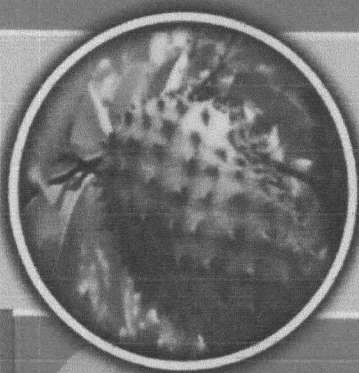
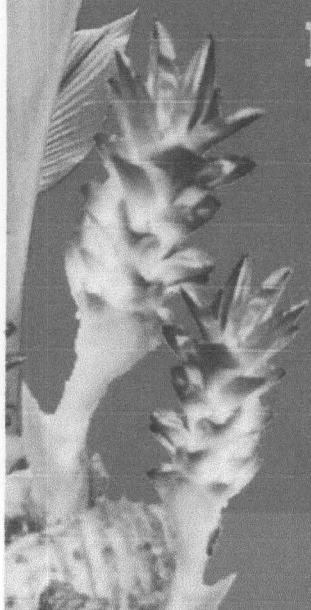


Proceedings



The 2nd International Symposium on Temulawak

The 40th Meeting of National Working Group on
Indonesian Medicinal Plant



**PROCEEDINGS OF
THE 2nd INTERNATIONAL SYMPOSIUM ON TEMULAWAK AND
THE 40th MEETING OF NATIONAL WORKING GROUP ON
INDONESIA MEDICINAL PLANT**



LAY-OUT

Titis Arifiana, SSi

December, 2011

ISBN No. 978-979-25-1209-0

ISBN 978-979-25-1209-0



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Preface

Alhamdulillah, thanks to God, the Almighty, finally we were able to publish the Proceedings of The Second International Symposium on Temulawak. It is now ready for distribution and circulation among the related researchers, industries, and scientists around the world.

On behalf of the Scientific's Team, we would like to express our sincere appreciation to all members of the Steering Committee and the Organizing Committee for their encouragement, contribution, and assistance during the symposium and made it as a successful one. We also grateful to our editors members for their cooperation and valuable contribution during the proceedings preparation.

This proceedings is consisted of 93 titles manuscripts, which are divided into 4 chapters, including botany, efficacy, technology, and toxicity. Almost of the manuscripts are reported as research results on medicinal plants, not only regarding the efficacy but also cultivation and harvesting, written by some local and international experts in their fields. Many of them are attracted the attention due to the information of huge contribution of temulawak both in the traditional and complementary medical systems, and it is intended to raise awareness on the prospects and usage of temulawak as an important alternative commodity.

We do believe that this Proceedings of The Second International Symposium on Temulawak would enrich our knowledge not only in *temulawak* (*Curcuma xanthorrhiza*) but also *takokak* (*Solanum torvum*), soursop (*Annona muricata*), etc., and turn out to be beneficial to mankind. We hope that it will serve as a useful reference for all concerned with the research and sustainable development of related industries.

Thank you very much.

Best regards,

The Editors

Remark from The Chairperson of Organizing Committee of

The 2nd International Symposium on Temulawak
the 40th Meeting of National Working Group on Indonesian Medicinal Plant

Praise and gratitude towards Allah who has given His Grace so that the Symposium with theme of globalization of Jamu Brand Indonesia could be conducted in IPB International Conference Center on May 24-29, 2011.

As Chairperson of the second International Symposium on Temulawak (*Curcuma xanthorrhiza*), which is also the 40th Meeting of National Working Group on Indonesian Medicinal Plant, I would like to welcome all of you in this symposium. It is my great pleasure that we successfully organized this even with other satellite meetings composing of workshop quality assurance, workshop on natural therapy, business meeting, and scientific output dissemination. We also successfully conducted *Batik* Design Competition and Temulawak Welcome Drink Formula competition. In conjunction with symposium presentation, there is the exhibition of Jamu Products held throughout the symposium period.

The symposium is organized by Bogor Agricultural University (IPB) in collaboration with various government and private institutions as well as foreign parties. The aim of the symposium is to promote the utilization and research development of temulawak as Indonesian medicinal herbal toward healthy life of global society.

The conference has brought 535 participants coming from Indonesia and overseas, 190 research paper with various topics coming from researcher of many institutions, 148 batik designs and 67 workshop participants, 14 invited speakers coming from Indonesia, Japan, Hungaria, India, Malaysia, and the Netherlands. There will be 48 oral presentations and 128 poster presentations coming from Indonesia and overseas. In this occasion we also launch the Indonesian road map Jamu and database of Indonesian jamu.

