Supplementary Information

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Cheilocostus speciosus extract-assisted and naringenin-encapsulated poly-€-caprolactone nanoparticles: evaluation of anti-proliferative activities

Bijuli Rabha[1], Kaushik Kumar Bharadwaj[1], Nizum Boro[1], Arabinda Ghosh[2], Sonit Kumar Gogoi[3] Rajender S. Varma[4], Debabrat Baishya[1]*

[1]*Department of Bioengineering & Technology, GUIST, Gauhati University Guwahati-781014, Assam, India*

[2]*Microbiology Division, Department of Botany, Gauhati University, Guwahati -781014, Assam, India*

[3]*Department of Chemistry, Gauhati University, Guwahati-781014, Assam, India*

[4]Regional Center of Advanced Technologies and Materials, Palacky University, Š lechtitelů 27, 783 71, Olomouc, Czech Republic.

Corresponding Author

Dr. Debabrat Baishya, Assistant Professor and Head i/c

Department of Bioengineering and Technology

Gauhati University, Guwahati-781014, Assam, India

Mobile: +91 9864012267

E-mail address:drdbaishya@gmail.com; dbaishya@gauhati.ac.in

ORCID: 0000-0002-29298539

Scopus ID: 14051603000

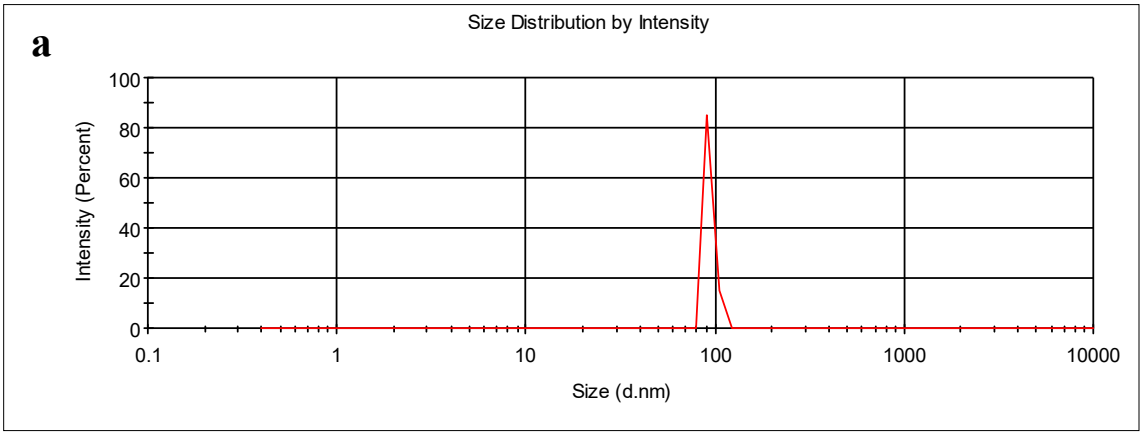
Researcher ID: H-5394-2017

Table. S1 Drug loading content (DLC), encapsulation efficiency (EE), and yield of production (YP) of synthesized nanoparticles.

Sample	DLC (%)	EE (%)	YP (%)
PCL-PE-NPs	------	------	47.56±3.42
PCL-PE-N-NPs	10.58±0.13	75.56±0.93	38.49±1.33

Table. S2 Mathematical models for naringenin release kinetics of PCL-PE-N- NPs

Model name	R^2	N
Zero-order	0.845	…..
First-order	0.561	…..
Higuchi model	0.945	…..
Korsmeyer-Peppas model	0.943	0.32



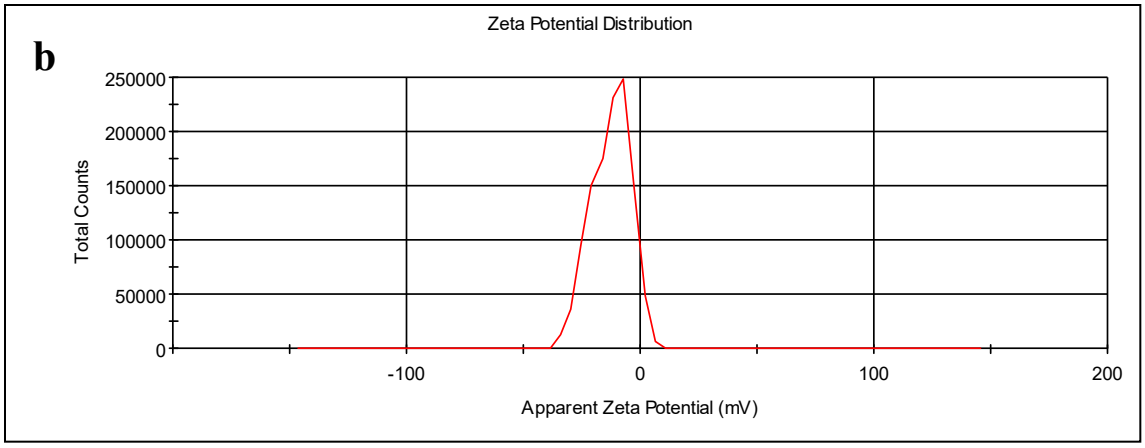


Figure S1. (a) Size distribution as determined by DLS , (b) Zeta potential Distribution of PCL-PE-NPs

