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Effect of microencapsulation and freeze-drying on the functional properties of *Spondia mombin* stem extract used in ethno-medical management of benign prostrate hyperplasia

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Results: The micromeritic studies showed improved flow properties for the microencapsulated extract with angle of repose < 27.9°, Hausner's ratio < 1.06, and compressibility index < 6.06 %. SEM analysis showed a larger particle sizes of 50–80 µm and >50 µm for microencapsulated and crude extract respectively. FT-IR and EDX analysis revealed relatively unchanged spectrum in the modified extract compared to the crude extract. These results are suggestive of chemical compatibility and physical agglomeration in the interaction of the crude extract with microencapsulation excipients. Dissolution studies over 60 minutes showed the microencapsulated extract releasing > 9.76 times the release from crude extract. The microencapsulated extract showed peak reduction in PSA at 100 mg / kg, compared to peak reduction of PSA of crude extract at 400 mg / kg. Serum testosterone levels decreased significantly in the microencapsulated extract.

Conclusion: Microencapsulation improved the processing ability and dissolution properties of *Spondia mombin* extract, and retained its pharmacological activities.

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Keywords: Dissolution studies; micromeritics; prostate index; prostate-specific antigen; serum testosterone

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Introduction

Spondia mombin is a member of the Anacardiaceae family, is a plant species that grows in Nigeria (Adeniran et al., 2021). The fruit is known by several names in the region: Iyeye or Yeye in Yoruba, Ngulungwu in Igbo, Ughighen in Urhobo, and Isada in Hausa (Aiyeloja & Bello, 2006; Aromolaran & Badejo (2014).

S. mombin extracts have been reported to selectively inhibit cell proliferation in the tumour cell line for prostate cancer (PC3) and did not significantly affect healthy cells (Guedes et al., 2020). It is one of the herbal remedies for BPH control that has been studied (Iwu et al., 2022). It is used in Leprosy, severe cough, diarrhoea, dysentery, dyspepsia, gastralgia, colic, constipation, haemorrhoids, gonorrhoea, leucorrhoea, dystocia, postpartum haemorrhage, and inflammation and antioxidant qualities (Iwu et al., 2022; Ogunro et al., 2023; Osuntokun, 2018, 2019, 2019). The *S. mombin* stem bark used for this study is presented in Figure 1

Different techniques, such a microencapsulation and freeze drying, have been used to improve the stability and bioavailability of biomaterials (Yousefi et al., 2019; Dominguez et al., 2021).

Based on our findings, no experimental data have compared the anti-BPH potential of both crude extract and microencapsulated particles of S. mombin

The goals of microencapsulation are to preserve and improve the stability of delicate materials, lessen activity loss, increase the applications of delicate materials, improve the bioavailability of biomaterials , incorporate certain particles into other materials, preserve flavours and aromas, change the consistency of materials from liquid to solid for navigating convenience, and shield particles from environmental influences, prevent oxidation and radiation-induced deterioration, slow the drying out of volatile chemicals, and other properties (Hendrawati *et al*., 2019; (Choudhury et al., 2021; Mehta *et al*., 2022).

Figure 1: The dried Bark of *Spondia mombin* used for this study

The prostate gland is located below the bladder in men and produces fluid components of semen. Benign prostatic hyperplasia or benign prostatic hypertrophy (BPH) is the non-cancerous enlargement of the prostate gland (Kim *et al*., 2016). . It is a normal part of aging for many men, and it is linked to hormonal changes specifically testosterone and dihydrotestosterone (DHT). Over half of men aged 60 and above have enlargement of the prostate gland (Lee *et al*., 2017; Xiong *et al*., 2020).

BPH can results partial to complete blockage of the urethra, difficulty, urgent urge to inability to urinate. Infections or and excessively weak bladder muscles may follow. Symptoms can permanently damage the kidneys.

Treatment options include: Life style modification; Drug treatment (such as 5 alpha reductase inhibitors and Alpha adrenoreceptor blocking agents); and Surgery (Open Prostectomy, TURP, TUIP, balloon dilatation) (Michael *et al*., 2025)

Methodology

- Plant collection and botanical authentication of *S. mombin plant*
- Extraction of of *S. mombin crude* extract using Hot continuous extraction method
- Modification of crude extract using microencapsulation and freeze drying technique.
- Characterisation of extracts using scanning electron microscopy (SEM), energy dispersive X-ray (EDX), and Fourier transform infrared spectroscopy (FTIR) analysis.
- Evaluation of crude and microencapsulated extracts for physicochemical properties using micromeritic determination and dissolution studies.
- Determination of *in vivo* pharmacological properties using prostate-specific antigen (PSA), serum testosterone, and prostate index (PI) analysis on male Wister rats.