We wish to thank the meeting sponsors and co-sponsors for their support, without which this symposium would not have been possible. Their support greatly facilitated the participation of several researchers from abroad, accommodation, exhibition, small/medium entrepreneurs, and farmers to joint this event.

Ladies and gentlemen, and all participants,

Due to our limitation, we do apologize for any inconvenience during the symposium. We are grateful for the advice and support of the Steering Committee. We wish to the guest speakers, papers contributors, participants and sponsors for their cooperation in organizing this symposium.

Finally, on behalf of the Organizing Committee, I would like to express my gratitude and hoping that all of you will have a nice symposium and enjoy the atmosphere of Bogor "Rain" City.

Thank you,

Dr Min Rahminiwati

*Acknowledgement from
Rector of the Bogor Agricultural University*



RECTOR SPEECH

The 2nd International Symposium on Temulawak
the 40th Meeting of National Working Group on Indonesian Medicinal Plant

Assalamualaikum wr. wb.

Salam sejahtera bagi kita semua
Good morning.

It is our pleasure today that we are all here to attend the important event so called 'Globalization of Jamu Brand Indonesia' which consists of several agenda such as the 2nd International Symposium on Temulawak (*Curcuma xanthorrhiza*); the 40th Meeting of National Working Group on Indonesian Medicinal Plant; Workshops, Business Meeting, Jamu Festival, 'Jamu' Batik Design Competition and Temulawak 'Welcome Drink' Formula Competition here in Bogor, Indonesia.

As most of us may still remember that 3 years ago, in 2008, I attended 'Gelar Kebangkitan Jamu Brand Indonesia' and the First International Symposium on Temulawak. The event was officially opened by the President of the Republic of Indonesia, in *Istana Negara*, Jakarta; while the symposium was held here in the same place, IPB International Conference Center. Now, we are here again to attend the Globalization of Jamu Brand Indonesia. For the two important events, held in 2008 and 2011, multisectors and international stakeholders are involved, and IPB is significantly taking parts. This clearly indicates that continuous improvement to achieve our goal, to make Jamu for the World Quality of Life, is really our concern.

We all know that Indonesia is the second largest countries in the world regarding biodiversity. The Indonesian people are used to their natural resources with knowledge they inherited from their ancestors. For example, medicinal plants, animals, and microbes are applied as preventive, promotive and curative alternatives. Jamu has long been known and applied by the society; and nowadays, there is also an increasing tendency of using herbal medicine that is popularly known as 'back to nature' for curing diseases worldwide. Jamu business keeps increasing, I heard that almost reach to 10 trillion IDR in 2010; and the support from Indonesian government becomes stronger and real actions have also been conducted, such as: Scientification of Jamu, and Roadmap of Jamu Development will be launched and used as a national guidance for Jamu development. So I believe that through the strong commitment from Jamu stakeholders, our vision that Jamu for the World Quality of Life can be achieved in the near future.

Distinguish Guest, Ladies, and Gentleman,

Bogor Agricultural University (IPB) with its vision to becoming a world class research university with core competences in tropical agriculture and bioscience with entrepreneur character, and one of our mission is to improve the welfare of human beings through the application of developed science and technology are clearly in line with our effort to improve Jamu development.

Within this context, Biopharmaca Research Center IPB keeps focusing its research development and optimizing its efforts to develop qualified biopharmaca products with the support of strong networking with international and national partners. IPB also contributes to the national policy development of Indonesian biopharmaca and committed its existence in education and research development in order to achieve national and international reputation. Currently, IPB is proposing the establishment of Indonesian Biopharmaca Center (IBC) through the support of Japan International Corporation Agency (JICA), and the visibility study of the project is now being conducted. The IBC is expected to be a center of excellence in Biopharmaca Research Development within the country and will contribute to international reputation.

Today as part of Globalization of Jamu Brand Indonesia, we also have an important international event, namely "The 2nd International Symposium on *Temulawak (Curcuma xanthorrhiza)*" which is conducted by Biopharmaca Research Center, IPB in collaboration with Indonesian government institutions, private sectors, and foreign partners. The theme of the symposium is utilization and application of *Curcuma xanthorrhiza* through scientific and technological approach toward better and healthy life.