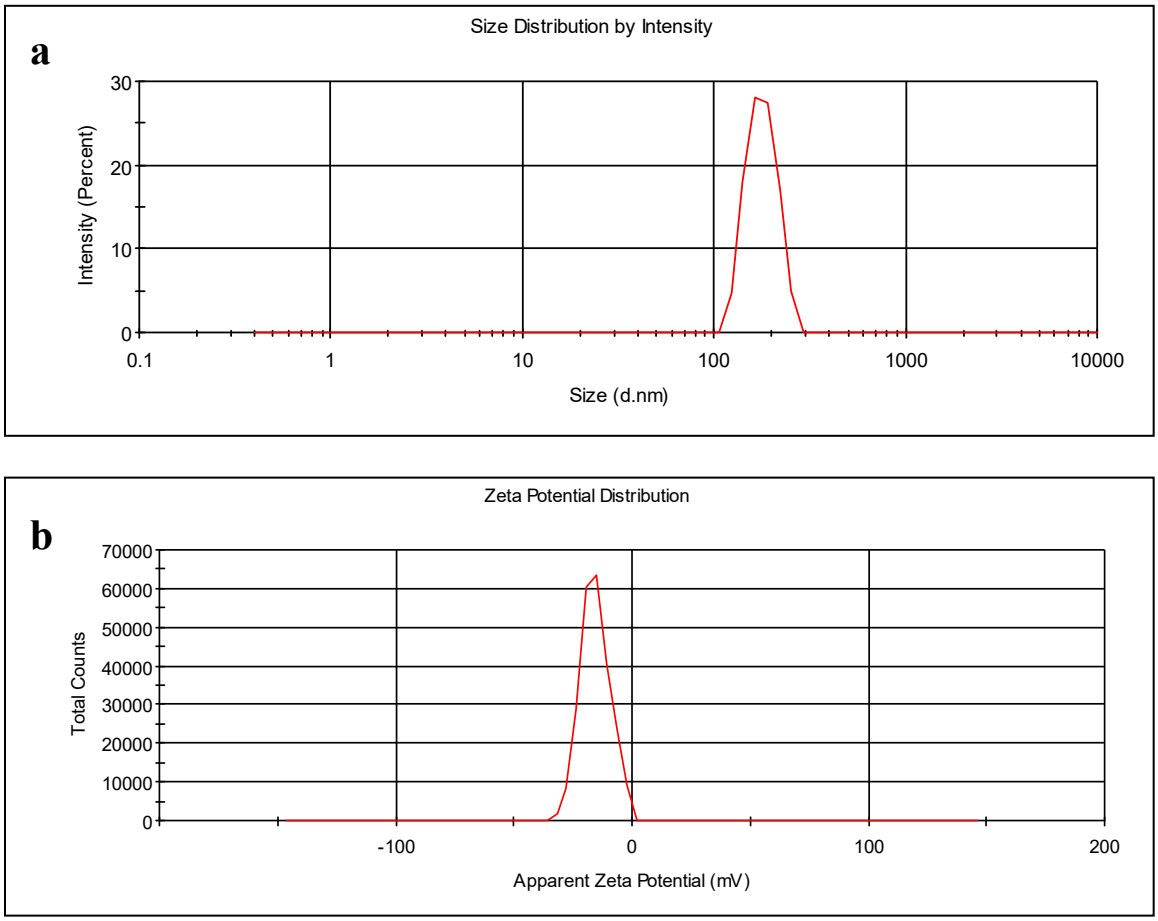
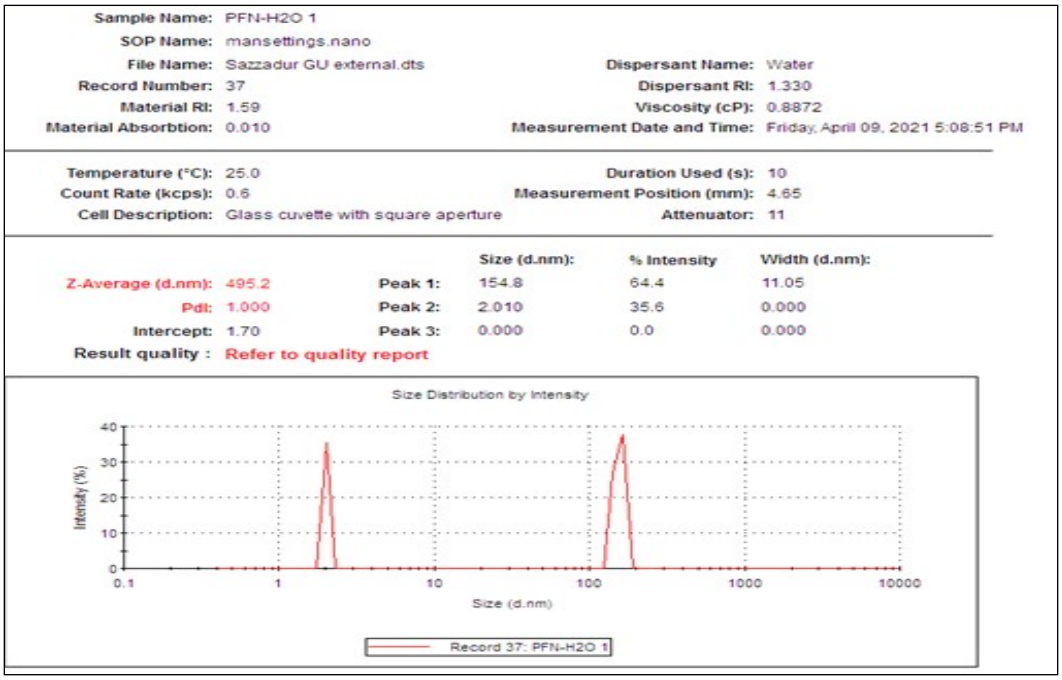


Figure S2. (a) Size distribution as determined by DLS , (b) Zeta potential Distribution of PCL-PE-N-NPs

Conventional synthesis of PCL nanoparticles by using PLuronic F68

(a)



(b)