Results and discussion

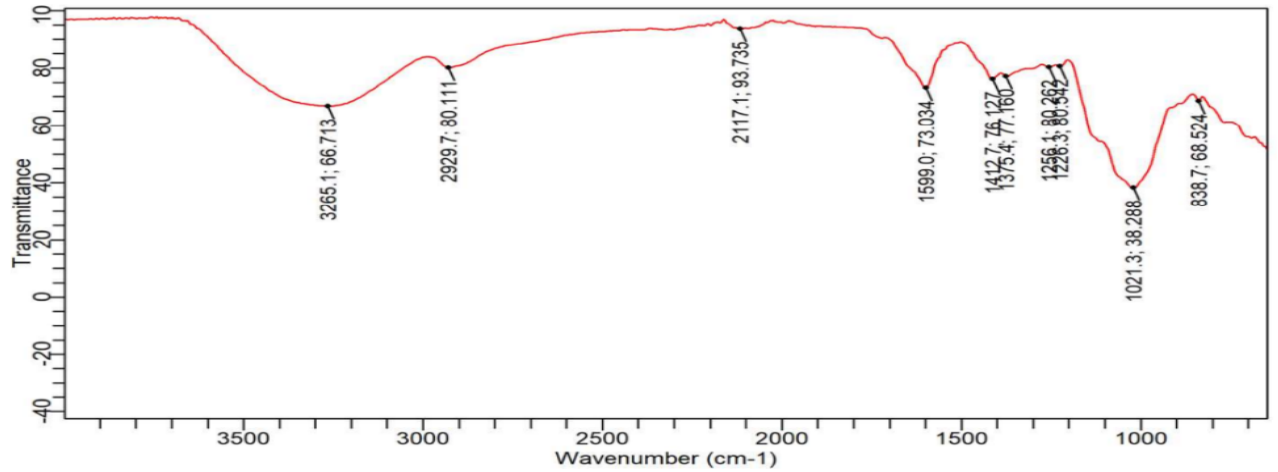
FT-IR analysis: The peaks of both the crude and microencapsulated extracts were near similar as presented in Figure 2. Peaks with wave numbers 2929.7 and 2117.1 were observed with blank and microencapsulated particles of S. mombin spectra, indicating changing intensity. In addition, peak wavenumbers of 1110.7, 1449.9, and 1364.2 with intensities of 42.699, 71.605, and 68.665, respectively were more peculiar to microencapsulated than crude extracts, this may reflect the addition of the encapsulating components to the active ingredient. This FTIR result revealed relatively unchanged spectrum and additional trace molecules in the modified extract.

SEM analysis: This is revealed in Figure 3 showed that the microencapsulated extract exhibited a granular texture and larger particle size of 50–80 µm compared to smooth and sharp-edged texture and smaller particle size of <50 µm for crude extract.

These results are presented in Figure 3, and are suggestive of chemical compatibility and physical agglomeration in the interaction of the crude extract with microencapsulation excipients.

EDX analysis: Calcium, observed in crude extract (Figure 4) was absent in microencapsulated extract (Figure 5) highlighting compositional distinctions. EDX microanalysis found both samples contained oxygen as the predominant element and sodium and carbon were present

