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Original Article

SYNTHESIS OF ENCAPSULATED *CHROMOLAENA ODORATA* LEAF EXTRACT IN CHITOSAN NANOPARTICLE BY USING IONIC GELATION METHOD AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

Objective: The aim of this study was to determine the antioxidant activity of *Chromolaena odorata*.

Methods: Encapsulation of *Chromolaena odorata* leaf extract by nano chitosan was synthesized by using chitosan and NaTPP as the crosslinking agent. The antioxidant activity was conducted by using the DPPH method.

Results: Nanoparticles of *Chromolaena odorata* leaf extract has an average diameter of 675±218 nm and+23.4±7.14 mV of zeta potential. The antioxidant activity of its extract was 0.86 ppm, while its nanoparticle has the better antioxidant activity of 0.21 ppm.

Conclusion: Nanoparticles of *Chromolaena odorata* have very strong antioxidant activity and the potential to be external antioxidants.

Keywords: Nanoparticle, Plant extract, *Chromolaena odorata*, Antioxidant activity

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INTRODUCTION

Degenerative diseases are associated with aging that affects the function and structure of organs or tissues [1]. Degenerative diseases occur when cells and tissues lose their optimal function. The presence of chronic inflammation is one of the causes of degenerative diseases [2]. Diabetes mellitus, cardiovascular disease, cancer, Parkinson's and Alzheimer's are degenerative diseases which are health problems in the world [3]. Oxidative stress is one of the causes of degenerative diseases due to DNA damage [4]. Oxidative stress occurs when there is an imbalance between reactive oxygen species and antioxidants [1].

Chromolaena odorata is a plant pest because it is a competitor of the surrounding plants in absorbing water and nutrients. However, on the other hand, this plant also has various potential benefits for human life, in agriculture, it can be used as organic fertilizer, biopesticides and herbicides. In the medical field, traditionally, it can be used as medicine for wounds, diabetes, coughing and stopping bleeding [5].

Nanoparticles are colloidal particles that can act as drug delivery according to the desired target. In its manufacturing process, widely used polymers are derived from natural sources, synthetic or semisynthetic, which can be biodegradable or not [6]. Chitosan is a natural polysaccharide derived from chitin (which is in crustaceans) through the process of deacetylation. Chitosan use has increased rapidly over the past few years, especially in the pharmaceutical and food fields. This is due to low production costs, biodegradability and biocompatibility [7, 8].

The technique that can be used to increase the biological activity of compounds is by encapsulating in nanometric sizes [7]. Previous studies have shown that curcumin diethyl disuccinate encapsulated by chitosan-tripolyphosphate nanoparticles has better antioxidant activity when compared to without encapsulation [9]. As well as clove essential oil [CEO] encapsulated in chitosan nanoparticles by using the emulsion-ionic gelation method has higher antioxidant activity than free CEO [10]. The same results also on Catechin Hydrate [CH] nanoparticles which have better antioxidant activity [67.01±0.15%] when compared to pure CH [65.69±0.34%] [11]. There was no one has reported the antioxidant activity of *Chromolaena odorata* loaded chitosan nanoparticles even though empirically, it has been widely used for treatment.

MATERIALS AND METHODS

Materials

The materials used is Chromolaena odorata leaf from Tasikmalaya, Indonesia. The other are ethanol 96% (Bratachem®), Chitosan (Bratachem® from shrimp, Deacetylation Degree of 99%), NaTPP (Bratachem®), vitamin C (Bratachem®), 1,1-diphenyl-2-picrylhidrazyl (DPPH) (Sigma Aldrich®), deionized water.

Methods

Extraction of *Chromolaena odorata* leaf

Chromolaena odorata leaf was extracted with 96% ethanol solvent using maceration method for 3 d while shaken occasionally, then extract was filtered. The result was collected and concentrated with a rotary evaporator at 60^{0}C to obtain a concentrated extract.