We know that temulawak is known as one of the Indonesian indigenous herbals, which mostly used as the main ingredient for traditional medicine or 'Jamu'. The popularity of temulawak is increasing along with its commercial use and research result applications. Many scientists have conducted research to reveal the secret of temulawak. Temulawak can be used for various purposes such as for maintaining human health, animal health, and supplement beverages to increase appetite and to keep fresh our stamina. As a continuation of our commitment, IPB is conducting various aspects of research based on temulawak, e.g. brain tonic, cardiovascular diseases, diabetic; further, our research output on avian flu has been registered for patent.

Nowadays, in the middle of modern lifestyle, temulawak is occupying place in our society's heart and with its various benefits, thus temulawak deserves to be Indonesian "ginseng" herbal. IPB fully support the three days activities involving researchers and scientists all over the world to share their experience and expertise which will be conducted in IPB International Convention Center.

We do hope that through Globalization of Jamu Brand Indonesia which are involving biopharmaca stakeholders within the country and abroad, modernization of Indonesian medicine/Jamu will be accelerated and generated benefits to increase our health and welfare.

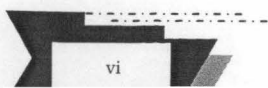
We would like to ask to the Minister of Coordinator of People's Welfare, Republic of Indonesia to officially open The Globalization of Jamu Brand Indonesia.

Finally, thank you to all of you who make this precious event possible.

Billahi taufik wal hidayah, wassalamualaikum wr.wb.

Bogor, May 26, 2011
Rector of IPB,

Prof Dr Herry Suhardiyanto, MSc



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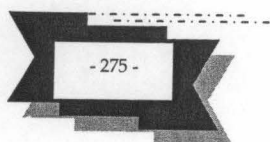
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Technology



ISBN No. 978-979-25-1209-0

Formulation and Physicochemical Characterization of *Curcuma xanthorrhiza* Roxb.-Loaded Chitosan Nanoparticle

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Abstract

Curcuma xanthorrhiza Roxb. is a traditional Indonesian medicine to nourish the liver. Due to poor water solubility of its major constituents such as xanthorrhizol and curcuminoid, its absorption upon oral administration could be limited. The purpose of this study was to discover the best method to prepare *C. xanthorrhiza* nanoparticles using ultrasonication-chitosan entrapment with tripolyphosphate anions. This research used variations of sonication time (30 and 60 minutes), usage of TPP, and addition process of *C. xanthorrhiza*. The *C. xanthorrhiza*-loaded chitosan nanoparticle were confirmed by scanning electron microscopy (SEM), Fourier transformed infrared (FTIR) spectroscopy, and X-ray diffractometry (XRD). Results have shown that the best method to formulate of *C. xanthorrhiza* nanoparticles was using addition of TPP and twice ultrasonication for 60 minutes. Thus, it would produce 222-3,500 nm sized particle. SEM imaging shown that chitosan was not loaded optimally, because the nanoparticles surface has been wrinkles and concave. The presence of *C. xanthorrhiza* in chitosan nanoparticle has been verified using FTIR, which showed the unique functional groups from major compounds in *C. xanthorrhiza*. The result of crystalline degree analysis using XRD showed amorphous structure of *C. xanthorrhiza* nanoparticles went down over ultrasonication time decreased from 60 minutes to 30 minutes.

Keywords: *Curcuma xanthorrhiza*, chitosan, nanoparticle, ultrasonication

Introduction

The tuber of *Curcuma xanthorrhiza* Roxb. is a commonly used as traditional Indonesian herbal medicine and food, especially as a tonic for the liver. In microbiological setting, *C. xanthorrhiza* has been used as antibacterial agents against *Streptococcus mutans* for the treatment of tooth caries (Hwang 2004, Rukayadi & Hwang 2006) and antifungal agents against *Candida sp.* for the treatment of candidiasis (Rukayadi & Hwang 2007). Previous studies have also investigated its pharmacological activities including anticancer (Choiet al. 2005, Kim et al. 2005), anti-aging (Lim et al. 2005), antihypercholesterolemia (Peschelet al. 2006), and antioxidants (Jayaprakasha et al. 2005).