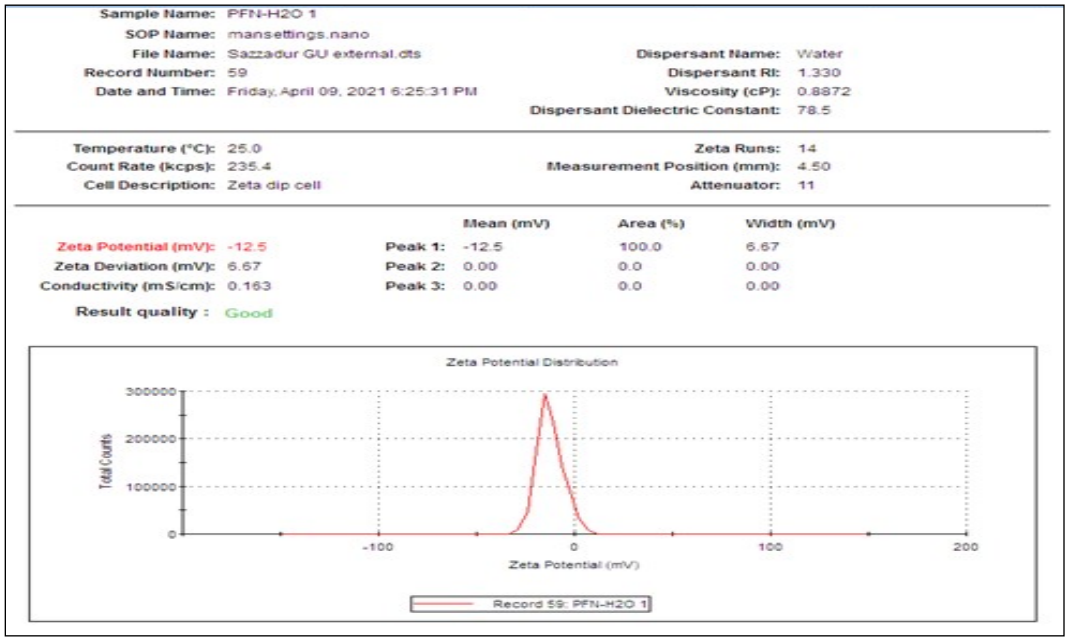


Figure S3. (a) Size distribution as determined by DLS , (b) Zeta potential Distribution of PCL-F68-N-NPs

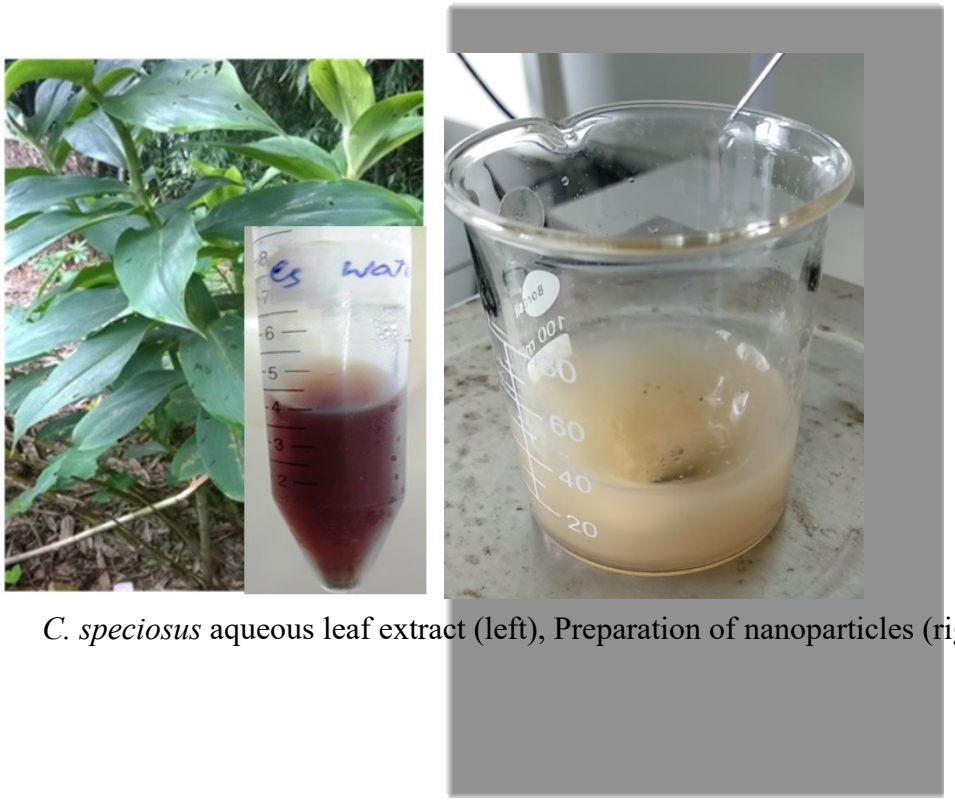


Figure S4. *C. speciosus* aqueous leaf extract (left), Preparation of nanoparticles (right)

