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# Physicochemical and Lipid Peroxidation Evaluation of Fabricated Extract from *Securidaca longipedunculata* Root Bark

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

## Abstract

**Background**: Modification of some crude extracts have improved the stability, functional properties and process ability of their bioactive.
**Objective**: To fabricate bioactive from *Securidaca longipedunculata* root bark and compare its activities with the crude extract.
**Methods**: Modified extract was fabricated from *Securidaca longipedunculata* root bark using microencapsulation and freeze-drying technique, and evaluated.
**Results**: Fourier Transform Infrared Spectroscopy evaluation showed similar leading peaks in the crude and fabricated extracts, indicating absence of chemical alteration from modification. Energy-Dispersive X-ray (EDX) of the fabricated extract revealed no new detectable element from the EDX of the crude extract, indicating chemical similarities in the crude and fabricated extracts. Scanning Electron Microscopy (SEM) of crude extract revealed flatbed surface with no observed particles, while SEM of fabricated extract revealed distinct, larger and sharp-edged particles, indicating structural changes from modification. In comparison to crude extract, fabricated extract showed controlled dissolution, starting with lower dissolution over 30 min, on to higher dissolution after 45 min. At 20 – 100 mcg / ml actives, Vitamin E showed 60 – 80 % lipid peroxidation, while crude and fabricated extracts both showed 12 – 20 % lipid peroxidation, indicating that modification did not significantly alter the antioxidant efficacy of *Securidaca longipedunculata* crude extract material.
**Conclusion**: The physicochemical and biological properties of fabricated *Securidaca longipedunculata* root bark extract showed promise in fast delivery of bioactive. There is need for further *in vivo* studies to ascertain the therapeutic potential of this fabricated form.

**Keywords**: Microencapsulation, freeze drying, chemical alteration, structural changes, dissolution.

## INTRODUCTION

Microencapsulation and freeze drying is a fabrication technique used in modifying bioactive to desired microstructure, shape, density, and surface properties, and to improve bioactive sterility, process ability, stability, delivery, and relevant functional properties (Yousefi *et al.*, 2019; Dominguez *et al.*, 2021). Fabrication by microencapsulation and freeze drying is carried out by casting wall materials over bioactive at low temperature. Here, the wall material shields and protects the bioactive from external interference and

degradation, and modifies its delivery properties. The low processing temperature by freeze drying in this technique helps to retain more wall material, sustain thicker and stronger wall cast, and reduce heat strain on the bioactive (Mehta *et al.*, 2022; Yan & Kim 2024). The micronized size of the resultant fabricated extract helps improve solubility and delivery of the biomaterials (Singh *et al*, 2010; Khan *et al.*, 2022).

Aphrodisiacs derived from plant biomaterials are gaining acceptance because they are known to stimulate sexual desire with fewer side effects compared to synthetic drugs (Melnyk & Marcone 2011; Nwindu *et al*. 2015; Jaradat & Zaid 2019). Ethno-medicinal use and preliminary studies suggest that *Securidaca longipedunculata* plant (Fig. 1), commonly found in tropical and subtropical Africa, possess aphrodisiac properties and other therapeutic potentials such as muscle relaxation, anti-inflammation, and anti-microbial properties (Abubakar *et al*, 2019; Namadina *et al*, 2020). The phytochemical and functional properties of *Securidaca longipedunculata* root extract have been characterised by researchers such as Namadina *et al*, (2020). To the knowledge of these researchers, there remains a gap in the understanding of how microencapsulation and freeze-drying fabrication technique might influence the pharmacological activities of *Securidaca longipedunculata* plant extract, particularly in relation to sexual health.

By micronizing and encapsulating the root bark extract of *Securidaca longipedunculata* plant in a wall material, this study will assess the potential benefits of microencapsulation and freeze-drying fabrication technique in preserving the bioactivity and delivering of the extract. To evaluate the structural and functional properties of the crude and fabricated extract, advanced analytical characterization analysis with Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDX), and dissolution testing will be conducted. EDX assesses the presence of trace elements in biomaterial (Rusli *et al*., 2022).To estimate the aphrodisiac potentials of the extracts, TBARS lipid peroxidation bioassays will be conducted. Lipid peroxidation measures the ability of actives to reduce oxidative stress. The degree of oxidative stress is a valuable marker and tool for assessing sexual status of individuals (Sheweita *et al*., 2020; Marin *et al.*, 2023). This study is justified by its dual focus on both the physicochemical and biological effect of crude extract fabrication by microencapsulation and freeze drying. The findings of this research is expected to provide insight into application of this fabrication technique in improving bulk processing of novel delivery natural-based herbal medicine that could offer alternatives to current synthetic aphrodisiac agents.

