JSMARTech
Journal of Smart Bioprospecting and Technology **p-ISSN**: 2686-0805 **e-ISSN**: 2714-7894
April 2022, VOL 03 NO 01 https://doi.org/10.21776/ub.jsmartech.2022.003.01.29

Microencapsulation Combination of *Nigella sativa* and *Cosmos caudatus* Kunth and In Vitro Protein Denaturation Inhibition Assay

Nadiyah Zuhroh[1], Anna Safitri[1,2]*

[1]Chemistry Department, Brawijaya University, Indonesia
[2]Research Centre of Natural Genetic Resources (SMONAGENES), Brawijaya University, Indonesia

Submission: 2 April 2022; **Revised**: 08 April 2022; **Accepted**: 26 April 2022

***Corresponding author**: Anna Safitri; **e-mail**: a.safitri@ub.ac.id

ABSTRACT. *Microencapsulation is a method of coating the active ingredients to form microparticles that can be used as a drug delivery agent. This study aims to determine the effect of sodium tripolyphosphate (Na-TPP) concentration and the stirring time in microencapsulation process of extract combination of N. sativa and C. caudatus K., as well as the ability to inhibit protein denaturation. Microencapsulation was carried out with various concentrations (w/v) of Na-TPP 0.2%; 0.4% and 0.6% and stirring time of 60, 90, and 120 min. The optimum conditions of microencapsulation were obtained at 0.2% Na-TPP concentration and a stirring time of 90 min, with mean diameter particle sizes of 37.68 µm. Characterization using FTIR showed that microencapsulation was successfully conducted from the absorption of the P=O functional group at wavenumber 1200 cm^{-1} which was a typical absorption of Na-TPP compounds and the presence of CN absorption at wavenumber of 1352 cm^{-1} which is an absorption peak of chitosan. In addition, SEM showed the surface morphologies of the resulted microcapsules were mostly spherical shapes. From the protein denaturation inhibition assay, the IC_{50} values were resulted 491.67 µg/mL, 113.37 µg/mL, and 90.96 µg/mL, for microcapsules, extracts only, and sodium diclofenas as a reference, respectively.*

Keywords: *microencapsulation, extracts combination, N. sativa, C. caudatus* K., *protein denaturation*

INTRODUCTION

The abundant potential of natural resources in Indonesia has led to many innovations based on the use of natural resources. In addition to its abundance, it is also due to the lack of side effects from natural resources produced. Several innovations have been proposed by combining herbal plants to improve their activity and potential as drug candidates. Black cumin (*Nigella* sativa) and *kenikir* (*Cosmos caudatus* Kunth) are herbal plants that are rich in benefits [1, 2]. But the combination of these two plants has never been carried out.

Nigella sativa is one of the herbal plants that are commonly found in Mediterranean countries and Southeast Asia, *i.e.* Indonesia [3]. Various studies on the immunomodulatory effects of *N. sativa* have also been investigated and it has been proven that *N. sativa* can enhance the immune response in humans [4].

Cosmos caudatus Kunth is a plant originating from the plains of America and currently its distribution is very wide, especially in tropical areas including Indonesia [5]. The active compound in *C. caudatus* K. shows its activity in inhibiting low-density lipoprotein (LDL) oxidation reactions in vitro, preventing oxidative damage and cell death by scavenging oxygen radicals, providing pharmacological effects as anti-inflammatory [6, 7].

Anti-inflammatory agent has an important role in the early prevention of a disease [1]. Traditional inflammatory therapy strategies mainly involve steroids and non-steroidal anti-inflammatory drugs, both of which have increasingly prominent side effects [8]. The combination of extracts form N. sativa and C. caudatus K has never been carried out, especially in assessment of anti-inflammatory activities.

Determination of the anti-inflammatory activity can be applied with the initial screening process, by conducting protein denaturation inhibition assay. Heating temperature is one of the factors that causes protein denaturation, since this affect hydrogen bonds and non-polar hydrophobic interactions in proteins [9].

Nevertheless, bioavailability is also an important factor before developing innovative plant combinations for a drug candidate. Bioavailability relates to the rate at which a drug affects absorption and when drug enters the membranes of the body's system. Various techniques are used to increase drug solubility through physical and chemical modification of the drug, including particle size reduction, crystallization, salt formation, solid dispersion, nanosuspension, and cryogenics [10, 11].