The chemical constituents of *C. xanthorrhiza* include the curcuminoid and xanthorrhizol (Sembiring et al. 2006). These compounds have assumed as the ones which responsible for the pharmacological activities observed from *C. xanthorrhiza* (Peschelet al. 2006, Rukayadi & Hwang 2007). Since these constituents belong to the group of compounds with poor water solubility, their absorption upon oral administration could be limited, especially when prepared in the aqueous extract of *C. xanthorrhiza*. In order to overcome the poor water solubility problem, we choose ethanol 96% to extract the active compounds of *C. xanthorrhiza*.

Recently, the nanonization of medicines has attracted much attention (Poole & Owen 2003). Nanoparticle (nanospheres and nanocapsules) are colloidal systems with particles varying in size from 1 nm to 1 μ m (Jain 2008). Nanoparticles systems of curcuminoid have been reported in literature, although with particle size above 100 nm (Tiyaboonchai et al. 2007). Nanonization possesses many advantages, such as increasing compound solubility, reducing medicinal doses, and improving the absorbency of herbal medicines compared with the respective crude drugs preparations (Paolino et al. 2007).

In the present study, the ethanolic extract of *C. xanthorrhiza* was prepared, and its nanoparticles were produced by ultrasonication method. Our aim was to obtain the best method in production of *C. xanthorrhiza* extract nanoparticles with ultrasonication and characterization of nanoparticles obtained. This research variables included the method of addition process of *C. xanthorrhiza* extract and the sonication time (30 and 60 minutes).

Materials and Methods

Materials

Tripolyphosphate (TPP), ethanol, and acetic acid were purchased from Sigma. The deacetylation degree of chitosan used was 80.45% and the average molecular weight was 800 kDa. Other reagents used were coater reagent for SEM, KBr for FTIR, and aluminum plate for XRD analysis.

Extraction of *C. xanthorrhiza*

The method of extraction were followed the BPOM (2005) method with some modification. The main roots of *C. xanthorrhiza* were obtained from Biopharmaca Research Center, Bogor Agricultural University, and stored as 100 mesh powder with water content below 10%. Fifty grams of *C. xanthorrhiza* were soaked in 500 ml ethanol 96% and stirred for 6 hours. Mixture was kept for 24 hours and filtered. The filtrates were concentrated by rotary vacuum evaporation. The extract were collected and stored at -20 °C freezer until being used.

Preparation of *C. xanthorrhizana* nanoparticles

The preparation of *C. xanthorrhizana* nanoparticles was carried out by ultrasonication method (Nakorn 2008 & Kim et al. 2006) using variation in the addition process of *C. xanthorrhiza* extract and different setting of sonication time (30 and 60 minutes), in order to obtain uniform particles and avoid clustering of particles. Briefly, 5% *C. xanthorrhiza* extract solution was made from *C. xanthorrhiza* extract in 70% ethanol, 2% chitosan solution was made from chitosan in 2% acetic acid, and 0.5% TPP solution was made from TPP in water. Then, 100 ml chitosan and 50 ml TPP solution was filled in to 2 Erlenmeyer flasks; 1 ml extract solution was added to the first flask to make *C. xanthorrhizana* nanoparticle in one step, the second one was not added by the extract (blank). Both flasks were sonicated using 130 W, 20 kHz, and 40% amplitude ultrasonicator for 30 minutes, and then samples were lyophilized using spray dryer.

The powder obtained from the second flask was further dissolved in 100 ml of 2% acetic acid and 50 ml of water. Mixtures were homogenized using magnetic stirrer. Subsequently, solution was resonicated for 30 minutes and lyophilized using spray dryer.

Particle Size Analysis

The particle sizes were determined by using JSM 6510 Scanning Electron Microscope (SEM) at BATAN, Tangerang. Both sample powders were laid in a double alloy stub which size 1 cm in diameter. The stubs were hold to stick together using sticky tape. After that, samples were coated with thin layer platinum using 30 seconds light ray, 2 Pa pressures, and 30 mA intensity. Sample pictures were taken under 10 kV electron voltages.

Spectroscopy Characterization

The Fourier Transform Infrared (FTIR) spectra of curcumin, chitosan nanoparticles, and *C. xanthorrhiza* extract-loaded nanoparticles were measured by using FTIR spectroscopy. Two milligrams of samples were mixed with 100 mg KBr and printed to pellet form using vacuum accessory. The scanning range was 400-4,000 cm^{-1} , and the resolution was 1 cm^{-1} .

X-ray Powder Diffractometry

X-ray diffraction (XRD) pattern were taken with X-ray diffractometer. Two hundred milligrams of samples were finely placed in a 2 x 2.5 cm aluminum plate and the crystalline degree was measured using Cu X-ray diffraction with 1.5405 Å wavelengths.