**Figure 1: *Securidaca longipedunculata* plant**

## METHODOLOGY

### Materials

The root bark of *Securidaca longipedunculata* plant was obtained from Okada market in Ovia Local Government of Edo, State Nigeria, and was identified at the herbarium section of the Igbinedion University, Okada. Goat liver was obtained from Okada Market abattoir and washed severally with an ice-cold saline solution and stored in 2 – 4 $^{o}$C awaiting use. All the chemicals used for the research work were of analytical grade purchased from Cayman Chemicals (East Ellsworth, Ann Arbor MI, USA) and Sigma, Merck and Fluka.

### Methods

*Preparation of plant extract*

Adapting the method of Osinubi *et al.* (2023), the root bark of *Securidaca longipedunculata* (SL) plant was sorted, washed, chopped into 2 cm pieces and air dried. The dried root bark grounded to coarse powder with a laboratory Kenwood miller with an attached grater and a 1 mm stainless sieve. A 2000 g of the powder was moistened and mixed with 5.0 L of equal volume of methanol in a clean container. The mixture was left to stand for 72 h at room temperature. The content was then filtered using a Whatmann No.1 filter paper and dried in lyophilizer freeze dryer (Harvest Right 110V Seven Tray Freezer Dryer with Stainless Steel Vacuum Pump, Model: HR-MEDPHRM) for 12 h. The dried blend was put through a 1.7 mm stainless steel screen, packed, labelled and stored for use. The

percentage yield of the extract was calculated using equation 1.

$$\% \text{ yield} = \frac{Weight\ of\ extract}{Weight\ of\ root\ bark\ powder} \times 100 \ldots. \text{ Eq. 1}$$

**Modification of extract**

Adapting the microencapsulation method of Soliman *et al.*, (2022), acacia coacervate was formed by progressively adding 400 ml of hot water to 40 g acacia with constant agitation on a hot plate magnetic stirrer (Model TK23, Kartel, Italy). The coacervate was agitated further for 2 h, and allowed to stand for 1 h to cool and swell. A 40 g extract powder was mixed with 40 ml anti-aggregating agent sodium sulphate solution, and the mix was added to the swollen dispersion and stirred for 1 h on the hot plate magnetic stirrer. At a rate of 1 ml / min drops of 99.8 % ethanol were injected at 5 cm height to the cold dispersion and stirred. The blend was transferred and dried in lyophilizer freeze dryer (Harvest Right 110V Seven Tray Freezer Dryer with Stainless Steel Vacuum Pump, Model: HR-MEDPHARM) for 12 h. The dried blend was put through a 1.7 mm stainless steel screen, packed, labelled and stored for use.

**Fourier transform infrared spectroscopy (FTIR) studies**

Using a Jasco FT-IR 4000/6000 series spectrometer (Jasco, Oklahoma, USA), the crude and fabricated plant extracts were examined for functional groups. A 2 mg crude or fabricated extract was made up to 200 mg with solid potassium bromide. The mixture was blended, pulverised and dried at 110°C for 2 hours in a hot air oven, and allowed to cool. The dried blend was compressed to pellet in a 13 mm diameter die at 8 tons pressure for 3 min. The FT-IR spectrum of the pellet was recorded using a Jasco FT-IR 4000/6000 series spectrometer (Jasco, Oklahoma, USA) set at 700 – 4000 cm−1 transmittance wavelength.

**Scanning electron microscopy (SEM) studies**

Samples of crude or fabricated extract were sprinkled onto a double-sided adhesive carbon conductive tape mounted on a microscope stub copper. The tape was sputter-coated with gold using an ion sputtering device of JEOL JSM-65 10LV scanning electron microscope. The scanning electron microscopy (SEM) photograph of the extract was captured at an acceleration voltage of 20 kv at x1000 and x2000 magnifications, and produced for analysis.