S. Mombin crude active



Microencapsulated *S. mombin active*

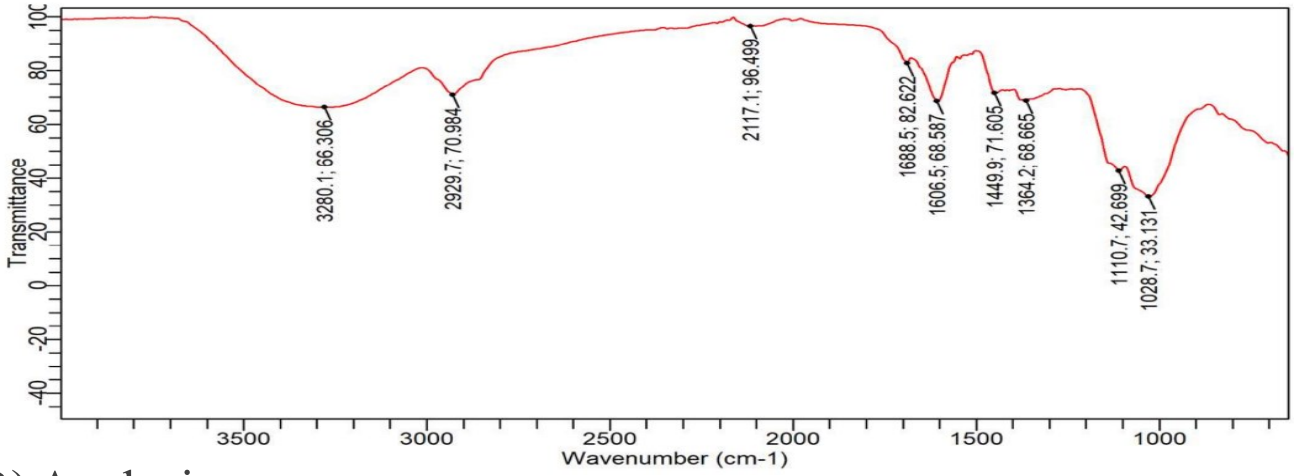
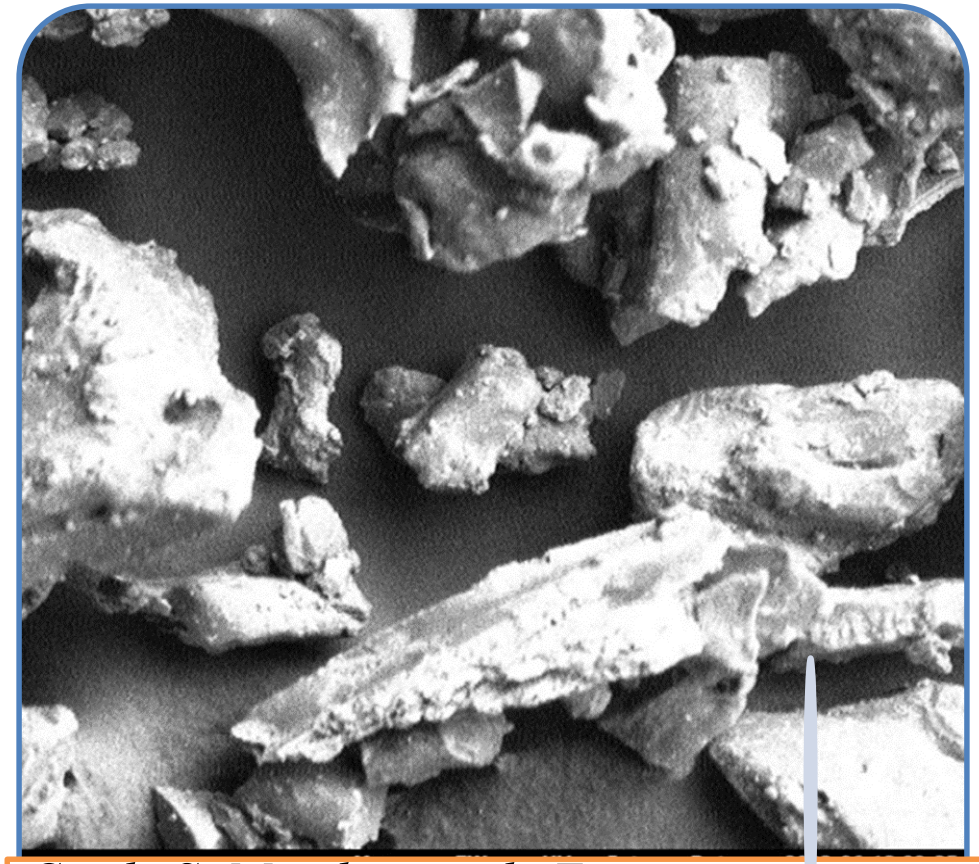
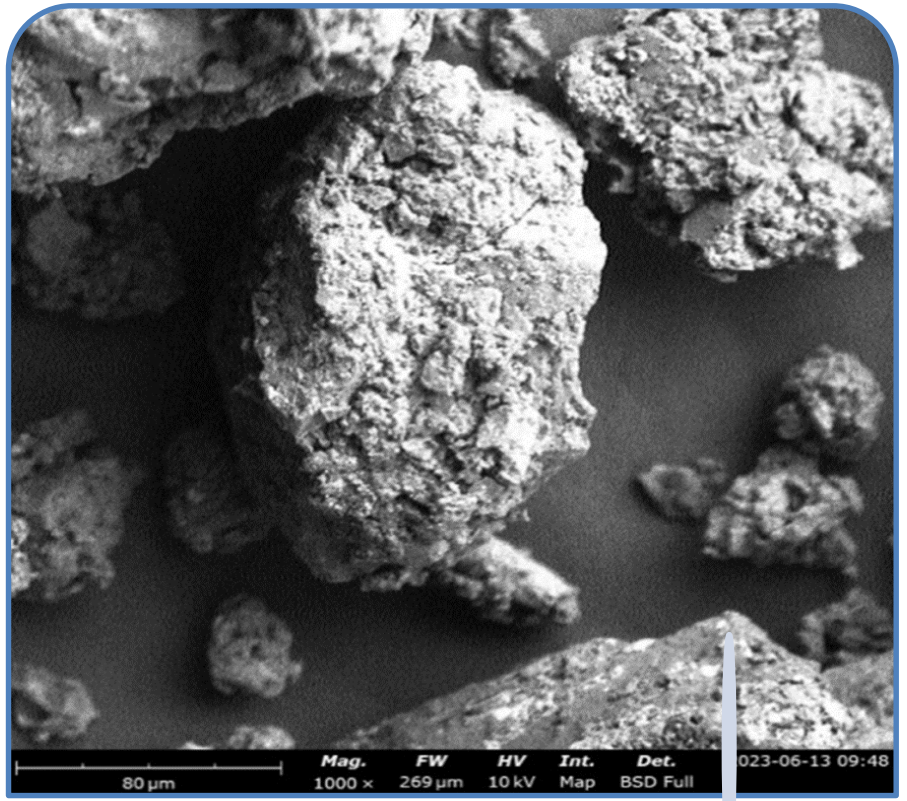


Figure 2: Fourier Transform Infrared (FT-IR) Analysis

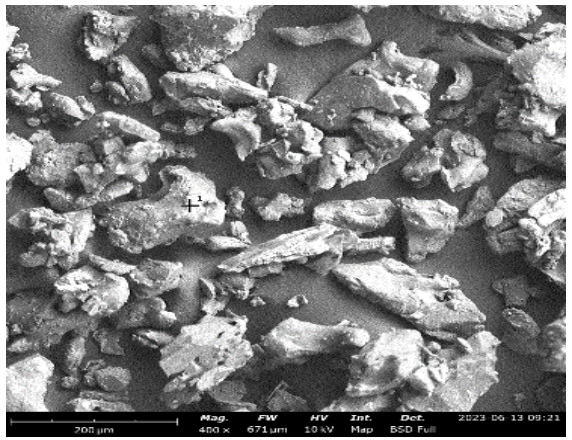


Crude S. Mombin crude Extract



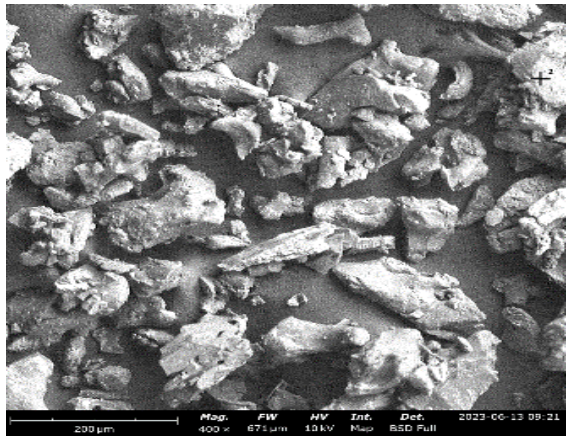
Microencapsulated *S. mombin Extract*

Figure 3: Scanning Electron Microscopy (SEM) Analysis



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	66.09	58.70
11	Na	Sodium	18.17	23.19
6	C	Carbon	8.89	5.93
16	S	Sulphur	6.84	12.18