Phytochemical screening of *Chromolaena odorata* leaf

Flavonoids

Simplicia powder was inserted into a test tube that has been filled with water. Then it was heated and filtered. The obtained filtrate was added magnesium and alcohol with hydrochloric acid by comparison 1:1. Mixture was shaken for 5 min. The formation of red, yellow or orange filtrate that can be pulled by amyl alcohol indicates the presence of flavonoids [12].

Tannins-polyphenols

Simplicia powder was put into a test tube that has been filled with water, it was heated, then filtered. Filtrate was divided into two parts. Solution of iron (III) chloride was added into the first part, the formation of blue-black color indicates the presence of tannins and polyphenols. Gelatin 1% was added into the second part; the formation of white sediment indicates the presence of tannins [12].

Saponin

Simplicia powder was extracted using water; then, it was heated and filtered. The obtained filtrate was cooled, then it was shaken firmly for a few minutes. The diluted hydrochloric acid added the formation of foam as high as 1 cm indicates presence of saponin compounds [12].

Steroids

Ether was added to the simplicia powder, then evaporated. After that, Liebermann-Burchad's reagents were dropped on residues,

purple color formation indicates the presence of steroid-triterpenoids [12].

Monoterpenoid-sesquiterpenoid

Simplicia powder was extracted using ether and then it was evaporated. Vanillin-H_2SO_4 or sulfate reagents were added to the residue. The presence of monoterpenoid and sesquiterpenoid compounds is indicated by the formation of color [12].

Synthesis of *Chromolaena odorata* nanoparticle

Nanoparticle of *Chromolaena odorata* was synthesized by using an ionic gelation method. 10 mg of extract produced was mixed with 3.5 ml of 0.05% chitosan. The mixture was homogenized at a speed of 1000 rpm for 24 h in a room temperature. After being homogeneous, 0.01% of NaTPP was added as much as 3.5 ml drop by drop then stirred again for 3 h. The nanoparticle produced was characterized by Particle Size Analyzer [PSA] to determine the particle size distribution and zeta potential to determine its stability [13].

DPPH radical scavenging assay

Antioxidant activity of extract and nanoparticle was determined by using the DPPH method. Test solution was mixed with DPPH at a ratio of 1:1 [volume]. It was incubated at room temperature in a dark place for 30 min. Then, it was measured for its absorbance at a wavelength of 517 nm using the UV-Vis spectrophotometer. The percentage of antioxidant activity was calculated by following:

$$\text{Inhibition [\%]} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of Blank}} \text{x100\% ..... [1]}$$

IC_{50} [Minimal concentration that can inhibit 50% of free radical] values can be calculated using a linear regression analysis from the graph [14].

RESULTS

Phytochemical screening of *Chromolaena odorata* leaf extract

Phytochemical screening aims to find out what group of compounds are found in *Chromolaena odorata* simplicia and extract. The result of phytochemical screening can be seen in table 1.

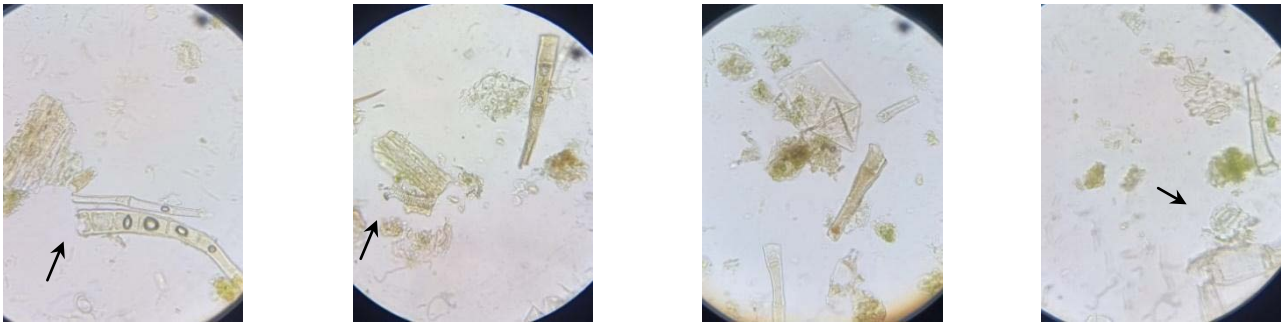
Table 1: Phytochemical screening of *Chromolaena odorata* leaf simplicia and extract