**Energy dispersive X-ray (EDX) studies**

Using the identical SEM apparatus (JEOL JSM-65 10LV type) that came with EDAX DX-4 eDXi System, version 2.11, the energy-dispersive X-ray of the microspheres of the extracts were analysed between 0 and 20 keV. A for 120 seconds and their spectra recorded.

**Dissolution studies**

A 200 mg extract powder was dispersed in 900 ml of 0.1N HCl at 37±0.5°. Using a USPXXIV dissolution tester with a rotating paddle set at a speed of 50 rpm, aliquot of the dispersion was removed at 5, 10, 20, 30, 45, and 60 min respectively, filtered and analysed for dissolution using a UV- spectrophotometer (Model 23D, Uniscope, England) at a wavelength (λmax) 245 nm. After each aliquot was removed, a dissolving media was introduced in an equal volume to keep the beaker in sink condition. The experiments were all conducted in triplicates.

**Lipid peroxidation assay**

The thiobarbituric acid reactive substance (TBARS) procedure of Dutta and Singh (2011) for lipid peroxidation assay was adapted. Using a Kenwood blender, 10 % w / v goat liver in 0.1 M phosphate buffer (pH 7.0) solution was blended to homogenate. The homogenate was centrifuged for 10 minutes at 8,000 rpm to obtain the supernatant. A 20, 40, 60, 80, and 100 mcg/ml crude or fabricated extract or positive control vitamin E or negative control distilled water was introduced into a screw capped test tube. A 0.5 ml liver supernatant was added to the test tube and the total volume adjusted to 1 ml with distilled water. Afterward, 0.07 M iron sulphate (FeSO4) was introduced to the test tube, blended and incubated 37˚ C for 1 hour to initiate lipid peroxidation. A 50 µL of 20 % ice chilled trichloroacetic acid (TCA), 1.5 ml 0.8 % thiobarbituric acid (TBA) in 1.1 % sodium dodecyl sulphate (SDS) solution, and 1.5 ml 20 % acetic acid were introduced into the reaction mixture, and boiled in water bath at 95 ºC for 60 min. The samples were kept to cool and 5 mL of butan-1-ol was thereafter added to each tube and centrifuged at 300 rpm for 10 min. The butan-1-ol layer taken and its absorbance measured using a UV- spectrophotometer (Model 23D, Uniscope, England) at a wavelength (λmax) at 532 nm. Using vitamin E as the reference/ control standard (C), the extent of inhibition of lipid peroxidation of the sample material (S), known as anti-lipid peroxidation (ALPO), was determined with the formula in equation 2.

$$\% \text{ ALPO} = 1 - (S/C) \times 100 \text{ ----------Eq. 2}$$

While, S = absorbance of sample material; C = absorbance of control material. The experiments were all conducted in triplicates.

## STATISTICAL ANALYSIS

The experiments were all conducted in triplicate and the mean was calculated. The mean was analysed using ANOVA and subjected to least significant difference, and recorded as Mean ±SD

## RESULTS

### Yield

The crude extract yield from *Securidaca longipedunculata* root bark was 10.87%.

### FTIR analysis

The crude and fabricated extracts of *Securidaca longipedunculata* are presented in Fig. 2a and 2b respectively. The spectra of crude and fabricated extracts showed similar curves and pronounced peaks at 3339.7 3276.3, 2922.2, 2855.1, 2318.4 $cm^{-1}$

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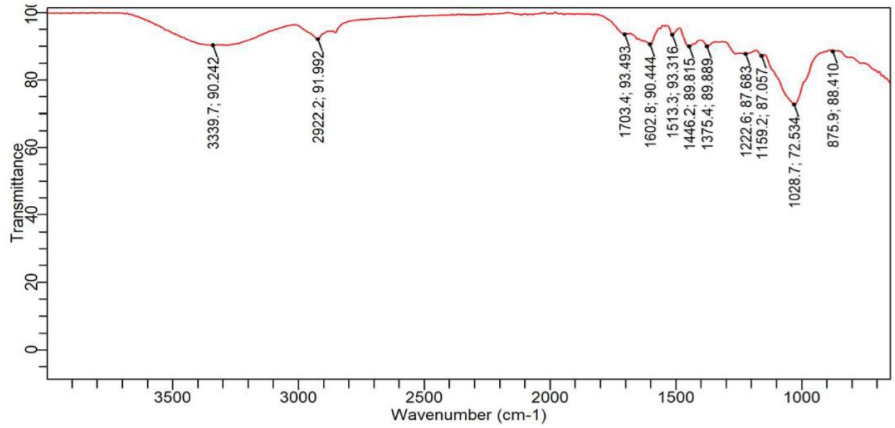