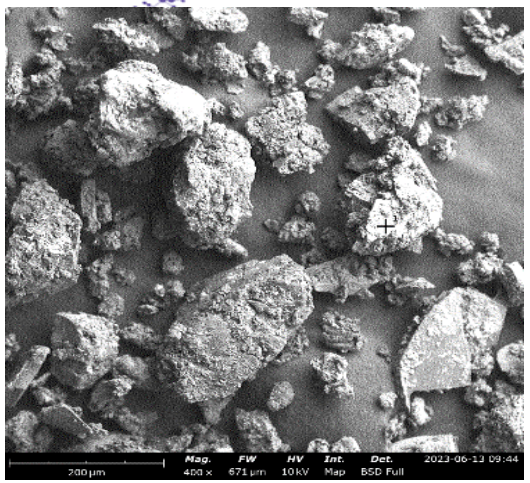
Constituent elements in Spot 1 of the EDX analysis of crude extract.



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	75.25	57.37
20	Ca	Calcium	12.60	24.06
16	S	Sulphur	12.15	18.57

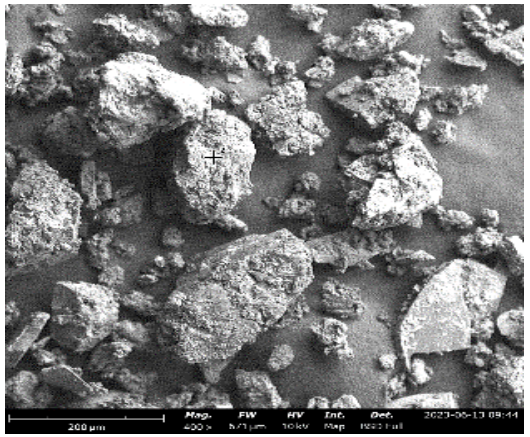
Constituent elements in Spot 2 of the EDX analysis of crude extract.

Figure 4: Energy-disperse X-ray (EDX) Analysis of Crude Extract



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	63.60	56.18
11	Na	Sodium	21.57	27.38
6	C	Carbon	8.86	5.87
16	S	Sulphur	5.97	10.57

Constituent elements in Spot 1 of the EDX analysis of microencapsulated extract



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
8	O	Oxygen	52.06	39.49
11	Na	Sodium	28.79	31.38
16	S	Sulfur	19.16	29.13

Constituent elements in Spot 2 of the EDX analysis microencapsulated extract

Figure 5: Energy-disperse X-ray (EDX) Analysis of Microencapsulated Extract

Physicochemical Properties

Micromeritics Properties: As presented in Table 1, the lower angle of repose in microencapsulated extract indicates reduced interparticle friction, which can be attributed to the differences in particle size, shape, or surface characteristics between the two samples (Shah *et al*., 2023). This finding is significant for potential applications in powder handling, where flowability is crucial (Shah *et al*., 2023).

A lower Hausner ratio and compressibility index typically indicates better flowability, lower susceptibility to caking and potentially better suitability for applications requiring precise dosage and consistent performance(Taylor & Aulton, 2021).

Dissolution studies profile: is presented in Figure 8. The profile revealed steady release of extract from the microencapsulated form over 60 minutes, and > 9.76 times increase in peak release compared to release from the crude extract.

The dissolution study results indicate that the microencapsulation of Spondia mombin significantly improves its dissolution profile compared to the crude microencapsulated formulation. The higher absorbance values for the Spondia mombin extract suggest enhanced solubility and possibly better bioavailability, which can be attributed to the microencapsulation process (Chen et al., 2019).

Microencapsulation is known to improve dissolution rates by reducing particle size, increasing surface area, and enhancing the interaction of the active ingredient with the dissolution medium (Jyothi et al., 2010; Kuang et al., 2010).

Table 1: Physicochemical properties of extracts

Parameters	Crude extract	Microencapsulated extract
The base of Cone (cm)	4.80	6.80
Height of Cone (cm)	1.50	1.80
Angle of Repose (°)	32.00	27.90
Bulk Volume (mL)	9.40	9.60
Tapped Volume (mL)	8.20	9.00
Weight of Powder (g)	6.68	8.88
Bulk Density (g/mL)	0.71	0.93
Tapped Density (g/mL)	0.81	0.99
Compressibility Index (%)	12.35	6.06
Hausner's Ratio	1.14	1.06

