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The synthesis of chitosan-sodium tripolyphosphate nanoparticles for delivery of *Ocimum africanum* extract

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Abstract. The aim of the present work is to obtain *Ocimum africanum* extract nanoparticles (OaE-NPs) that have been crosslinked via ionic gelation with chitosan (chi) and sodium tripolyphosphate (TPP). Nanoencapsulation will enable investigation of the morphology profile and size of OaE-NPs. The OaE-NPs produced via ionic gelation exhibited a 6.4 nm in size and a low polydispersity index (PDI) of 0.177. Additionally, SEM observation revealed that the surface shape of OaE-NPs was a loose aggregate with an uneven surface. These findings provide a simple and reliable approach for generating nanoparticles from *O. africanum* coated with chitosan/TPP.

1 Introduction

Recent advances in a wide range of scientific fields have provided the groundwork for nanotechnology, which draws on fields as diverse as chemistry, engineering, biology, and physical sciences [1]. Numerous researchers have regarded it as a promising topic owing to its wide range of practical uses, including biosensing materials, automobiles, electronics, personal care products, and antimicrobials [2–5]. There has been a demand for a less expensive, less harmful, and more environmentally friendly alternative to the current methods of synthesizing nanoparticles because of the many problems caused by the high cost and toxicity of these methods [6,7].

Aside from the utilization of metals, there has been growing interest in the new trend of nanoparticle generation using polymers. Biopolymeric utilization increases the therapeutic effect by minimizing costs, avoiding adverse effects, and controlling drug distribution [8,9].

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Chitosan/TPP nanoparticles are desirable as possible drug delivery vehicles because of their biocompatibility [10], muco-adhesiveness [11], and easy manufacturing techniques [12]. Proteins encapsulated by these nanoparticles can protect against denaturation and retain their bioactivity [13,14]. The size of nanoparticles determines their biological fate; hence, managing their average size and polydispersity has garnered considerable interest [15,16]. Currently, medicinal plants are gaining interest in the green synthesis of nanoparticles because they can accelerate the conversion of metal ions into biologically active nanoparticles in an eco-friendly manner [17]. Medicinal plants with high levels of alkaloids, phenolics, and tannins offer low toxicity, high yield, cost-effectiveness, and flexibility, which can be used to synthesize nanoparticles [18].

Ocimum is the most important genus of the Lamiaceae family. The genus *Ocimum* has approximately 65 species that have a long history of use in the traditional medicine [19], fragrant [20], and pharmaceutical industries [21]. *O. basilicum* is the most studied among other *Ocimum* species, such as *O. gratissimum*, *O. tenuiflorum*, *O. sanctum*, *or O. africanum* (Table 1). *O. basilicum* contains essential chemicals, including phenolic acids, sesquiterpenes, phenylpropanoids, anthocyanins, and others, which are well known and serve as antioxidants, anticancer, anti-inflammatory, UV-ray protecting effects, as well as positive effects on reproductive health, such as protection of spermatozoa and an increase of testosterone levels [22–27]. Also, numerous evidence reported that several researcher have successfully built nanoparticles from *O. basilicum* [28–32]. This indicated that other species of the *Ocimum* genus might possess the capability to be utilized as nanoparticles.

O. africanum (lemon basil) is commonly found in the Southeast Asia region as a side-dish vegetable in several cuisines. A previous study reported that *O. africanum* contains phenols, flavonoids, and terpenes [33]. The essential oils of *O. africanum* are predominantly neral, geranial, (*E*)-caryophyllene, germacrene, and alpha-humulene [34]. However, *O. africanum* is still underutilized compared with *O. basilicum* [35]. In the present work, we prepared nanoparticles of *O. basilicum* in chitosan cross-linked with TPP using the ionic gelation method. Therefore, we measured their morphology and size after the nanoparticle preparation.

Table 1. A summary of research was done using the genus *Ocimum*.

No.	Contribution	Source	References
1.	Antidiabetic activity prediction through Keap1/SIRT1/ NF-κB Signalling Pathway	*O. basilicum*	[25]
2.	Improve spermatozoa quality on mice exposed to cigarette smokes	*O. basilicum*	[36]
3.	Copper oxide nanoparticles (CuO-NPs) synthesized at room temperature (under 70 nm range in size)	*O. basilicum* plant extract	[28]
4.	Titanium dioxide nanoparticles (TiO_2-NPs) by the green synthesis method (50 nm in size)	*O. basilicum*	[29]
5.	Zinc oxide nanoparticles (ZnO-NPs) with hexagonal in shape (9 to 18 nm in size)	*O. basilicum* leaf extract	[30]
6.	Identification of essential oils from different parts of plants	*O. africanum*	[33,34]
7.	Antiproliferative activity against the MCF-7 cell line	*O. sanctum*	[37]
8.	No inhibitory effect on the reproductive function and fertility of adult male albino rats	*O. gratissimum* methanol and oil extracts	[38]
9.	Extend the storage time of chilled dog semen	*O. gratissimum* ethanolic leaves extract	[39]

2 Materials and methods

2.1 *O. africanum* extraction

Ocimum plants were obtained from a local market in Malang, East Java, and Indonesia. The plant was identified as *O. africanum* in the UPT Herbal Laboratory, Materia Medica Batu, with ID number 067/1503/102.20/2023. Fresh leaves of *O. africanum* were washed three times with distilled water. The leaves were then air-dried for seven days and ground (or pulverized) with a blender (Miyako, Japan). The powder of *O. africanum* leaves (200 g) was then macerated with 750 mL of methanol, stirred, and kept standing overnight. The *O. africanum* extract (OaE) was then filtered and concentrated using a vacuum evaporator. The filtrate was stored at -20°C until use.