Figure 2a: FTIR of *Securidaca longipedunculata* crude extract

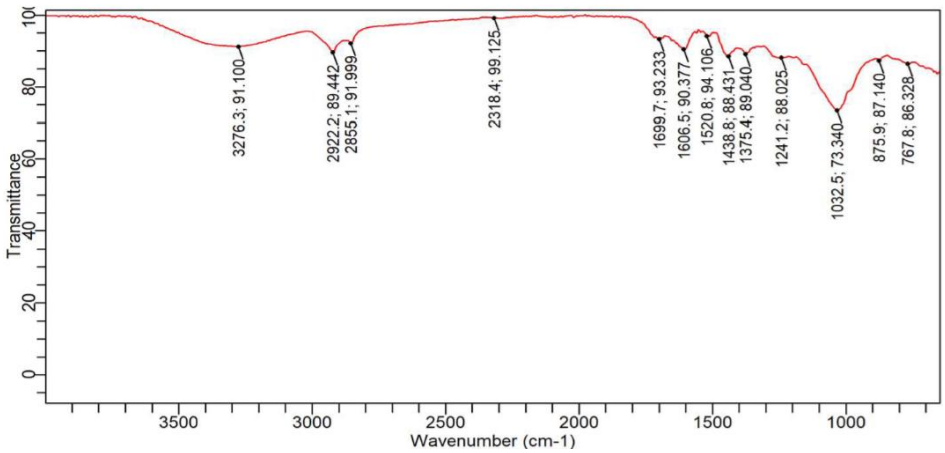


Figure 2b: FTIR of *Securidaca longipedunculata* fabricated extract

### SEM analysis

The SEM images of crude and fabricated extracts at x1000 and x2000 magnifications are presented in Fig. 3. The crude extract at ×1000 magnification, showed no observable particles, presenting a flatbed surface. In contrast, the fabricated extract displayed rough, large particles with sharp edges, about 100 µm in length and 60 µm in diameter, irregular in shape. This morphology remained consistent at ×2000 magnification, with the fabricated particles retaining their rough, sharp-edged characteristics and irregular shapes.