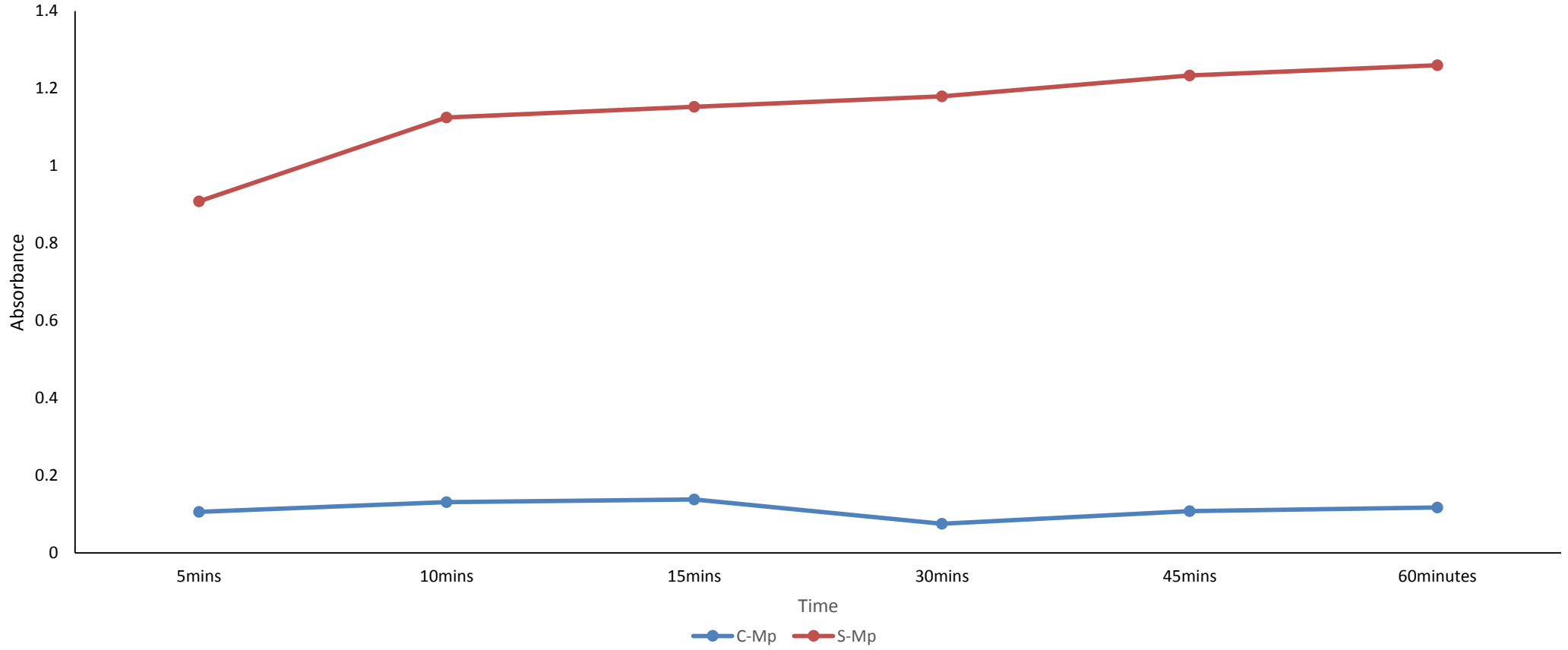
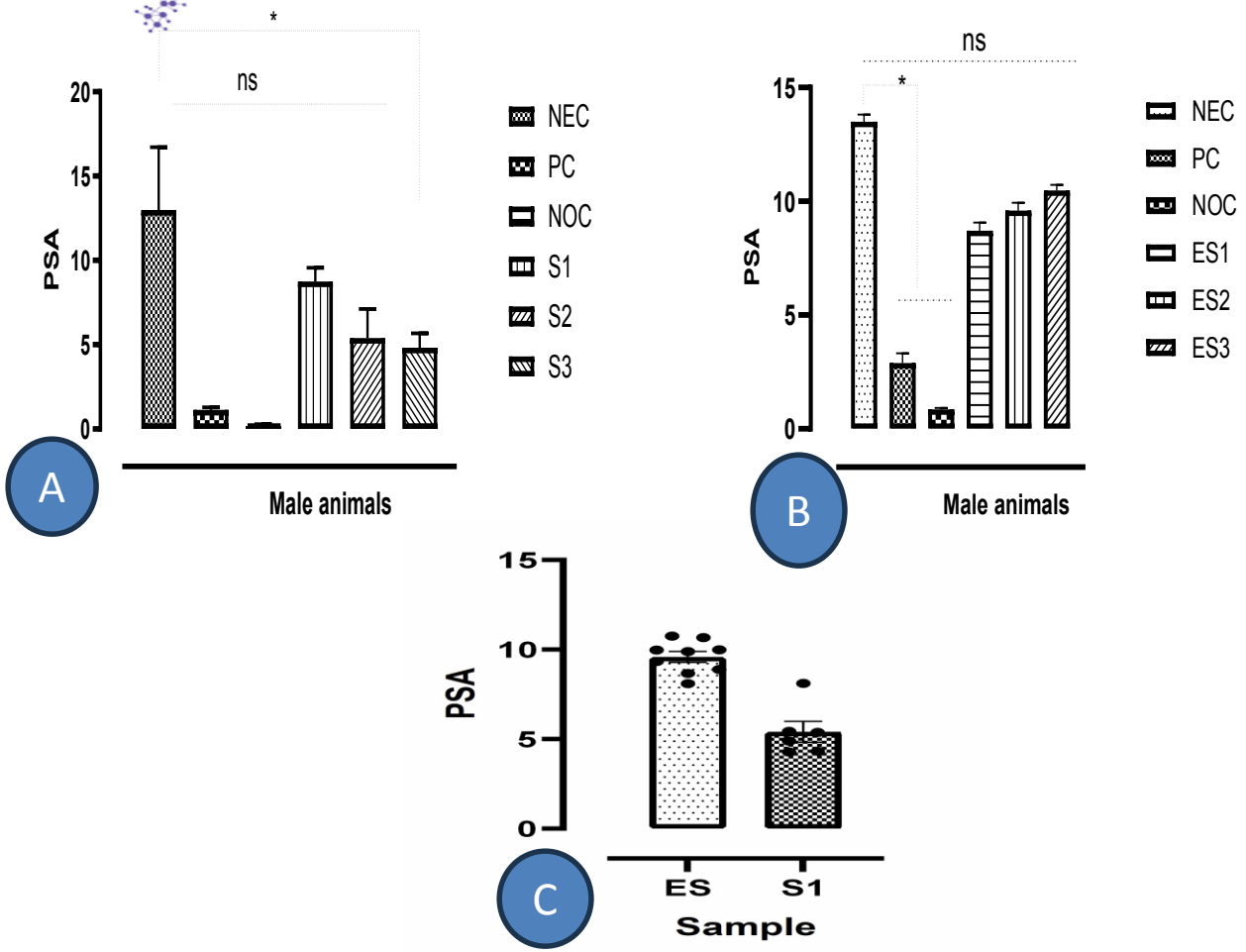