Among those various techniques, physical modification of drug products such as reduction of particle size and modification of crystal characteristics is a common approach to increase drug solubility. One of the methods is performing microencapsulation [12].

Microencapsulation assists in increasing a drug's distribution based on the particles' sizes. Microencapsulation is the process of coating the core materials in the form of solid particles, liquids, or gases using coating materials [12]. In addition, microencapsulation can increase the absorption of drugs when they enter the body, thus they can function optimally.

The ionic gelation method is a common method in microencapsulation, this method involves the use of polymer and crosslinker compound, like chitosan and sodium tripolyphosphate (Na-TPP) [13]. The principle of ionic gelation is an electrostatic interaction between the positively charged amine group of chitosan and the negatively charged Na-TPP polyanions (14).

Research on the concentration of Na-TPP used in the preparations of microcapsules has also not been widely carried out. In addition, duration of stirring time in the manufacture of microcapsules will also affect the particle sizes. Therefore, it is important to conduct microencapsulation the combination of *N. sativa* and *C. caudatus* K. extracts, under the influence of the Na-TPP concentration and stirring time on the microencapsulation process, also to determine the biological activity of the microcapsules as anti-inflammatory agent, by performing protein denaturation assay.

RESEARCH METHODS

Materials and Methods

Nigella sativa and *C. caudatus* L. dried powder were obtained from UPT Lab. Herbal Materia Medica, Batu, East Java. The chemicals used in this research were purchased from Merck: ethyl alcohol (99.7%), glacial acetic acid (secondary pharmaceutical standard), alpha-amylase from Aspergillus oryzae (≥150 units/mg protein), chitosan (low-molecular weight, 50.000-190.000 kDa), sodium tripolyphosphate (Na-TPP, technical grade, 85%), aluminium chloride ($AlCl_3$), 3,5-dinitrosalicylic acid (DNS) reagent (≥98%, HPLC grade), 2,2-diphenyl-1-picrylhydrazyl (DPPH, reagent grade), soluble starch (from potato, ACS grade), and bovine serum albumin (≥96%)). The instrumentations used in this research were Shimadzu UV-Vis spectrophotometer, FTIR spectrometer from Shimadzu Prestige 21, CILAS 1090 PSA (particle size analyzer), and SEM TM 3000 Hitachi.

Preparations of *N. sativa* and *C. caudatus* K. extracts

The dried powder of *N. sativa* and *C. caudatus* K. extract was immersed separately in 400 mL of ethyl alcohol for 3x24 h. The extracts were filtered using filter paper and concentrated with a rotary evaporator. For extracts combination, 1 g of *N. sativa* and *C. caudatus* K. extract was dissolved in 5 mL of ethyl alcohol separately. The mixture was concentrated with a rotary evaporator to obtain a thick mixture of *N. sativa* and *C. caudatus* K. extracts.

Microencapsulation of Combination of *N. sativa* and *C. caudatus* K. Extracts

The microencapsulation of *N. sativa* and *C. caudatus* K. extracts was carried out by weighing 500 mg of extract mixture. The extract mixture was then dissolved in 17.5 mL of ethanol, then 50 mL of chitosan solution in 1% (v/v) glacial acetic acid solution was added. Next, 200 mL of Na-TPP was gradually added with various concentrations of 0.2%; 0.4% and 0.6% (w/v) while stirring at a steady speed for 90 minutes. The suspension resulting from microencapsulation of chitosan and Na-TPP was then freeze-dried they became powder. The procedures were repeated under different stirring times of 60, 90, and 120 min. Using PSA, the optimum concentration and stirring time were determined based on the smallest particle size.

Protein Denaturation Inhibition Assay

Protein denaturation inhibition test was carried out based on [15] with modifications. The microencapsulated of extract mixture were prepared with various concentrations of 100 to (g/mL. Diclofenac sodium as a standard anti-inflammatory drug was used as a positive control, the concentrations used were 20 to 100 (g/mL. A total of 400 mL of 1% (w/v) solution of bovine serum albumin in PBS solvent was added to 100 mL of the tested sample. Then, the solution was incubated at 37 °C for 30 minutes, heated to 51°C for 5 minutes, and heated to 51°C for 5 minutes. The mixture was cooled at room temperature for 10 min, the absorbance of the sample was measured at a wavelength of 278 nm using a UV-Vis spectrophotometer. The calculation of the percentage inhibition of protein denaturation is calculated using the following equation:

% Inhibition =(Abs sample – Abs control)/(Abs sample) x 100%

The results on these calculations are reported as IC_{50} values, which was calculated by drawing linear regression equation between concentration (x-axis) and % inhibition (y-axis).