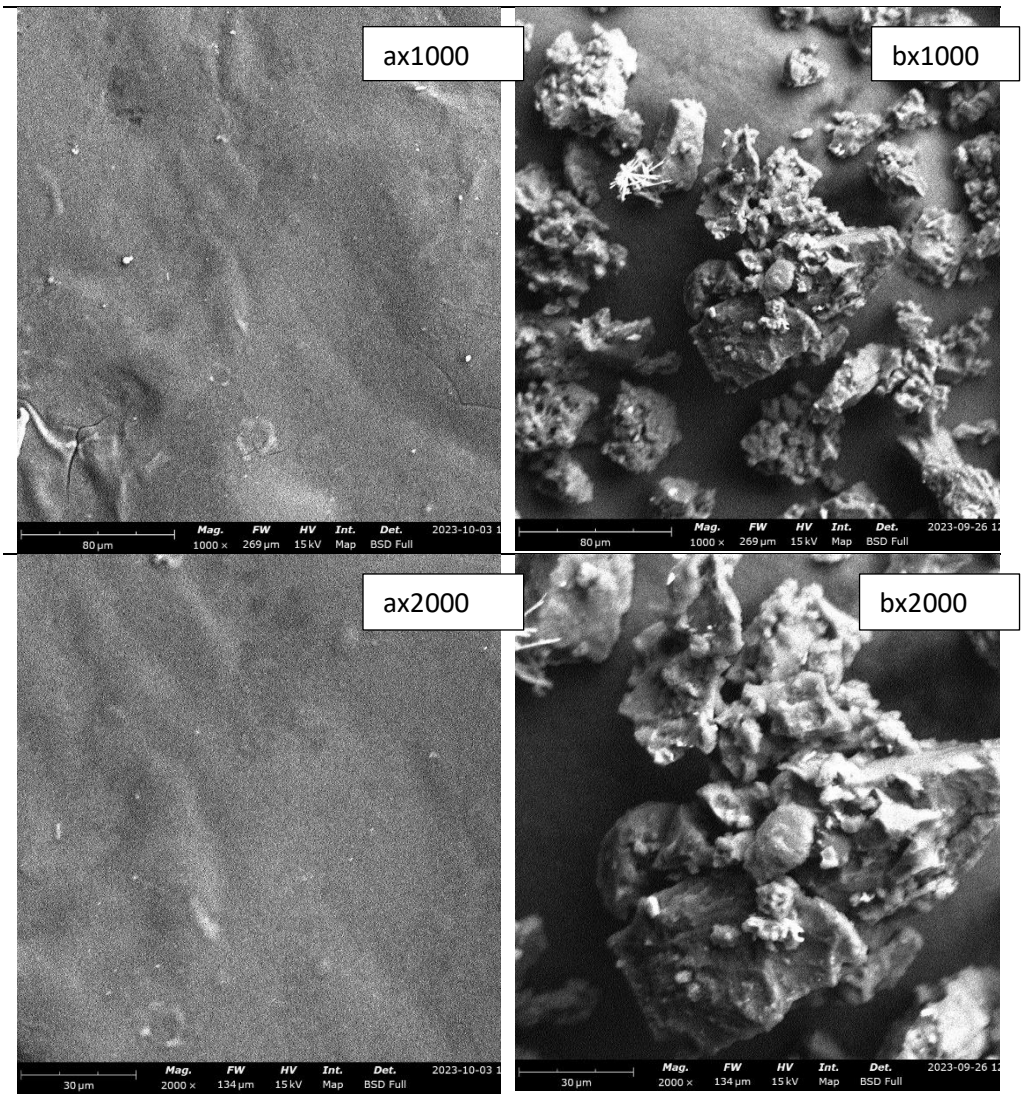


Figure 3: SEM images of *Securidaca longipedunculata* crude (a) and fabricated (b) extracts at x1000 and x2000 magnifications

**Table 1**: EDX elemental composition of solid surfaces of *Securidaca longipedunculata* extracts

| | Crude | | | | Fabricated | | | |
|---|---|---|---|---|---|---|---|---|
| Element | Spot 1 (%) | | Spot 2 (%) | | Spot 1 (%) | | Spot 2 (%) | |
| Name (no) | Atomic % | Weight % | Atomic % | Weight % | Atomic % | Weight % | Atomic % | Weight % |
| Oxygen (8) | 74.35 ± 2.03 | 79.43 ± 1.54 | - | - | 71.4 ± 1.04 | 76.88 ± 2.87 | | |
| Carbon (6) | 25.65 ± 0.43 | 20.57 ± 0.54 | | - | 28.6 ± 0.17 | 23.12 ± 0.67 | | |

**EDX analysis**

The EDX results of crude and fabricated extracts are presented in Table 1 and Fig. 4.

In both the crude and fabricated extracts, Spot 1 showed Oxygen and Carbon, with Spot 2 revealing no detectable elements.

**Dissolution analysis**

The dissolution rate of crude and fabricated *Securidaca longipedunculata* extracts is presented in Fig. 5 below. The fabricated extract showed steady increase in dissolution over 60 min, with absorbance increasing from 0.019 at 5 minutes to 0.070 at 60 min. The crude extract showed modest dissolution over 60 min, with absorbance starting at 0.026 at 5 min and reaching 0.034 at 60 min.

**Lipid peroxidation analysis**

The effect of *Securidaca longipedunculata* crude and fabricated extracts on lipid peroxidation compared to positive control (vitamin E) is shown in Fig. 6. Vitamin E demonstrated the highest lipid peroxidation across all concentrations, achieving 60% inhibition at 20 mcg/ml and increasing to 80% at 100 mcg/ml. This consistently high performance underscores its effectiveness as an antioxidant. The crude extract showed low but consistent inhibition rates, starting at 12% at 20 mcg/ml and peaking at 20% at 100 mcg/ml. The fabricated extract replicated the performance of the crude extract, maintaining similar inhibition across all concentrations.