Figure 6: Dissolution Profiles of Crude and Microencapsulated *S. mombin* Extract



NEC = distilled water

PC= positive controls = 100 mg/kg Finasteride,

NC= Normal controls (no induction of BPH)

S1, S2 and S3 = Crude *S. mombin* extract- 100, 200 & 400 mg/kg.

ES1, ES2 and ES3 = microencapsulated *S. mombin* extract - 100, 200, & 400 mg/kg.

The oral administrations of the samples were done for each group for 28 consecutive days.

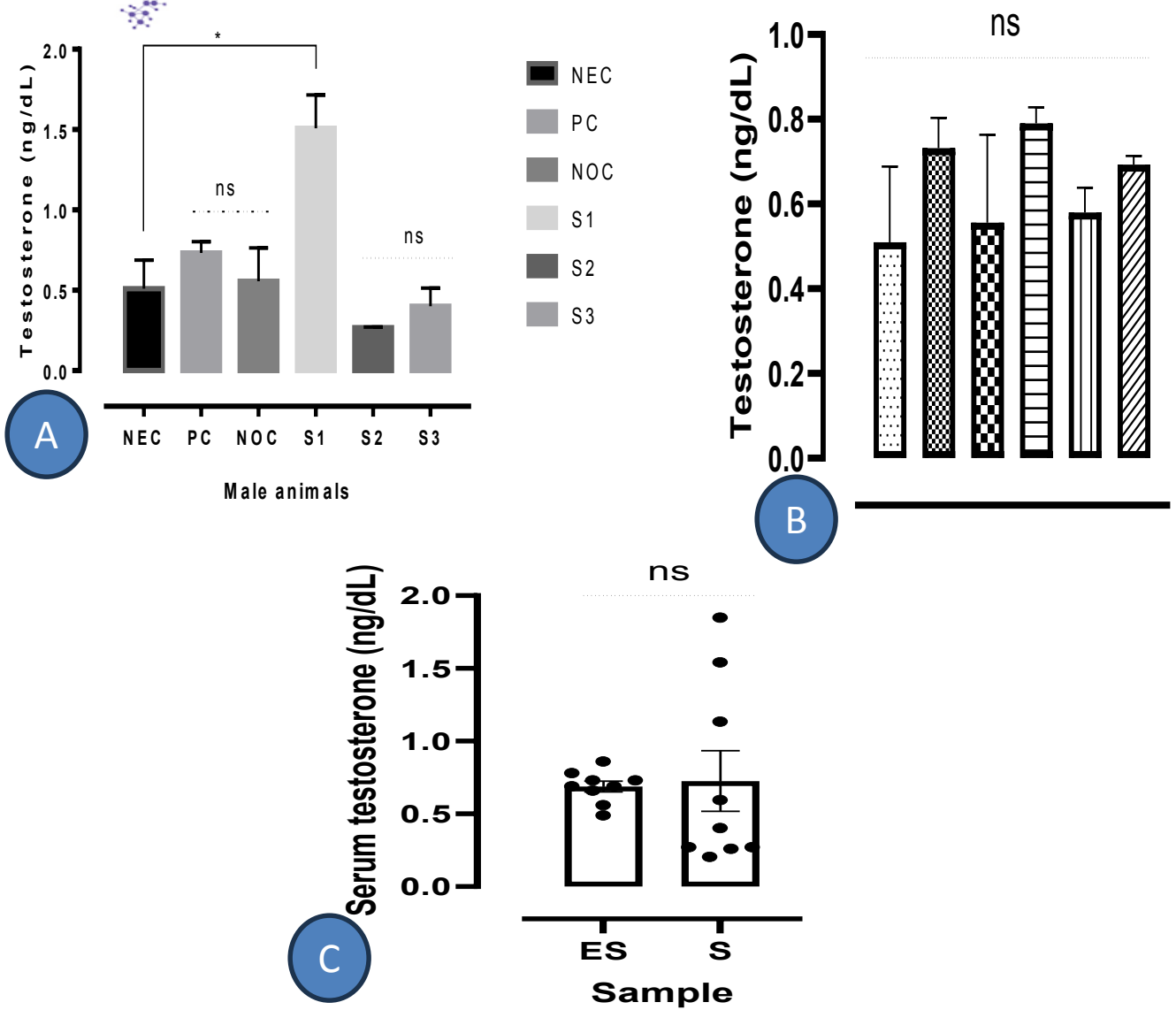
Figure 7: Effect of Crude (A) and Microencapsulated (B) *S. mombin* Extracts, Comparison of Crude and Microencapsulated *S. mombin* Extracts at 400 mg/kg on Serum Prostate Specific Antigen (PSA) in BPH-induced Male Wister Rats