Results and Discussion

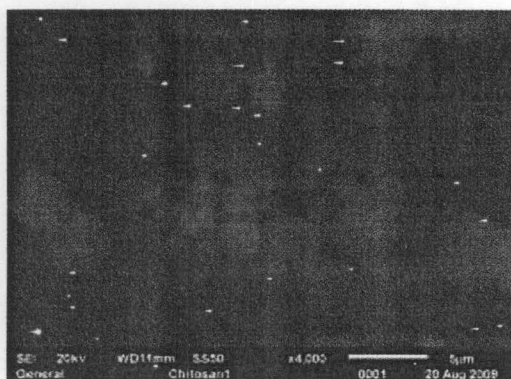
The concentrate of *C. xanthorrhiza* resulted from rotary vacuum evaporation were in thick paste form. Subsequently, it was spray dried to obtain a yellow dry powder. Table 1 showed the percentage of powder obtained comparing to its raw material.

Table 1. The yield of spray dried powders of *C. xanthorrhiza*

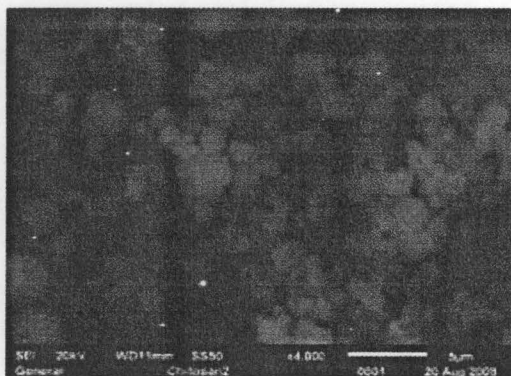
Samples	Powder Yields
Chitosan, TPP, and <i>C. xanthorrhiza</i> extract mixtures (one step ultrasonication)	37,48 %
Nanochitosan and <i>C. xanthorrhiza</i> extract mixtures (two step ultrasonication)	5,47 %

The nanotechnology of extract formulation has several advantages, i.e. enhancing the solubility of poor water soluble extract molecule, improving extract therapeutic effectiveness, ameliorating the bioavailability, and decreasing the dosage required for the same effects, compared with the crude extract (Yen et al. 2008). Chitosan is a polymer that has been widely used as emulsifier and suspension stabilizer in oral, liquid, and topic dosage forms (Nakorn 2008). Furthermore, chitosan could provide sufficient stabilization to the nanoparticles system (Ambarsari et al. 2009).

Scanning Electron Microscope (SEM) measurement is an effective method to provide the surface morphology and size. Figure 1 showed a representative SEM of dried *C. xanthorrhizana* nanoparticles which indicated that the shape of the particles formed was round. The approximate particle sizes using one and two step ultrasonication method were determined ranging from 400 to 5,000 nm and 400 to 2,500 nm respectively. Meanwhile, as showed in the Figure 2, particles size formed from 30 minutes and 60 minutes two step ultrasonication were determined ranging from 333 to 4,600 nm and 222 to 3,500 nm correspondingly.

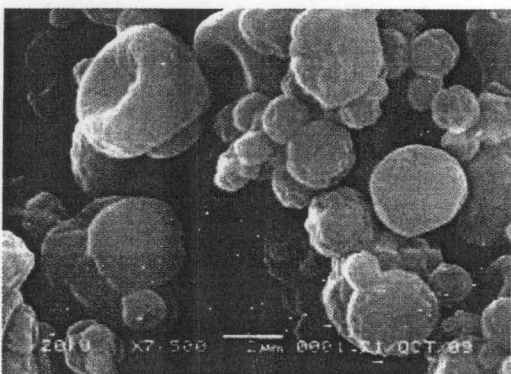


a

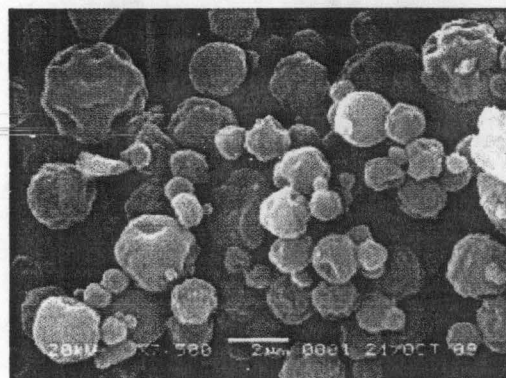


b

Figure 1. Scanning Electron Microscope images of *C. xanthorrhizana* nanoparticles using 30 minutes (a) one step ultrasonication and (b) two step ultrasonication methods at 4,000 times enlargement



a



b

Figure 2. Scanning Electron Microscope images of *C. xanthorrhizana* nanoparticles using (a) 30 minutes and (b) 60 minutes two step ultrasonication methods at 7,500 times enlargement