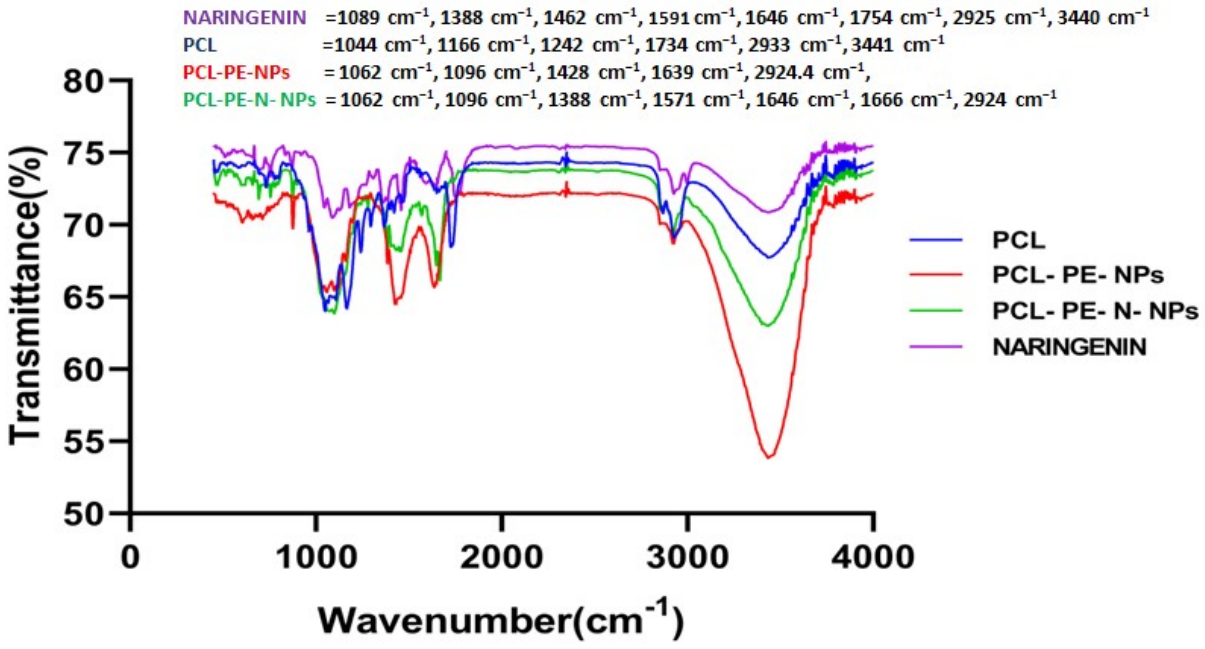


Figure S5. FTIR spectra of PCL, PCL-PE-NPs, PCL-PE-N-NPs and pure free naringenin

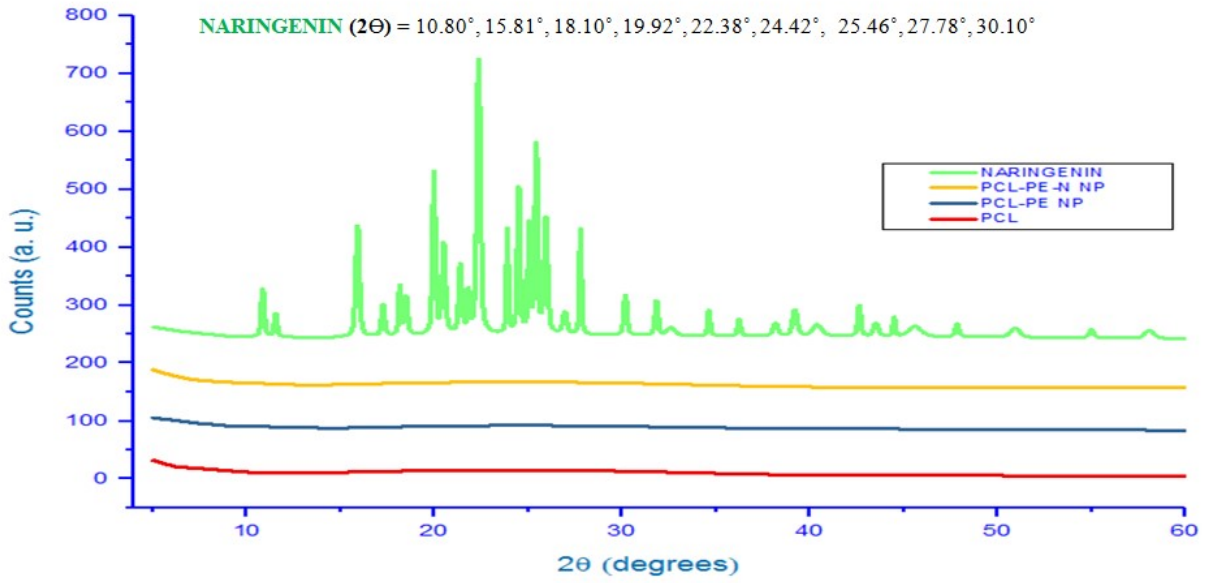


Figure S6. XRD pattern of PCL, PCL-PE-NPs, PCL-PE-N-NPs and pure free naringenin.