In Vitro Pharmacological Evaluation

Prostate Specific Antigen (PSA) Reduction: *S. mombin* extracts demonstrated a dose-dependent reduction in PSA as presented in Figures 7(A, B and C). The crude extract, as presented in Figure 7A, exhibited increasing reduction in PSA with increase in dose concentration. The maximum reduction in PSA for crude extract is at 400 mg/kg. The microencapsulated extract, as shown in Figure 7B, showed less reduction in PSA with increase in dose (diminishing efficacy higher doses). The maximum reduction in PSA for microencapsulated extract is at 100 mg/kg. Generally, the crude extract exhibited superior PSA reduction activity than microencapsulated extracts with the most significant PSA reduction effect observed in Figure 11 at 400 mg/kg of the crude extract.

Serum Testosterone reduction: The extracts extracts are presented in Figures 8 (A, B, and C). They showed appreciable increase in testosterone levels at 100 mg/kg, compared to the positive control. A reduction in serum testosterone below the testosterone reduction of the positive control was observed upon increase in dose concentration for both the crude and microencapsulated extracts of S. mombin. expecially at 200 mg/kg as shown in Figures 8 A and B respectively.

Increase in testosterone level in positive control is an indication with 5-alpha-reductase drug (finasteride) in treating BPH. This drug blocks the conversion of testosterone to dihydrotestosterone (DHT), helping to keep testosterone (like testosterone replacement therapy) to improve quality of life, address inflammation, and reduce symptoms of BPH (Rastrelli et al., 2019). Alpha-blockers and 5-alpha reductase inhibitors are two common treatments for BPH in conventional medicine (Lepor, 2016; Kim et al., 2018)



NEC = distilled water

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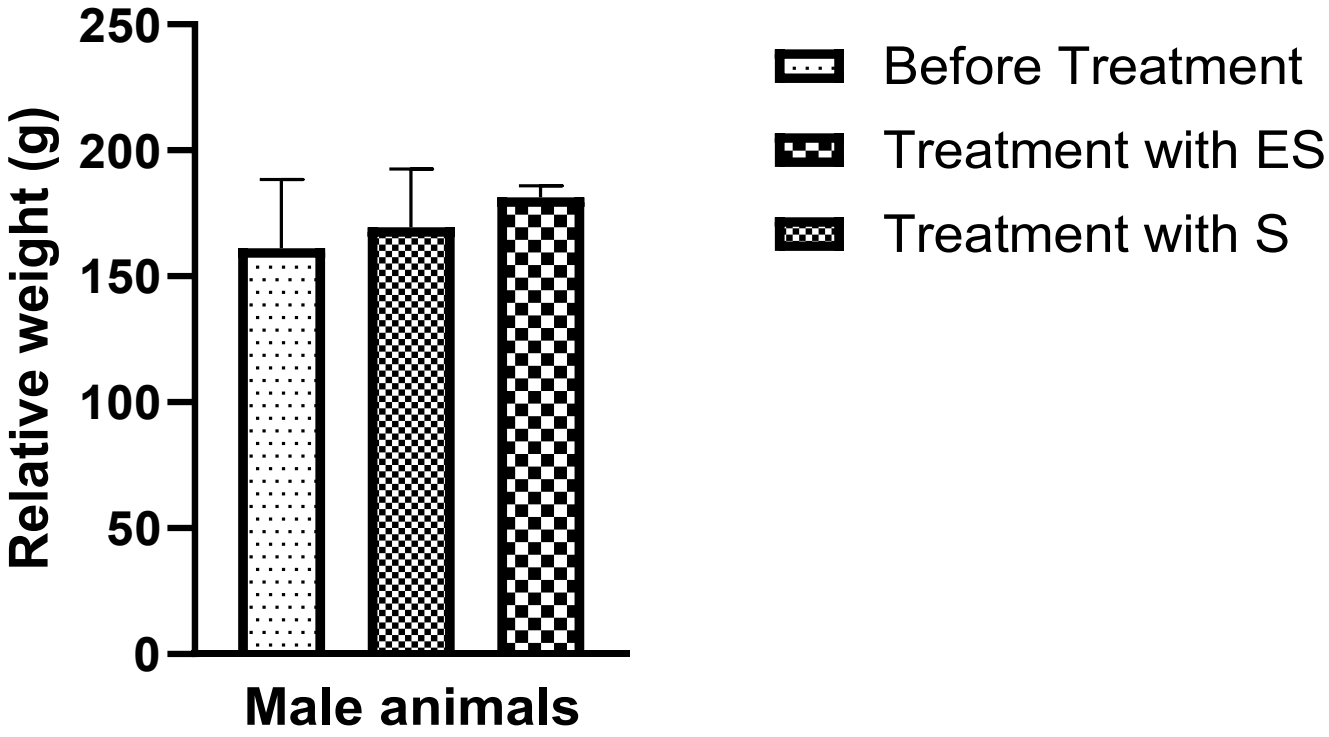
ES1, ES2 and ES3 = Microencapsulated *S. mombin* extract - 100, 200 & 400 mg/kg,

Mean of six replicates. n=6. Values with superscripts * indicate a significant difference relative value of negative control p<0.05 using Dunnett´s test

Figure 8: Effect of *S. mombin* Crude (A), and Microencapsulated (B) Extracts, and Comparison of Crude and Microencapsulated Extract (C) on Serum Testosterone Level in BPH-induced Male Animals



D - relative weight of the male animals before and after treatment with S and ES)

Figure 9: Animal Weight Before the Induction and After the Treatment of induced BPH in Male Animals

Weight reduction and Percentage Prostate index

Weight reduction: Both crude and microencapsulated extracts treated rats with induced BPH showed relative increase in animal weight, with the crude extract inducing less weight gain (Illustrated in Figure 9). The varied effects on weight may be attributed to differences in formulation and drug release profiles.

