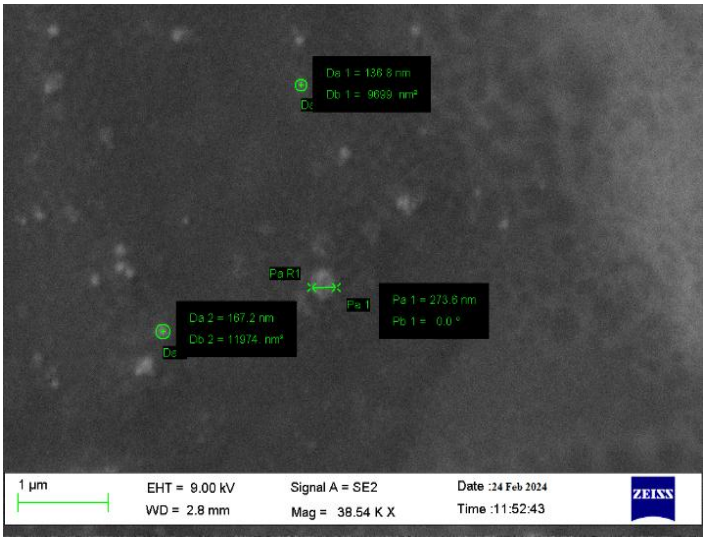

Figure 9: SEM (Scanning Electron Microscopy).

Scanning electron micrograph of the prepared nanoparticles at 38.54 KX magnification showed that the nanoparticles were porous with a smooth surface morphology and spherical shape.

3.4 Stability study

Table 6: Stability Study of Nanoparticle F4 formulation.

S.No	Time (Days)	25^0C±2 ^{0}C and 60 ± 5% RH		40^0C±2 ^{0}C and 70 ±5% RH	
		Particle size (nm)	Zeta potential (mV)	Particle size (nm)	Zeta potential (mV)
1.	0	97.23	-8.7	97.23	-8.7
2.	30	97.07	-8.3	98.03	-8.8
3.	45	96.83	-8.4	98.11	-8.7
3.	60	96.96	-8.9	97.18	-8.7
4.	90	97.01	-8..9	97.01	-8.9

DISCUSSION

According to ICH guideline [Q1A(R2)Guideline] Solid lipid nanoparticle formulation were found to be stable, both physically and chemically, for a period of 3 months at accelerated stability conditions (25^0C±2 ^{0}C and 60 ± 5% RH) and (40^0C±2 ^{0}C and 70 ±5% RH).

5. CONCLUSION

The formulation of solid lipid nanoparticles loaded with *Cassia alata* extract was successfully developed and evaluated. The methanolic extract demonstrated a higher percentage yield and richer phytochemical profile compared to petroleum ether extract, making it more suitable for nanoparticle preparation. Among the five formulations, SLN3 and SLN4 exhibited desirable particle size and PDI values, essential for effective drug delivery. SLN4, with the smallest particle size (97.23 nm), and SLN3, with the lowest PDI (0.212), showed excellent potential in terms of particle uniformity and bioavailability. Zeta potential studies indicated that all formulations maintained sufficient surface charge to ensure colloidal stability. Furthermore, SEM imaging confirmed the spherical and smooth morphology of the SLNs, and the 90-day stability study of SLN4 affirmed its structural and chemical integrity under both standard and accelerated conditions.

These findings suggest that SLNs are a promising delivery system for enhancing the therapeutic efficiency of *Cassia alata* extract. The optimized formulations, particularly SLN3 and SLN4, hold potential for further in vivo studies and future development into stable, effective herbal-based nanomedicines for treating infections, inflammation, and related conditions.

6. REFERENCES

1. Chew, Y. L., Khor, M. A., Xu, Z., Lee, S. K., Keng, J. W., Sang, S. H., ... & Ming, L. C. *Cassia alata*, *Coriandrumsativum*, Curcuma longa and *Azadirachtaindica*: food ingredients as complementary and alternative therapies for atopic dermatitis-a comprehensive review. *Molecules*, 2022; *27*(17): 5475.
2. Ghaffari, S., Alihosseini, F., Sorkhabadi, S. M. R., Bidgoli, S. A., Mousavi, S. E., Haghighat, S., ... & Kianvash, N. Nanotechnology in wound healing; semisolid dosage forms containing curcumin-ampicillin solid lipid nanoparticles, in-vitro, ex-vivo and in-vivo characteristics. *Advanced pharmaceutical bulletin*, 2018; *8*(3): 395.
3. Ravi, P. R., Aditya, N., Kathuria, H., Malekar, S., & Vats, R. Lipid nanoparticles for oral delivery of raloxifene: optimization, stability, in vivo evaluation and uptake mechanism. *European Journal of Pharmaceutics and Biopharmaceutics*, 2014; *87*(1): 114-124.
4. Rajendran, N. K., Kumar, S. S. D., Houreld, N. N., &Abrahamse, H. A review on nanoparticle based treatment for wound healing. *Journal of Drug Delivery Science and Technology*, 2018; *44:* 421-430.
5. Landén, N. X., Li, D., & Ståhle, M. Transition from inflammation to proliferation: a critical step during wound healing. *Cellular and Molecular Life Sciences*, 2016; *73:* 3861-3885.
6. Rifkowaty, E. E., & Wardanu, A. The Physicochemical Characteristic and Inhibition Zone of Eschericia coli in Ketepeng (*Cassia alata* L.) Leaf Extract Transparent Soap. In *Proceedings of the 4th International Conference on Applied Science and Technology on Engineering Science*, 2021; 786-793.
7. Saha, K., Proma, R. Z., & Khan, N. Phytochemical screening of plant extracts and GC-MS analysis of n-hexane extract of the leaves of Cassia alata Linn. *The Journal of Phytopharmacology*, 2020; *9*(5): 342-347.
8. Rahman, Z., Zidan, A. S., & Khan, M. A. Non-destructive methods of characterization of risperidone solid lipid Nanoparticles. *European journal of pharmaceutics and biopharmaceutics*, 2010; 76(1): 127-137.
9. Sarangi, B., Jana, U., Sahoo, J., Mohanta, G. P., & Manna, P. K. Systematic approach for the formulation and optimization of atorvastatin loaded solid lipid nanoparticles using response surface methodology. *Biomedical Microdevices*, 2018; *20:* 1-12.
10. Narayan, S., & Choudhary, M. A review on stability studies of pharmaceutical products. *International Journal of Applied Pharmaceutical and Biological Research*, 2017; *2*(3): 67-75.