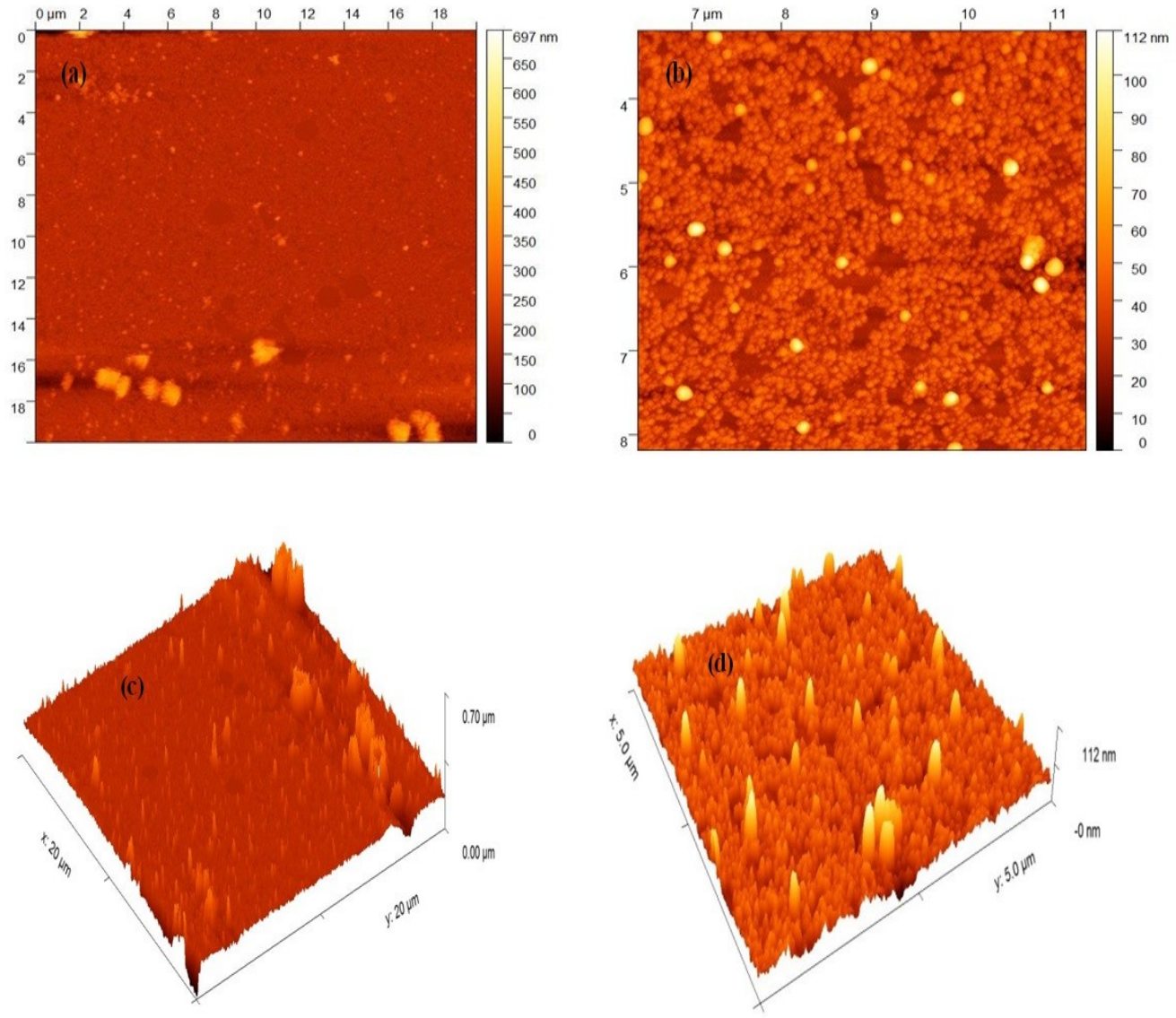


Figure S7. AFM 2D image (a) PCL-PE-NPs, (b) PCL-PE-N-NPs; and AFM 3D image (c) PCL-PE-NPs, (d) PCL-PE-N-NPs.

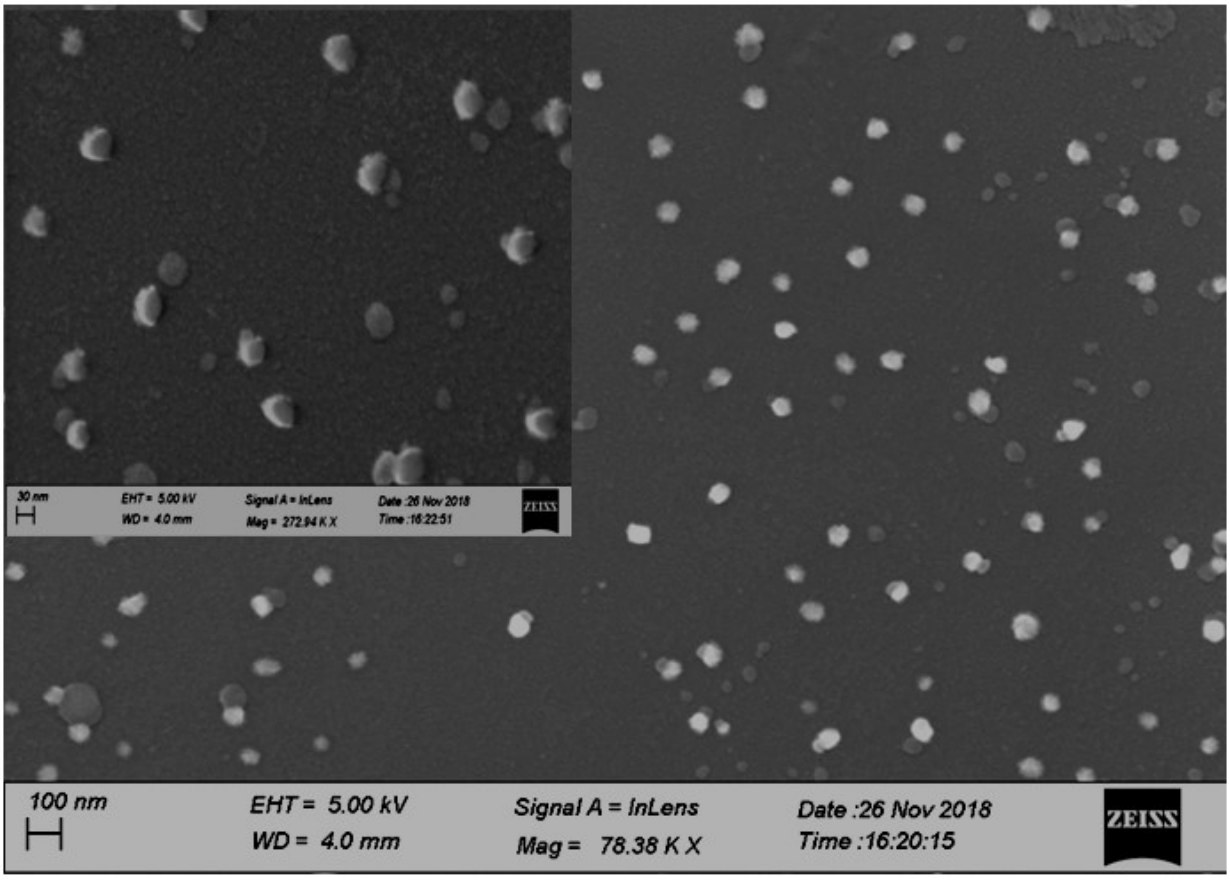


Figure S8. FESEM image PCL-PE-NPs.

