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Microencapsulation of bioactive leaf extracts of *Eucalyptus camaldulensis* by freeze drying technology using sodium alginate and sodium carboxymethyl cellulose as coating materials

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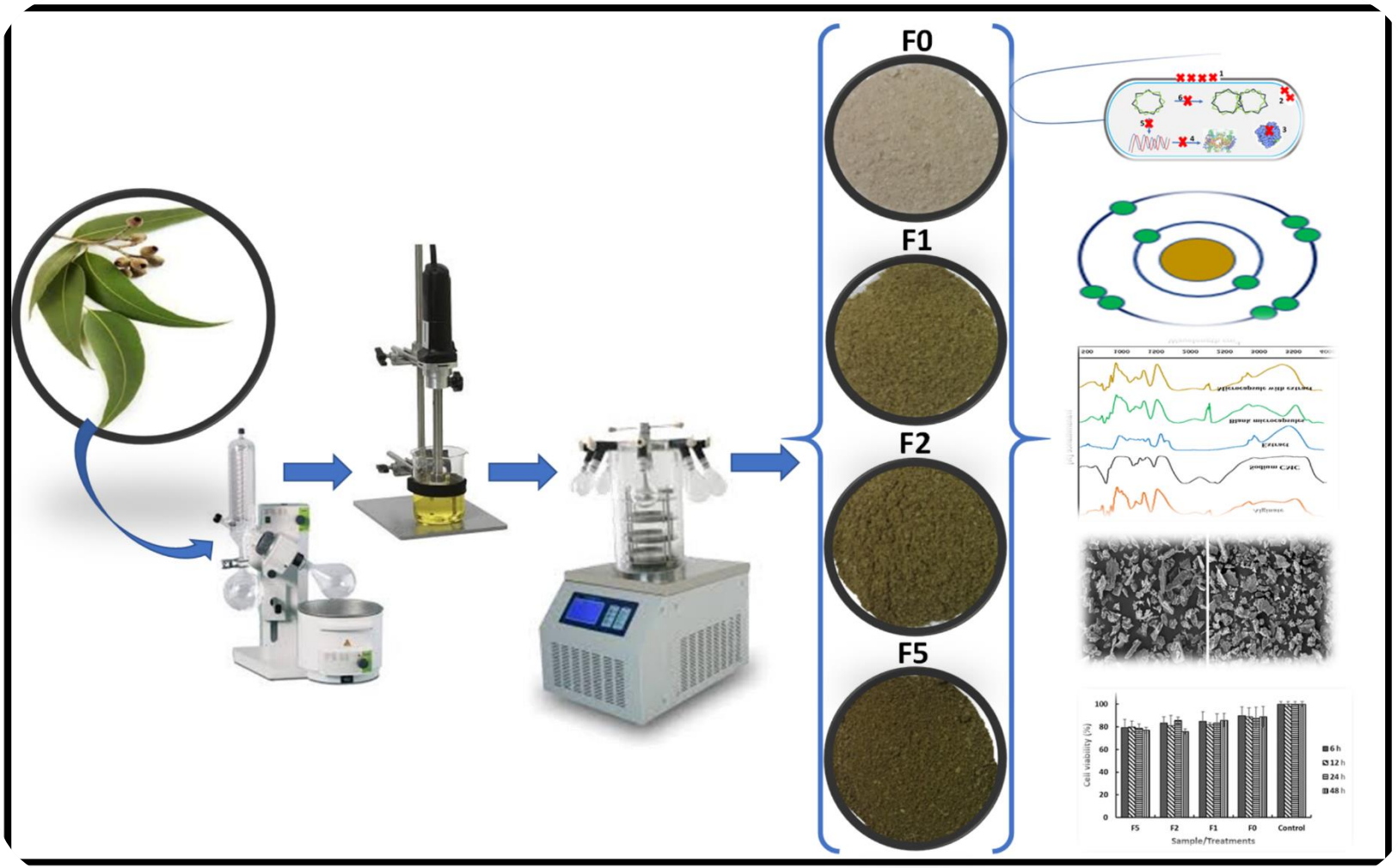