Phytochemical	Simplicia	Extract
Flavonoids	+	+
Polyphenols	+	+
Tannins	-	-
Saponin	-	-
Steroids	+	-
Seskuiterpenoid	+	+
Monoterpenoid	+	+
Quinone	+	+

The results in table 1 shows that both simplicia and extract contain flavonoids, polyphenols, and seskuiterpenoid, monoterpenoid and quinone compounds. Steroids exist on simplicia but they are not found on extracts. Macroscopic and microscopic analysis of *Chromolaena odorata* Leaf are shown in table 2 and fig. 1.

Table 2: Analysis macroscopic of *Chromolaena odorata* leaf extract

Characteristics	Extract
Color	Dark Green
Odor	Distinctive Smell
Taste	Bitter
Shape	Thick



Cover hair **Vessels with spiral thickening** **Calcium oxalate crystals** **Stomata**

Fig. 1: Analysis of microscopic *Chromolaena odorata* leaf

The extraction method used was the maceration method using 96% of ethanol as solvent. The result of the maceration process obtained liquid extract, which is evaporated to obtain concentrated extract. The result obtained from this maceration process was 7.26% as seen in table 3.

Table 3: The results of *Chromolaena odorata* leaf extraction

Chromolaena odorata leaf powder	Concentrated extract	Result (%)
900 g	65.33 g	7.26 % w/v

Characterization of *Chromolaena odorata* leaf extract loaded chitosan nanoparticle

Particle size analysis and zeta potential

Encapsulated *Chromolaena odorata* in Chitosan Nanoparticle [ECC] were prepared by using an ionic gelation method with NaTPP as a crosslinker. Distribution of particle size and zeta potential can be seen in fig. 2 and fig. 3 respectively. Particle analysis shows that the average diameter was 675.1±218 nm. Nanoparticles produced has a zeta potential value of+23.4±7.14 mV.

Antioxidant activity of *Chromolaena odorata* leaf extract loaded chitosan nanoparticle

The ability of nanoparticles to act as free radical scavengers was measured by the DPPH method. This method is widely used to observe the antioxidant activity. Extract of *Chromolaena odorata* Leaf (EC), Encapsulated *Chromolaena odorata* in Chitosan Nanoparticle (ECC) and vitamin C, as a comparison, have been measured for their antioxidant activity. The results can be seen in table 4