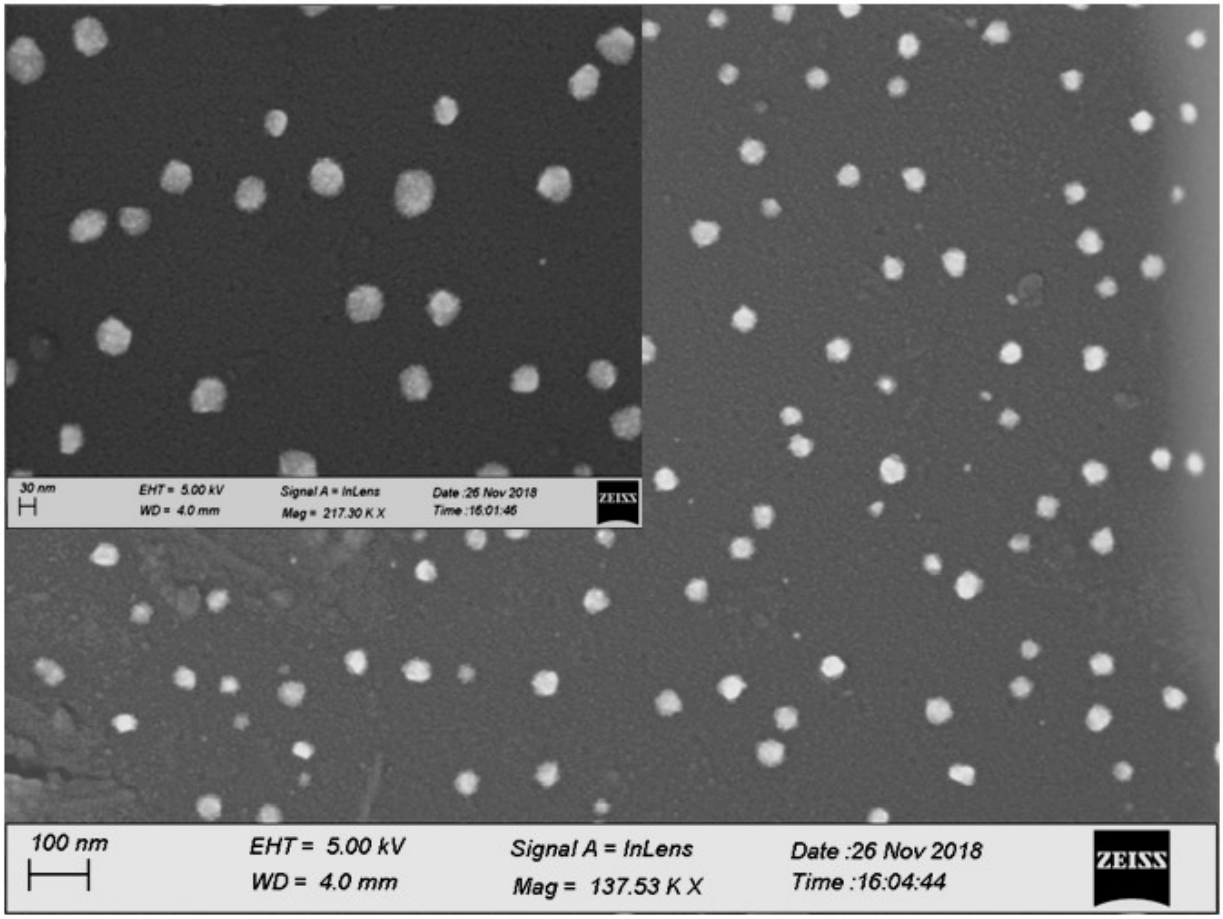
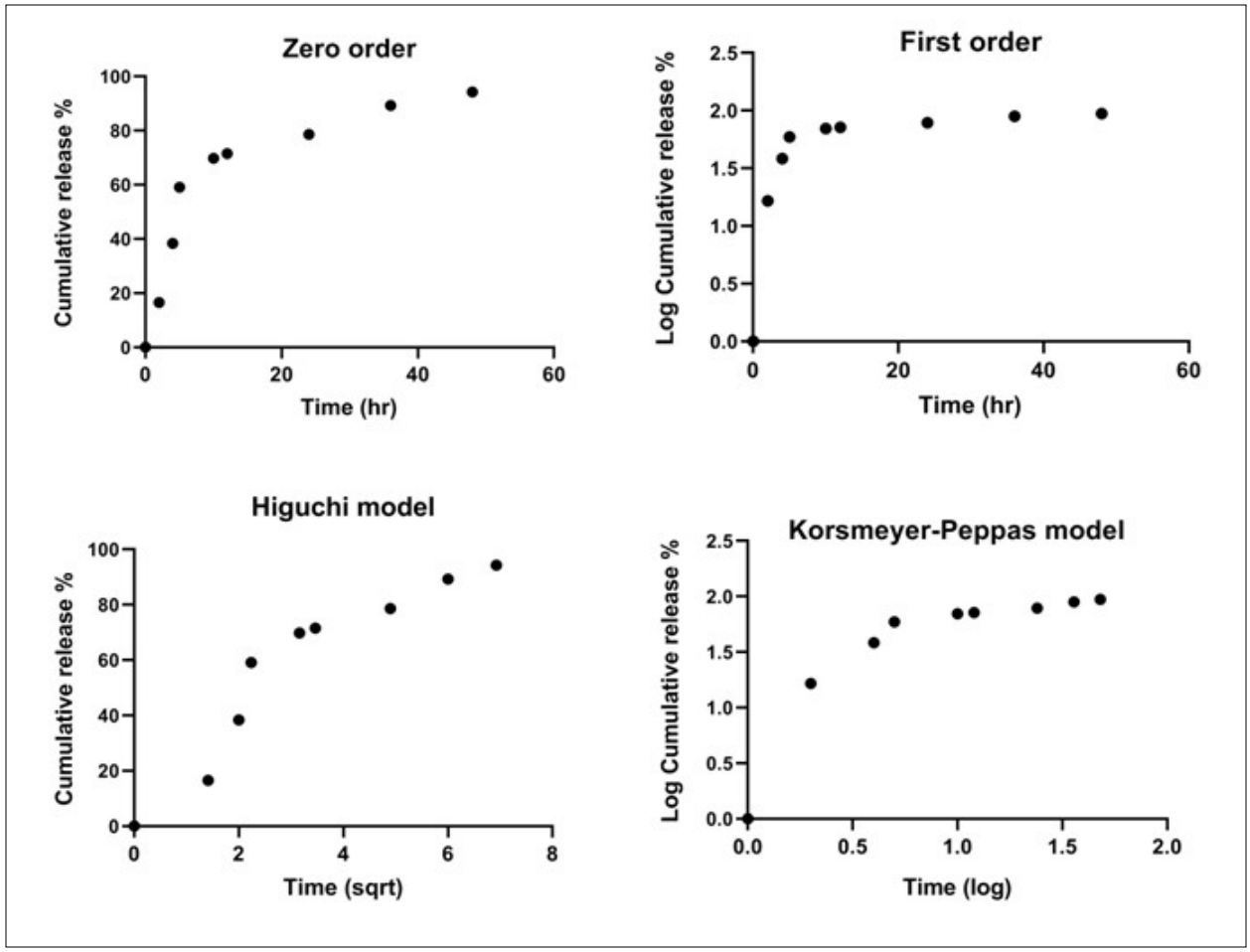