Abstract:

Bioactive crude ethanolic extracts of *Eucalyptus camaldulensis* was encapsulated with alginate–CMC using freeze drying technique. The microcapsules were characterized for particle size, morphology, physicochemical parameters such as solubility, swelling index, and micromeritics properties. FTIR was used to evaluate the interactions of the polymer and the extract. Antioxidant and antimicrobial activities of the microcapsules were also demonstrated. Results revealed and irregular shaped microparticles with mean diameter ranging from 6.7–26.6 µm. Zeta potential and polydispersity index ranged from -17.01–2.23 mV and 0.344–0.489 respectively. Percentage yield and encapsulation efficiency ranged between 70.4–81.5 % and 74.2±0.011 – 82.43±0.772 %. In addition, the microcapsules exhibited high swelling index with poor solubility. Antioxidant activity of the microcapsules evaluated using DPPH and ABTS assays increased with increase in the concentration of the extract. Minimum inhibitory and minimum bactericidal concentrations of the microcapsules against gram-positive foodborne pathogens ranged from 0.19–3.12 mg/mL and 0.19–12.25 mg/mL respectively. Moreover, the microcapsules at concentration of 1 mg/mL did not show cytotoxic effects on human colon cell CaCo-2. Alginate–CMC showed good encapsulation properties that preserved the bioactivity of the extract, thus might be employed for application of natural products in processing systems.

Keywords: ***Microencapsulation; Freeze–drying; Eucalyptus camaldulensis; Bioactive; Cytotoxicity***

Introduction

- Plants are excellent source of bioactive, natural and healthy compounds that could be used in product formulation
  - However, the inert nature of most of these bioactive compounds limits their usage
- Microencapsulation as a technique preserves plant component active compounds, resulting in prolonged activity, as well as regulated release
  - Alginate and carboxymethyl cellulose are both biocompatible polymers with wide usage in the food industry

- Eucalyptus a plant native to Australia and the Southeast Asia contains various phytochemicals and is a rich source of medicinal essential oil

- Leaf extracts of the plant have been reported to exhibit good antimicrobial and antioxidant activities

- Leaf extracts of the plant was microencapsulated by freeze-drying technique, characterized and assessed for bioactive activities

Results and discussion

TABLE 1

Formulations	Particle Size (µm)			
	D_{10}	D_{50}	D_{90}	Span
F0	3.07	6.70	14.63	1.73
F1	11.85	26.59	59.70	1.79
F2	4.99	10.03	20.15	1.51
F5	4.41	9.06	18.61	1.56

Table 1, shows the particle size of microcapsules reported based on equivalent sphere concept (D_{10}: 10th percentile of cumulated volume distribution, D_{50}: median particle diameter (50th percentile) of cumulated volume distribution and D_{90}: 90th percentile of cumulated volume distribution) and span.

The D_{50} values of the microcapsules ranged from 6.7–26.6 µm, with the blank microcapsules presenting the lowest particle size of 6.7 µm.

TABLE 2

Formulations	% Yield	Encapsulation efficiency (%)	Swelling index (%)	Polydispersity index	Zeta potential (mV)	Solubility (%)
F0	80.7	NA	84.0	0.450	-11.01	22.2±1.1
F1	76.5	74.2 ± 0.011	81.4	0.489	-17.01	22.1±1.6
F2	81.5	80.11 ± 0.008	71.8	0.344	2.23	19.9±0.1
F5	70.4	82.43 ± 0.772	54.4	0.370	-2.45	18.8±0.2

From Table 2, a high percentage yield (70.4–81.5%) was observed, demonstrating a minimal loss of material. Similarly, encapsulation of the extract using alginate–CMC demonstrated %EE of 74.2±0.011–82.43±0.772%, suggesting the formation of stable interactions between reactive sites of the polymers and the extract.