Data Analysis

The calculation results of protein denaturation inhibition assay were analyzed using the One-Way ANOVA test with a 95% confidence level ($\alpha = 0.05$) to determine the effect of the IC_{50} value on the test sample. The Kolmogorov-Smirnov test first analyzed the data for normality and homogeneity of data variance. Statistical tests were carried out with the normal distribution of data and homogeneous data variations. Data analysis was performed using SPSS v.25 software.

RESULTS AND DISCUSSION

In the manufacture of microcapsules, the combination of *N. sativa* and *C. caudatus* K. extracts was carried out under the influence of the concentration of Na-TPP as a cross-linker agent and the stirring time. The microencapsulated of extracts combination results were in the form of green powder which was then characterized using PSA to determine the optimum results based on smallest particle sizes. Particle size is one of the parameters for the successful microencapsulation process. One of the aims of microencapsulation is to facilitate a drug in the distribution process in the circulatory system. The smaller the particle sizes, the easier the drug distribution process to the target system.

Table 1 PSA Results of microencapsulated extracts combinations of *N. sativa* and *C. caudatus* K. under the influence of Na-TPP concentration

Na-TPP concentration (w/v)	Diameter 10% (µm)	Diameter 50% (µm)	Diameter 90% (µm)	Mean Diameter (µm)
0.2%	4.42	28.71	85.54	37.68
0.4%	48.90	136.73	307.08	158.73
0.6%	58.35	157.35	419.28	206.54

Based on Table 1, the particle sizes of N. sativa and C. caudatus K extract combination microencapsulated nanoparticles at the Na-TPP concentration variation were getting smaller as the Na-TPP concentration decreased. This means that the concentration of Na-TPP in the manufacture of microencapsulation affects the particle sizes. In this study, at 0.2% Na-TPP concentration with the smallest particle size, with a mean diameter of 37.68 µm. In addition, from the distribution spectrum of PSA results, the 0.2% Na-TPP concentration (Fig 1a) had a homogenous particle sizes distribution compared to other concentrations (Fig 1b and 1c).

The resulting optimum particle sizes are expected to carry the optimal amount of drug with a smaller dose, therefore more efficient in its application. Particle sizes are one of the factors that determine the efficiency of absorption and affects the release of active compounds. The smaller microencapsulated size provides better drug release [16]. If the concentration of Na-TPP is too high, this can cause the active compounds to be more challenging to diffuse out from the microcapsules [14]. The increase in the Na-TPP concentration may also cause an increase in the viscosity of the active compound dispersion system due to the production of large particles that require high stirring strength and a longer stirring time to break the particles and a perfect coating process (13).

Table 2 PSA Results of microencapsulated extracts combinations of *N. sativa* and *C. caudatus* K. under the influence of stirring time

Stirring time (min)	Diameter 10% (µm)	Diameter 50% (µm)	Diameter 90% (µm)	Mean Diameter (µm)
60	3.31	31.35	98.17	42.24
90	4.42	28.71	85.54	37.68
120	6.58	36.59	106.92	48.46