Figure S9. FESEM image PCL-PE-N-NPs.



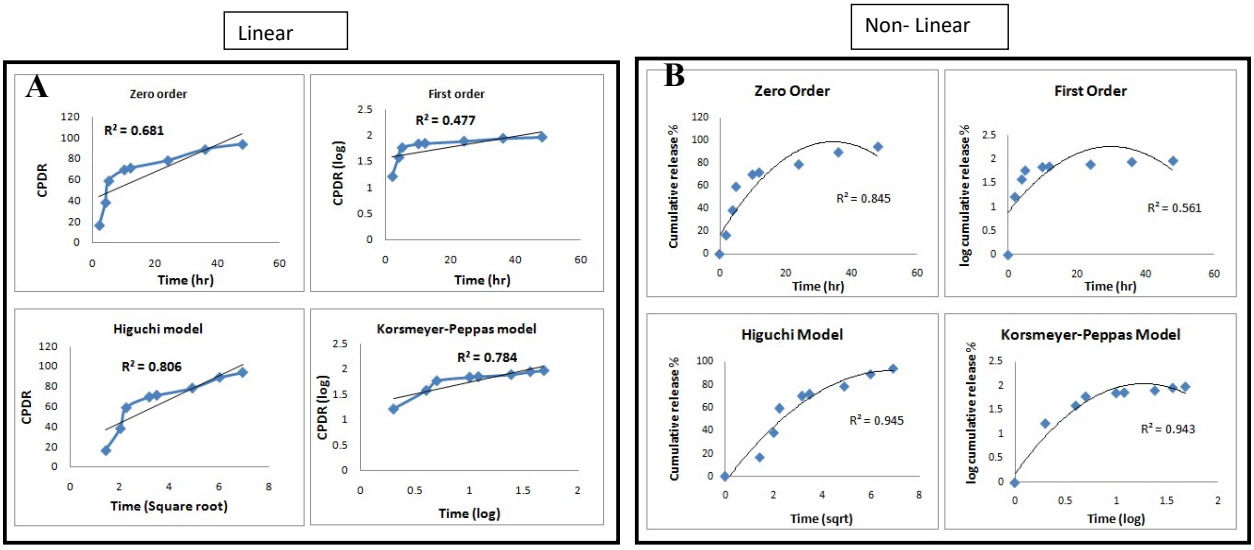


Figure S10. Evaluation of naringenin release from PCL-PE-N-NPs with various mathematical models and presentation of various linear and non-linear models fit.

Experiment sections

Preparation of *Cheilocostus speciosus* (CS) plant extracts (PE)

Plant samples were collected from local areas of Assam and authenticated in the herbarium from Department of Botany, Gauhati University (GUBH). Leaves were cleaned and dried at 25 ºC and ground to make a fine powder. Twenty grams of this powdered sample was dissolved in 200 mL of distilled water and kept for maceration for 24 h at 25 ºC. The samples were then filtered and diluted with deionized water (1:29 V/V) prior to its use for the synthesis of PCL nanoparticles.

Identification of saponin in *Cheilocostus speciosus* (CS) plant extracts (PE)

To identify the saponin in *Cheilocostus speciosus* leaf aqueous extract (PE), diosgenin was taken as standard compound and analyzed for its qualitative determination.