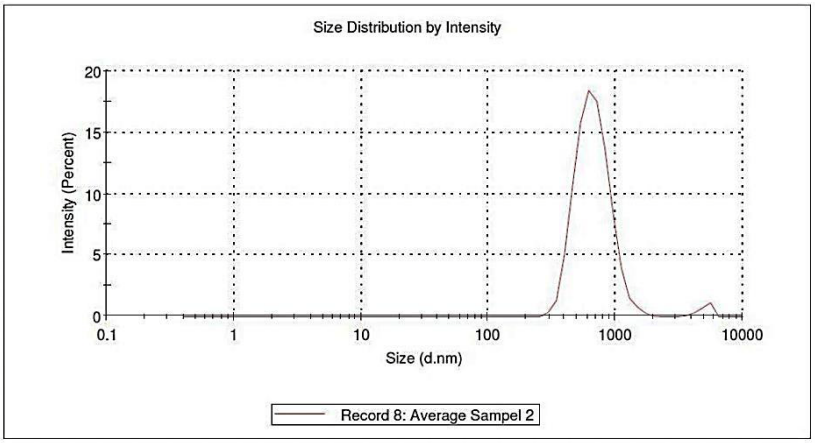


Fig. 2: Particle size analysis of nanoparticles

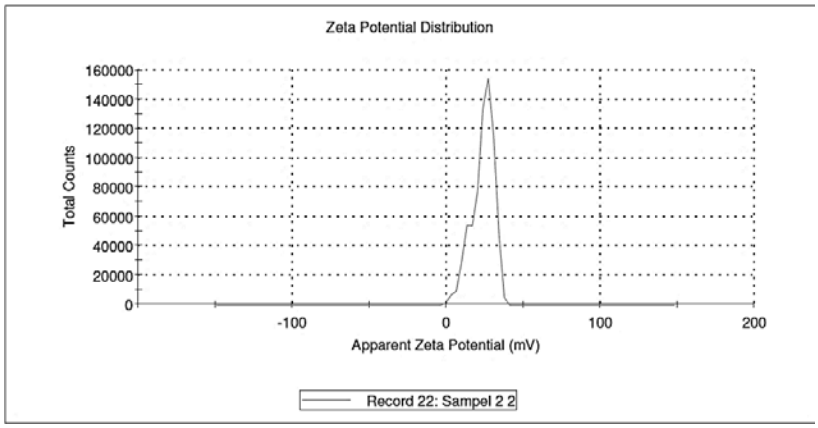


Fig. 3: Zeta potential analysis of nanoparticles

Table 4: IC_{50} value of EC, ECC and vitamin C

IC_{50} [ppm]		
EC	**ECC**	**Vitamin C**
0.86	0.21	0.14

DISCUSSION

The maceration method was chosen because it uses simple tools and processes, 96% of ethanol solvent is used because the target compound is a flavonoid that is soluble in ethanol. Flavonoids and polyphenols are compounds contained in *Chromolaena odorata* leaf that can provide antioxidant activity. Flavonoids have the ability to reduce free radicals and act as antioxidants. The mechanism of flavonoids as antioxidants is by donating hydrogen ions so that they can neutralize the toxic effects of free radicals. Phenolic compounds also have antioxidant activity because they can reduce free radicals by donating electrons through the hydrogen atoms of the hydroxyl group [15]. Steroids compounds are not found in extract but they do exist in simplicia. It indicates that the steroid is not attracted by ethanol solvents at the time of the maceration process. Ionic gelation is the easiest technique to produce nanoparticles. This technique is made through electrostatic interaction between cationic amino groups in chitosan with anions from tripolyphosphate [16]. Suspension is called nanoparticles when it has size of 10-1000 nm [17], so the extracts produced are already in the nanometer range. The nanoparticle has a polydispersity index of 0.380, indicating a homogenous dispersion because the value is between 0 to 1 [17].

Besides the determination of particle size, the measurement of potential zeta value is very important in producing nanoparticles. Zeta potential value shows the stability of nanoparticles, it is said to be stable if the value is lesser than-30 mV and more than+30 mV [18]., so it is still possible to become aggregates.

It was observed that the IC_{50} value of ECC is lower than EC, which means that its antioxidant activity is stronger and close to vitamin C. Flavonoid compounds contained in EC, as shown in the results of phytochemical screening, encapsulated by chitosan nanoparticle have shown enhanced antioxidant activity [11, 19].

This result was consistent with the research of Karimirad *et al.* stated that encapsulation of essential oil by chitosan nanoparticles can extend shelf life and there are more antioxidant enzymes than controls [7]. The increased antioxidant activity is also caused by chitosan which has hydroxyl and amine groups which can prevent free radical chain reactions [13, 20, 21].

Nanoparticle of *Parkia speciosa* leaves extract has IC_{50} value of 15.26 ppm [22], nanoparticle of *Garcinia mangostana* L. leaves extract has IC_{50} of 0.81 ppm [18] and nanoparticle of *Annona Muricata* L has bigger IC_{50} value, it was 46.88 ppm [17]. It means that nanoparticles of *Chromolaena odorata* leaves extract have better antioxidant activity when compared to the results of other previous studies.

CONCLUSION

Chromolaena odorata leaf extract encapsulated by nanochitosan has been successfully synthesized by using ionic gelation method. It has

an average diameter of 675±218 nm and+23.4±7.14 mV of zeta potential. Antioxidant activity of its extract is 0.86 ppm while its nanoparticle has the better antioxidant activity of 0.21 ppm.

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Nil

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

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