In addition, the alginate–CMC encapsulated extract showed a zeta potential of -11.01, -17.01, 2.23 and -2.45 for F0, F1, F2 and F5 respectively with , PDI ranging between 0.344–0.489, indicating a wide size dispersion.

TABLE 3

Isolates	MIC/MBC (mg/mL)				
	Blank	F1	F2	F5	Extract
Bacillus cereus	>24	0.39/1.56	0.19/0.39	0.19/0.19	0.064/0.128
Listeria monocytogenes	>24	1.56/6.25	0.39/6.25	0.19/0.78	0.128/0.512
Staphylococcus aureus	>24	3.12/12.25	1.56/6.25	0.39/1.56	0.128/0.256

Table 3, shows the antimicrobial activities of the encapsulated extracts of *E. camaldulensis* against foodborne pathogenic bacteria. The microencapsulated extracts demonstrated antimicrobial effects with minimum inhibitory concentrations and minimum bactericidal concentrations ranging from 0.19–3.12 and 0.19–12.25 mg/mL.

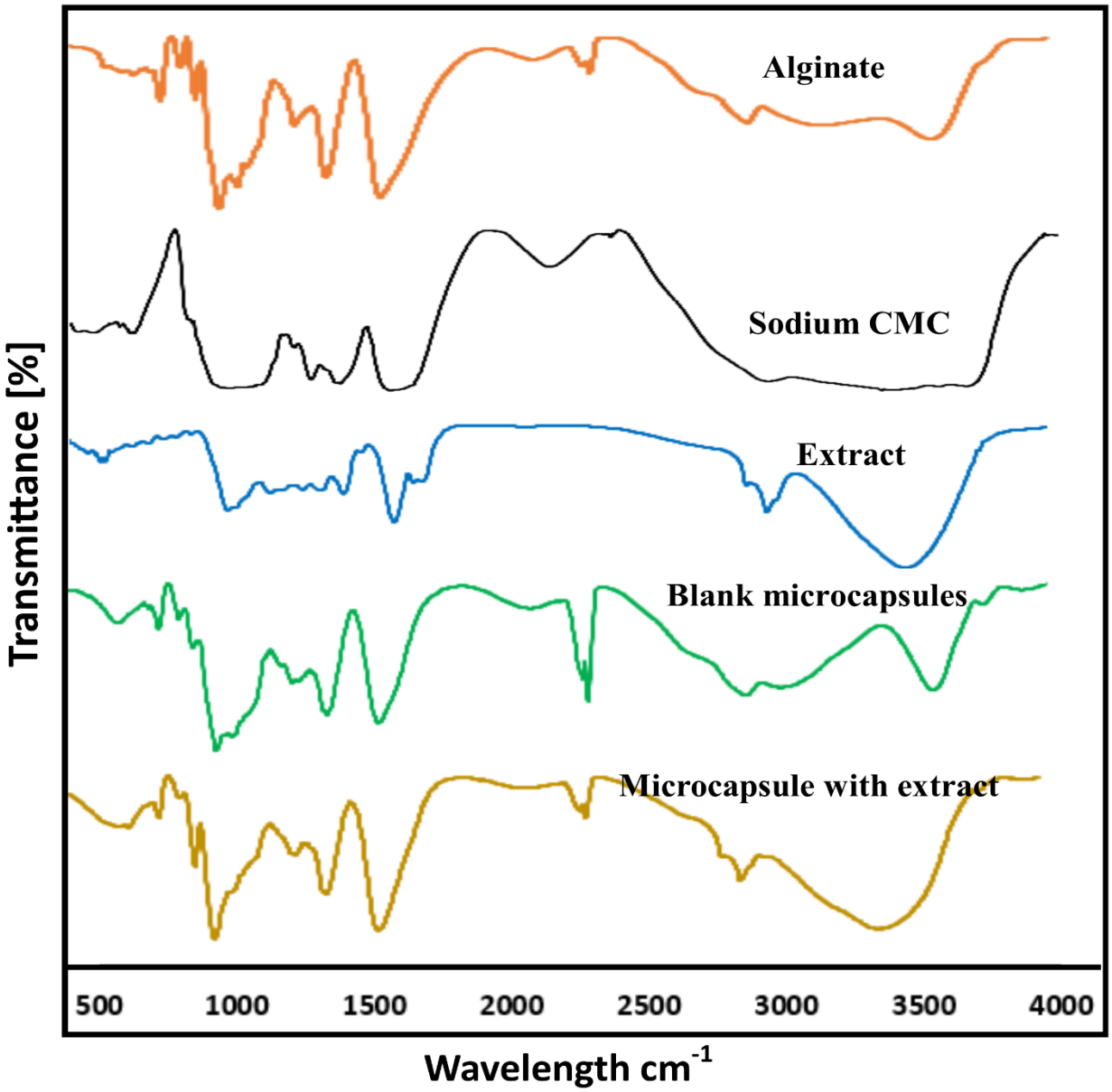


FIGURE 1

The FTIR spectra showing interactions at the C-H peak located at 2928 cm^{-1} and 2950 cm^{-1} in the extract and blank capsules, resulting in a minor shift to 2926 cm^{-1}.

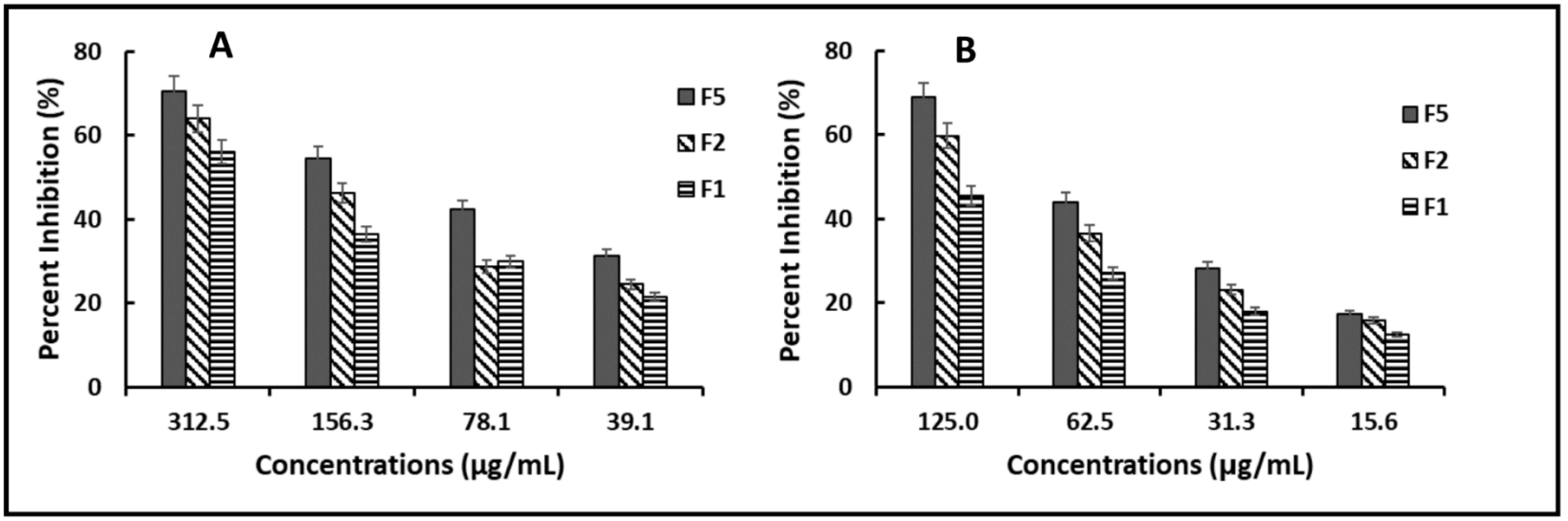
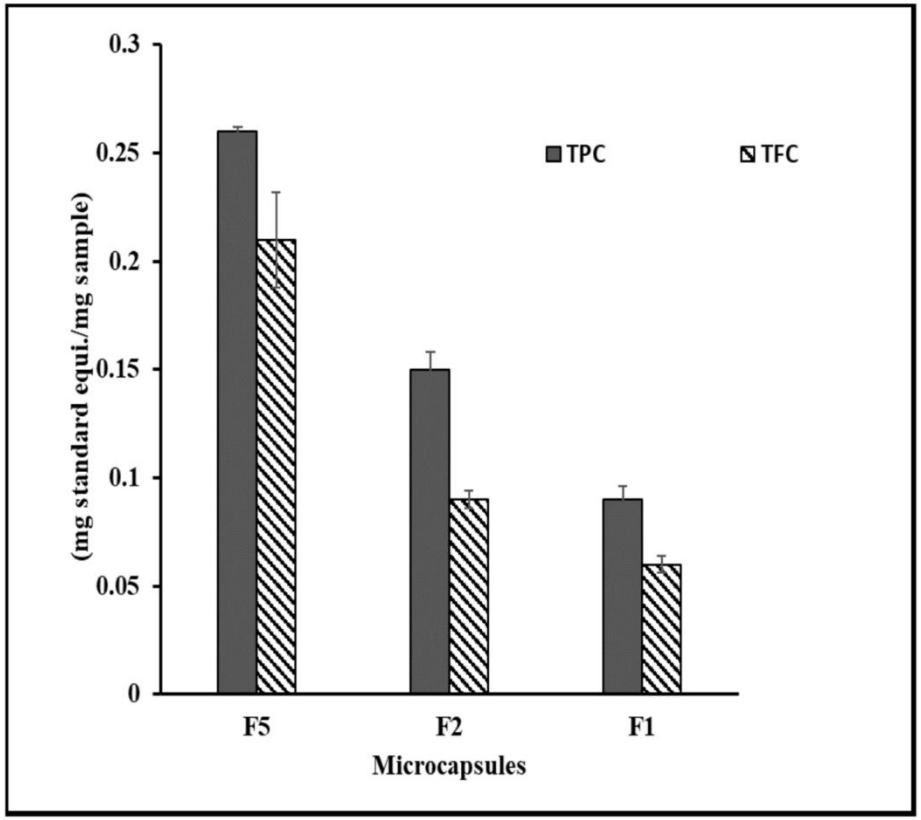