HPLC:

The qualitative analysis/detection of Sapogenin Diosgenin in the CS leaf extract was performed by HPLC method. The analysis was carried out using Agilent/1260 Infinity with a mobile phase containing acetonitrile: water (90: 10, v/v) at the flow rate of 1 mL/min and a temperature of 25 ^{0}C by injecting 20 µl of samples. Detection was performed at 202 nm.

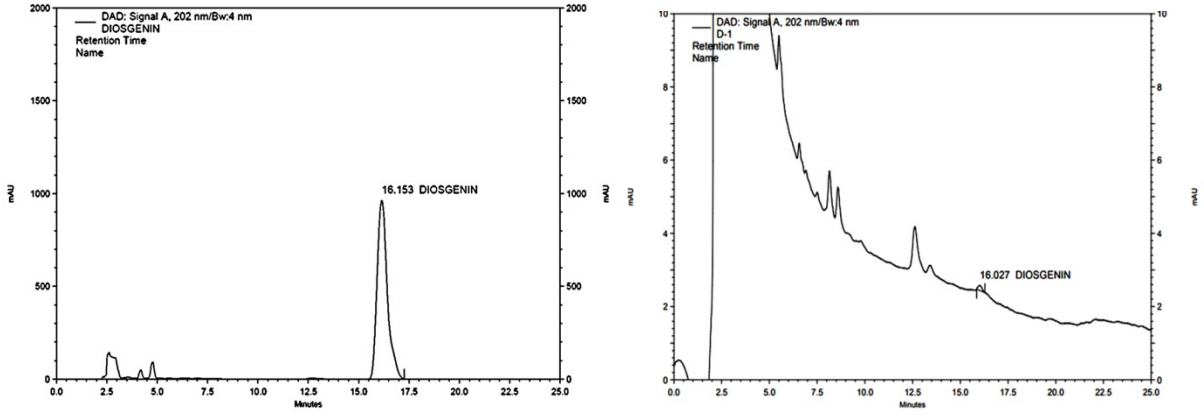


Figure S11. HPLC chromatogram. Standard Diosgenin (Left) and Diosgenin from *Chielocostus speciosus* leaf aqueous extract (Right).

The identification of diosgenin in aqueous plant extract was analyzed by HPLC method as is simple, precise, specific, sensitive and accurate.

The presence of steroidal saponin Diosgenin in the leaf aqueous extract was confirmed by comparison of their retention times and overlaying of ultraviolet spectra with standard

Diosgenin. The typical chromatogram showed retention time for standard Diosgenin at 16.153 minute. The aqueous extract showed retention time of 16.027 minute that determined the presence of Diosgenin.

Surface tension measurements

The relative Surface tension measurements of the *Cheilocostusspeciosus* leaf aqueous extract (PE) and non- ionic surfactant pluronic F-68 were carried out with stalagmometer by drop count method. All measurements were carried out thrice at 24 ± 0.1 C. The measured value of PE was compared with the surface tension obtained for synthetic surfactant pluronic F-68. The measurement was calculated using the following formula:

$$\gamma_{\text{sample}} = \frac{\rho\ sample\ X\ n1}{\rho\ water\ X\ n2}\ \text{X}\ \gamma_1$$

Where,

ρ = density
n1= Average number of drops of water
n2 = Average number of drops of sample
γ1= Surface tension of water

Table. S3 Surface tension measurements

Liquid	**Number of drops**	**ρ (g/mL)**	**γ (N/m)**
water	40	0.997	72.8
Pluronic F-68	56	1.022	53.15
PE	44	1.047	66.18

Surfactants play important role in formation of nanoparticles by reducing interfacial tension, Laplace pressure between both outward and inward side of droplet as well as minimize coalescence of new droplets. The relative surface tension measurement of our PE and synthetic surfactants in water is shown in above table. The reduction of surface tension by surfactants is governed by the adsorption at air-water interface. The standard surface tension of water at 24 ± 0.1 ˚C is 72.8 N/m. At 1% concentration of pluronic F-68 showed minimum surface tension of 53.15 N/m in comparison with 1:30 PE which also showed a lower surface tension of 66.18 N/m in relation to water. This proved that the aqueous leaf

extract possess the surfactant property which can be utilized in the formulation of nanoparticles. As it is already well known that the PE contains saponin which is naturally occurring bio-based surfactants.

Table. S4 A literature review of nanoparticle size, PDI, zeta potential, encapsulation and drug loading –efficiency, use of different surfactants as compared to our study.

Nanoparticle formulations	Surfactant	Size	PDI	ZETA POTENTIAL(mV)	EE	DLC	References
nar-gel-c-PCL NPs	Pluronic F127	213.7	0.27	20.7	86.75%	8.68%	1
NAR-SFNs 1:1		180.1 ± 2.6	0.22 ± 0.01	−30.5 ± 0.7	21.81 ± 0.30	21.82 ± 0.40	2
NAR-PCL-NP	Tween-80	222 ± 0.36 nm	0.077 ± 0.0011	−19.54 ± 0.015	75.8 ± 0.23	3.92 ± 0.012	3
CSDS-Nar		337.2	.355	−34.4 ± 7.45	94.32 ± 0.24	4.72 ± 0.01	4
PCL-F68-D-NPs	Pluronic f68	245 nm	.367	−11.5	80.8 ± 0.26	10.3 ± 0.31	5
NP-SFB-Ab	TPGS	99.1 nm	0.16	-16.6	76.3	9.5	6
PCL-PE-N-NPs	Aqueous extract	169.7 nm	0.23	−15.6	75.56%	10.58%	Our study

References

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