In the present study, the *C. xanthorrhiza* was successfully nanonized with nanochitosan to produce *C. xanthorrhiza*-loaded nanoparticles using two step ultrasonication method compared to single step ultrasonication method (Figure1). The possible explanation in generating *C. xanthorrhiza*-loaded chitosannanoparticles is that the hydrophobic portion of curcumin in *C. xanthorrhiza* extract interpenetrated into chitosan nanoparticles during the second sonication process and formed a nanocapsules system. On the other hand, the powder resulted from the two step sonication process was less than single step sonication process (Table 1). It possibly there was water molecule trapped inside the *C. xanthorrhizana* nanoparticles from single step ultrasonication process.

As presented in Figure 2, 60 minutes two step ultrasonication method produced smaller particles size compared to the 30 minutes two step ultrasonication process. It appears that the nanocapsules were uniform and round in shape with rough surfaces in both the two images. The results indicated that the length sonication time correspond linearly to the smaller of particles size.

As shown in Figure 3, for the spectra of *C. xanthorrhiza*-loaded nanoparticles with 30 and 60 minutes two step ultrasonication methods, the characteristic bands of free curcumin disappeared, while the absorption peaks assigned to chitosan appeared. It can be concluded that *C. xanthorrhiza* was not present on the surface but included inside the nanoparticles as nanocapsules.

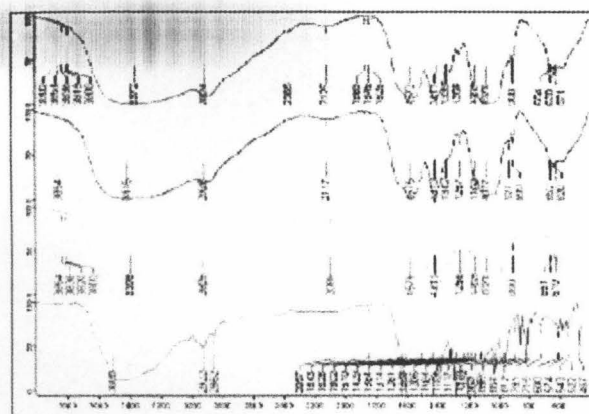


Figure 3. FTIR spectra of (a) *C. xanthorrhizana* nanoparticles with 60 minutes two step ultrasonication, (b) chitosan nanoparticles, (c) *C. xanthorrhizana* nanoparticles with 30 minutes two step ultrasonication, and (d) curcumin standard

During this experiment, a FTIR spectroscopy was adopted to detect the absorption features on the surfaces of the powder (Figure 3). The FTIR spectra of the *C. xanthorrhiza*-loaded nanoparticles showed no characteristic peaks of free curcumin, associated with -OH bending (3509 cm^{-1}), C-H stretching (2922 cm^{-1}), C=O (1628 cm^{-1}), C=C (1602 cm^{-1}), C-C (1429 cm^{-1}), C-O (1281 cm^{-1}), and C-H (812 cm^{-1}) bending (Colthup *et al.* 1975). It can be concluded that *C. xanthorrhiza* extract was not present on the surface but included inside the nanoparticles and formed nanocapsules.

According the X-ray diffractogram of chitosan powder, *C. xanthorrhizana* nanoparticles resulted from 30 and 60 minutes sonication showed crystalline degree 21.94%, 22.24%, and 23.58%, respectively. XRD analysis of chitosan and *C. xanthorrhiza*-loaded chitosan nanoparticles using 30 and 60 minutes two step ultrasonication method. X-ray diffractogram showed that the sonication time corresponded linearly to the crystallinity of the nanoparticles.

Acknowledgments

The authors are grateful to Mr. Taufiqurrahman Sidqi and Ms Tyas for their excellent technical assistance. We also thank Department of Biochemistry, Department of Physics, and Biopharmaca Research Center, Bogor Agricultural University for providing facilities and other needs in the research.

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ISBN 978-979-25-1209-0



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