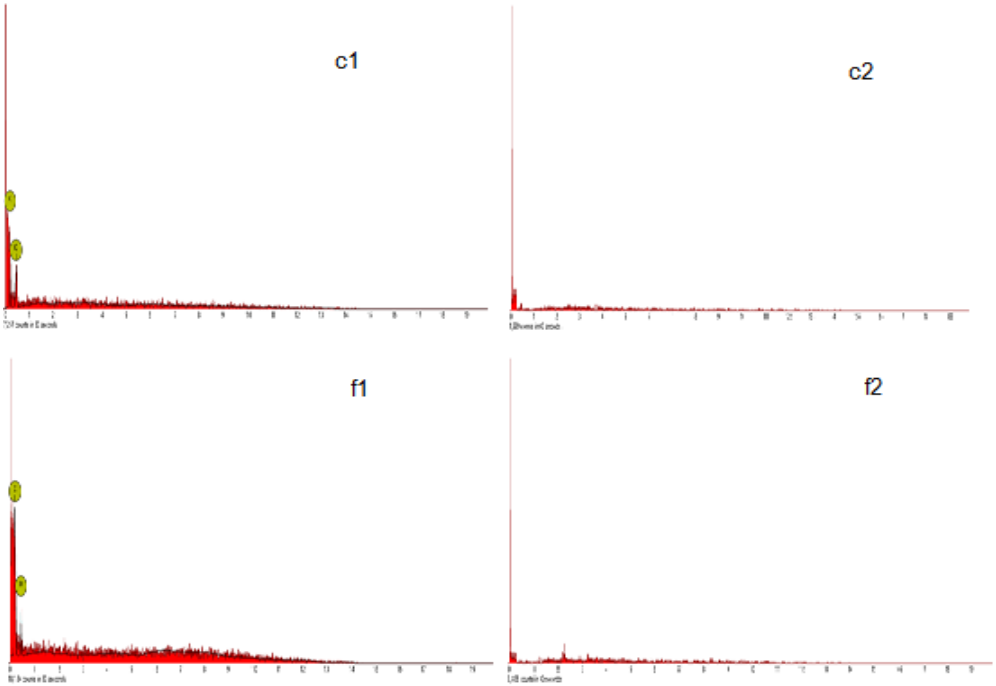


**Figure 4: EDX Spectra of Crude (c) and fabricated (f) *Securidaca longipedunculata* solid surface at spots 1 and 2.**

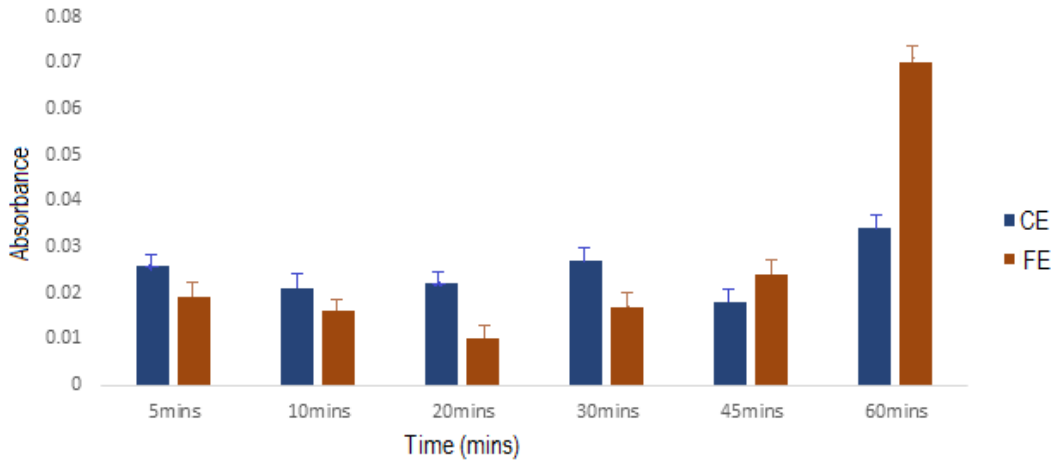


**Figure 5: Dissolution absorbance of *Securidaca longipedunculata* crude and fabricated extracts.**

*****CE = crude extract. FE = fabricated extract

The values are expressed as Mean ± SEM, (n = 3). Values with superscript * indicate significant difference at $p < 0.05$