FIGURE 2

- The antioxidant activities of the microcapsules, demonstrated using the (A) DPPH and (B) ABTS assays. The capsules showed a concentration dependent inhibition with F5 showing the highest antioxidant properties and F1 the least antioxidant activity

FIGURE 3



The total phenolic and flavonoid contents of the microcapsules are presented in (Figure 3). The results indicate a TPC content of 0.26, 0.15, and 0.09 mg garlic acid equivalent/mg sample for formulation F5, F2 and F1 and a TFC of 0.21, 0.09 and 0.06 mg catechin equivalent/mg sample for F5, F2 and F1

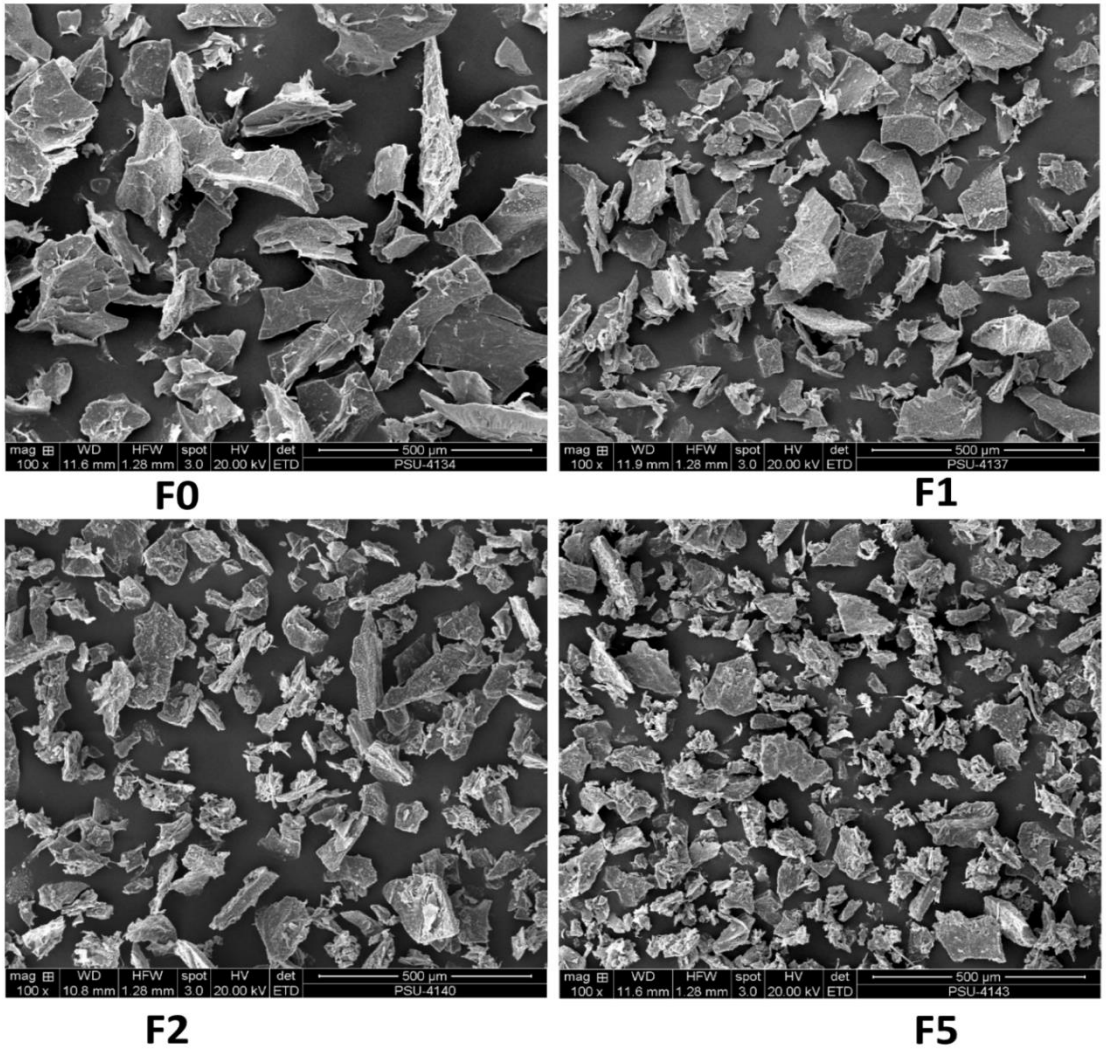
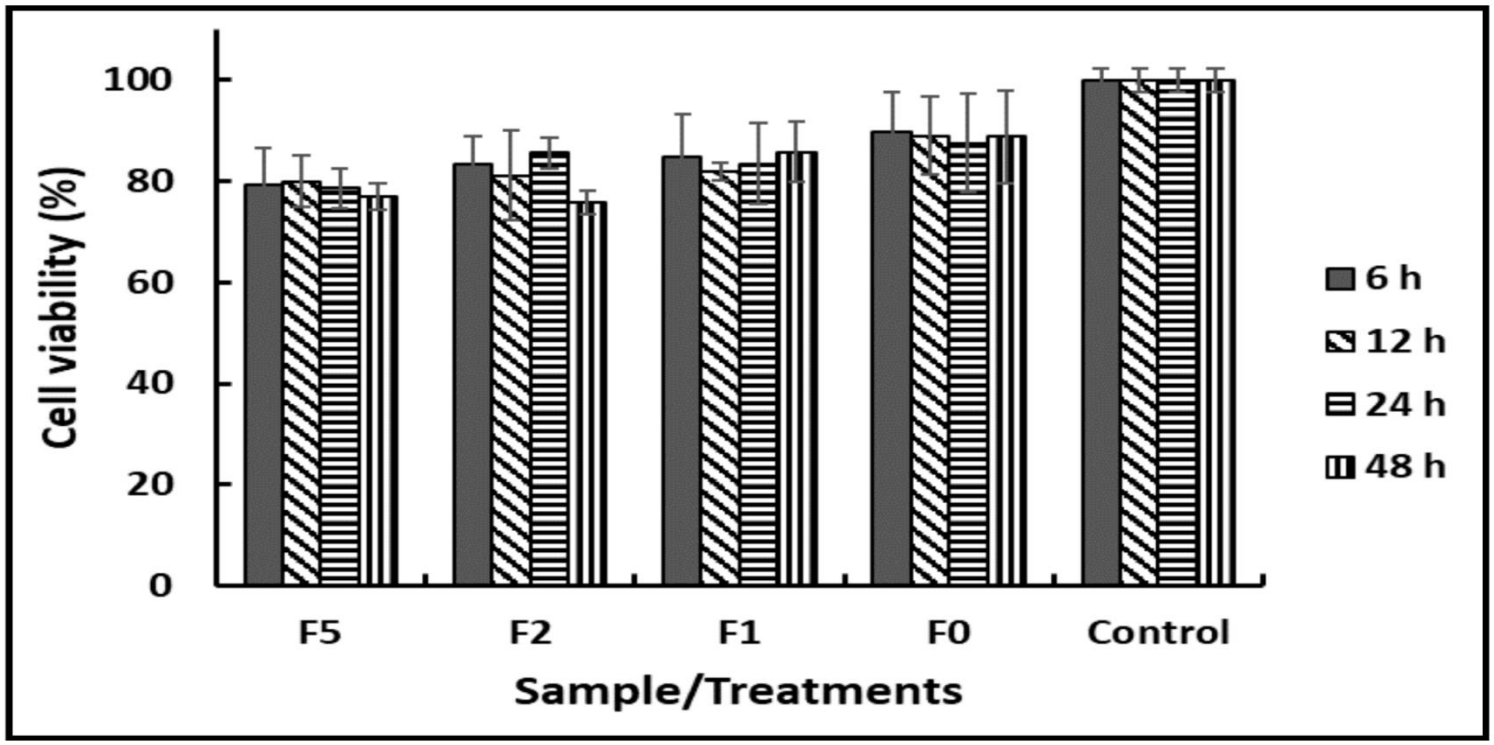


FIGURE 4

Morphological examination of the microcapsules by scanning electron microscopy showed irregular shape and compact structure similar to previously observed micrograph for freeze dried microcapsules.

FIGURE 5



Cytocompatibility testing of microcapsules

The cytotoxicity evaluation of eluates obtained from microcapsules at various time intervals against human embroyonic colon cell Caco–2 showed >80% cell viability when compared with the control (100%). Encapsulation of active compounds can reduce the adverse effects through regulated slow release with prolonged activity.

Conclusions

- Freeze drying methods yielded microcapsules with good properties
- Sodium alginate and sodium carboxymethyl cellulose co-polymers should good properties as encapsulants with excellent encapsulation efficiency and percentage yield
- The capsules should high retention of the core as shown by the TPC and TFC contents
- The microcapsules exhibited antimicrobial and antioxidant properties reflecting the release of the core material

Conclusions

- Scanning electron microscopy revealed irregular microcapsules

- The microcapsules showed excellent compatibility with human embryonic colon cell Caco-2

Acknowledgments

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