Percentage prostate index: The percentage prostate indices result presented in Figure 10 showed that crude and microencapsulated *S. mombin* extracts induced a reduction in the percentage prostrate index, although the percentage reduction is not significant $p<0.05$

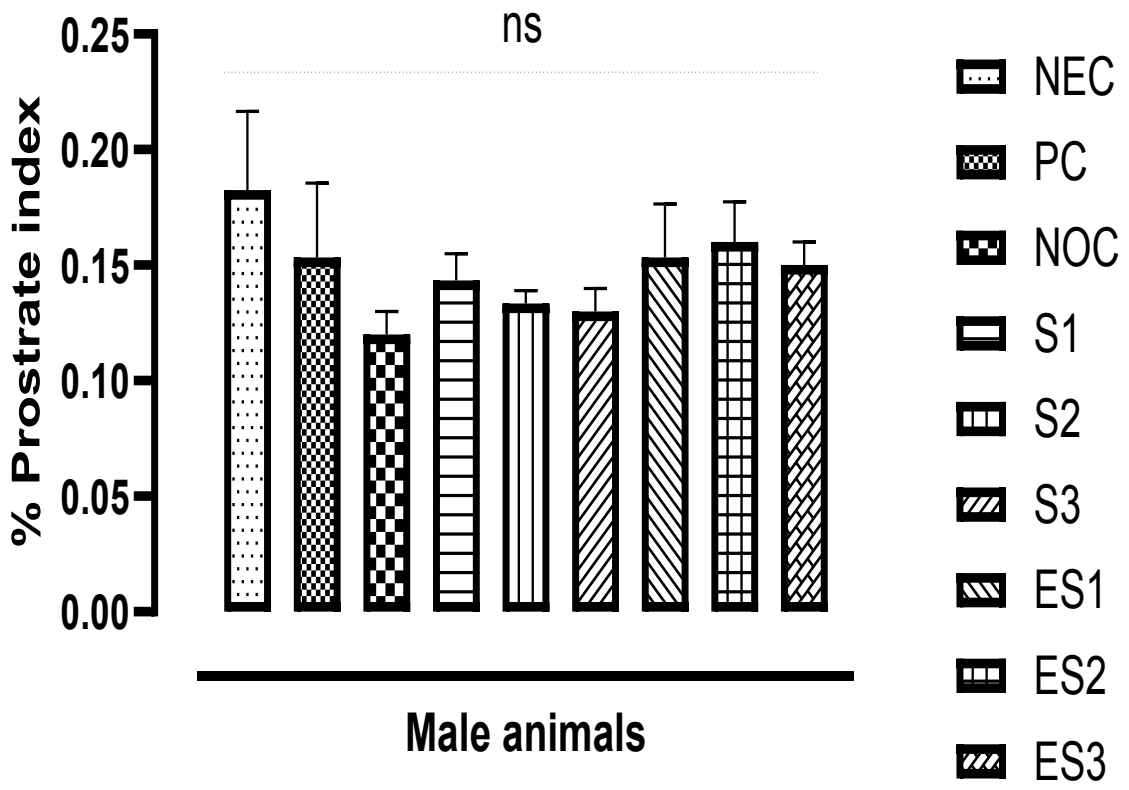


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Figure 10: Percentage prostrate index of the animal induced with BPH

DISCUSSION

- The micromeritic studies showed improved flow properties for the microencapsulated extract with angle of repose < 27.9°, Hausner's ratio < 1.06, and compressibility index < 6.06 %. SEM analysis showed a larger particle size of 50–80 µm for microencapsulated extract compared to >50 µm of crude extract.
- FT-IR and EDX analysis revealed relatively unchanged spectrum and additional molecules respectively in the modified extract compared to the crude extract. These results are suggestive of chemical compatibility and physical agglomeration in the interaction of the crude extract with microencapsulation excipients.
- Dissolution studies revealed steady release of extract from the microencapsulated form over 60 minutes, and > 9.76 times increase in peak release compared to release from the crude extract.
- The microencapsulated extract showed peak reduction in PSA at 100 mg / kg, compared to peak reduction of PSA of crude extract at 400 mg / kg. Serum testosterone levels decreased significantly in the microencapsulated extract.

Conclusions

The variations in efficacy between the crude and microencapsulate *S. mombin* extracts, especially in PSA reduction, serum testosterone levels, and percentage prostate index, suggest that the encapsulation process influenced the bioavailability and pharmacological anti-BPH effect of *S. mombin*. This reduced anti-BPH effect in microencapsulated *S. mombin* extract might be linked to the delayed release of the plant extract by the acacia gum matrix.

Microencapsulation and freeze drying significantly improved the processing ability and dissolution properties of *Spondia mombin* crude extract, and retained its pharmacological activities. There is a need for optimization to improve the anti-BPH activity, and also to conduct human clinical trials to further explore the potentials of this microencapsulated extract.

Acknowledgments

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