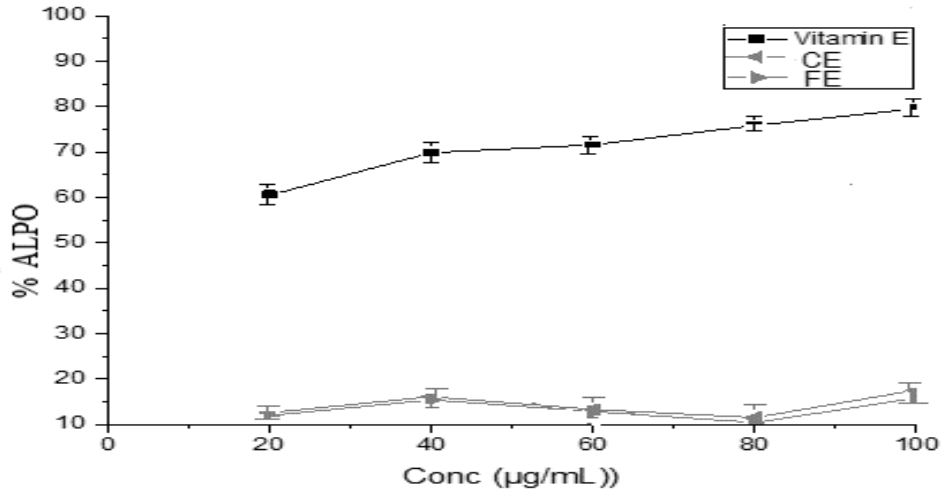


**Figure 6:** Effect of crude and fabricated *Securidaca longipedunculata* extracts on lipid peroxidation.
NB: ALPO = anti-lipid peroxidation. CE = crude extract. FE = fabricated extract
The values are expressed as Mean ± SEM, (n = 3). Values with superscript * indicate significant difference at $p < 0.05$

## DISCUSSION

This study seeks to prove that modification of crude extract of *Securidaca longipedunculata* root bark enhances its structural, absorption and antioxidant properties without compromising the chemical structure of the crude extract. The 10. 87 % crude extract yield from its root bark falls within the range from previous researches such as Osinubi *et al*. (2023) on *Securidaca longipedunculata* root bark extraction. Using the explanation in Hinterstoisser and Salmen (2000), the similarity in the spectra of the crude and fabricated extracts is a pointer to similar functional groups of the same compound. This result suggests that fabrication of the crude extract did not alter the chemical nature of the crude bioactive. The absence of observable particles in the SEM images of the crude form at both magnifications, compared to the distinct particles in the fabricated form, underscores the significant structural changes introduced by modification. Ali *et al*. (2023) had demonstrated that modification alters the morphology and structure of plant extract. The absence of additional element in the EDX of the fabricated extracts suggests that the fabrication process did not introduce new elements into *Securidaca longipedunculata* extract. The steady increase in dissolution of the fabricated extract from the dissolution result highlights the effectiveness of the fabrication technique in enhancing the solubility of the extract. The enhanced dissolution observed in this study is a positive indicator for potential improvement in bioavailability, especially for oral drug formulations. This result supports the findings of researchers such as Mardani *et al.*, (2024) on the efficacy of microencapsulation in enhancing release of bioactive plant extract. The absence of significance difference in the lipid peroxidation of the crude and fabricated extracts from the lipid peroxidation analysis may indicate that fabrication did not significantly alter the known antioxidant efficacy of *Securidaca longipedunculata* root bark.

## CONCLUSION

The fabricated extract from *Securidaca longipedunculata* root bark showed improved structural and dissolution properties, and similar functional chemical or lipid peroxidation biological properties to the crude extract. The result from this study on the effect of microencapsulation and freeze-drying fabrication technique on the properties of the modified extract is limited by the absence of *in vivo* studies. We recommended that extensive biological and other in vivo evaluation be conducted to optimise, and understand the physiological and clinical effect of the fabricated extract as it compares with the crude extract.

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