2.2 Nanoparticle synthesis

Chitosan/TPP/OaE was formed using the ionic gelation method. Approximately 5 mg of OaE was added to 0.2% chitosan solution at pH 4.5 and mixed gently. Next, a 0.1% TPP solution (pH 4.5 was added to the mixed solution of OaE-chitosan. The mixture was maintained under constant magnetic agitation (1500 rpm at 50°C) for two hours.

2.3 Nanoparticle of *O. africanum* confirmation

The particle sizes of the OaE nanoparticles were measured using a Particle Size Analyzer (VASCO Flex^{TM}). The morphology and particle surface were observed using scanning electron microscopy (SEM) (Hitachi Flexsem 100, Japan). Briefly, OaE nanoparticles (OaE-NPs) were coated with gold by fine coating (Quantum®) under vacuum. The OaE-NPs were observed at 500 × and 10,000 × magnifications.

3 Results and discussion

3.1 Size and visualization of *O. africanum* nanoparticles

Nanoparticles are molecules with sizes ranging from 1 to 1000 nm. The PSA results suggested that nanoparticles were successfully produced (Figure 1). OaE-NPs were created by ionic gelation using a polymer matrix of chitosan and tripolyphosphate. PSA analysis revealed three peaks with average particle sizes of 6.4 nm and polydisperse indices of 0.177 (Figure 1). The OaE-NPs contained tiny nanoparticles, and the sample's polydisperse index was less than 0.5, indicating good results. Agglomeration can cause non-uniform particle sizes and distributions by combining smaller particles. This can be caused by several factors, including the chitosan and TPP combination formula, pH of the solution, and speed at which the stirrer is used [40,41].

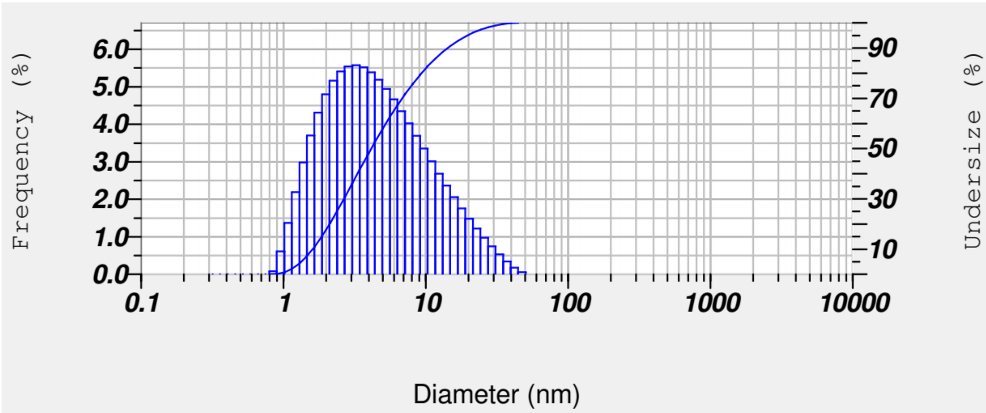

Fig. 1. PSA results of OaE-NPs.

The SEM-based characterization of TPP-chitosan nanoparticles (Figure 2A-B) revealed their shape and topography with clearly visible surface pores. The SEM characterization results demonstrated that the surface of the OaE-NPs was a loose aggregate with an uneven surface. Figure 2 shows the SEM results.

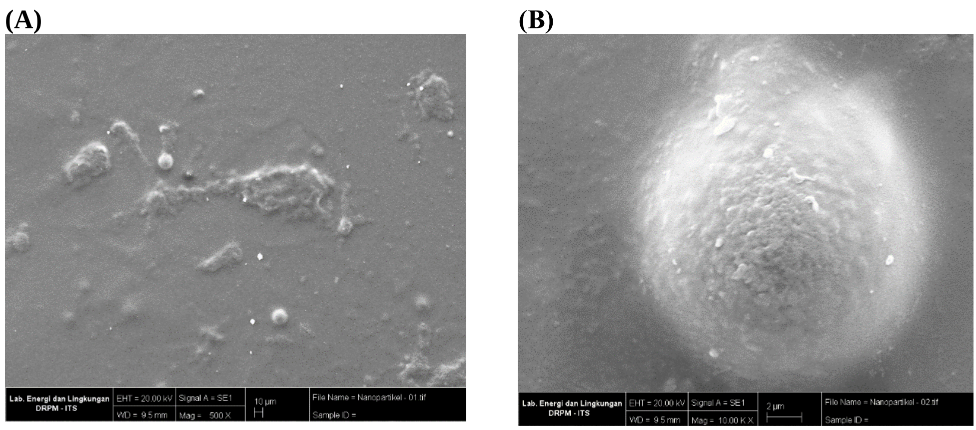

Fig. 2. SEM observations of OaE-NPs. (A) 500x, (B) 10.000x.

4 Conclusion

In summary, the present study successfully generated nanoparticles of *O. africanum* extract coated with chitosan and TPP. The size of the OaE-NPs was in the range of the nanoparticle size (6.4 nm) with a polydisperse index of less than 0.5. SEM observations suggested that the OaE-NPs were loose aggregates with uneven surfaces. Further in vitro and in vivo studies are required to investigate the mechanism of action of OaE-NPs.

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