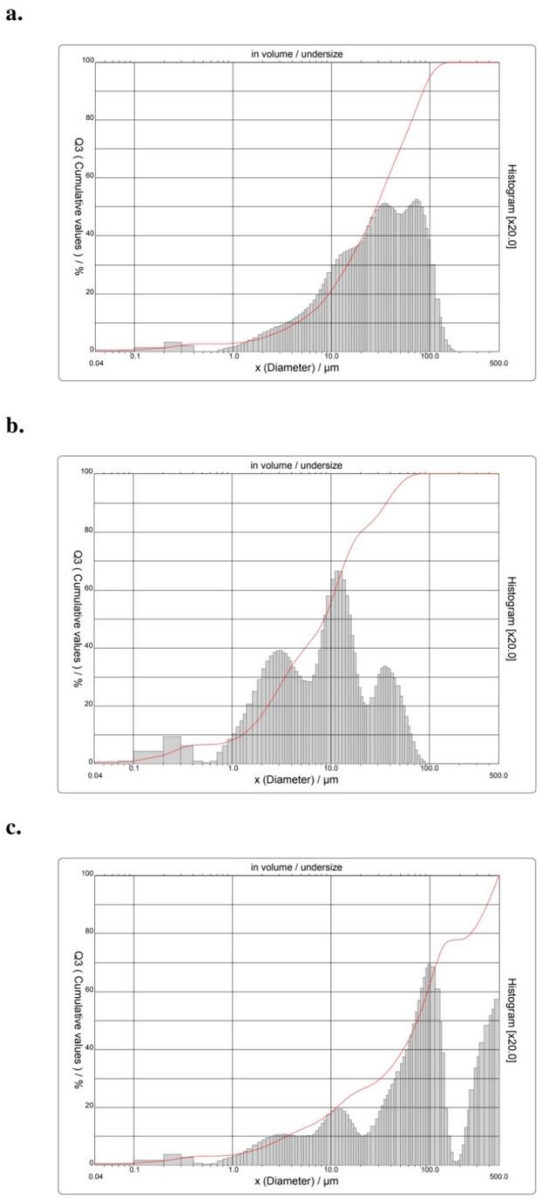

Figure 1. The PSA results of microencapsulated *N. sativa* and *C. caudatus* K. extracts under Na-TPP concentration variations of: a. 0.2%; b. 0.4%; and c. 0.6%.

Stirring in the manufacture of microencapsulation is a reaction process for the formation of encapsulates where homogeneous stirring can help the crosslinking reaction run perfectly. In addition to the concentration of the cross-linker material, in the manufacture of microencapsulation is also influenced by stirring time. In addition, the collision process in stirring produces an energy that will affect the size or surface of a particle [17].

In this study, the optimum stirring time achieved at 90 min. This can be seen from the size of the particles

formed based on the results of PSA analysis (Table 2). The mean diameter of the microencapsulated particles with a stirring time of 90 minutes was 37.68 µm. The PSA spectra as shown in Figure 2 also support that 90 min had the most uniform particle sizes distribution (Fig 2b) among other stirring time (Fig 2a and 2c).

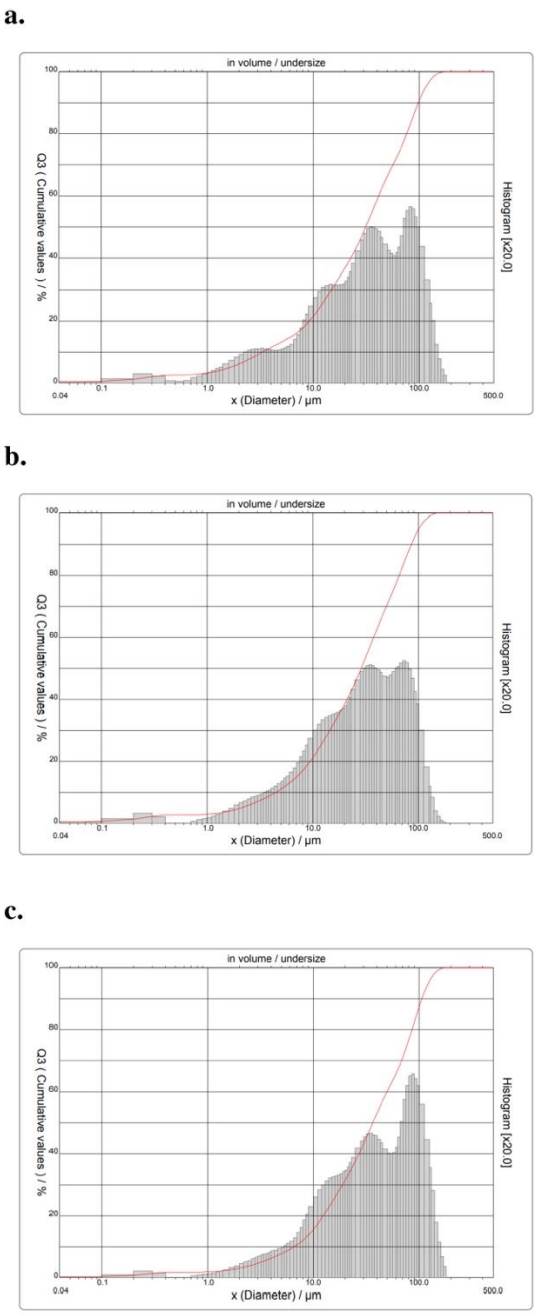

Figure 2. The PSA results of microencapsulated *N. sativa* and *C. caudatus* K. extracts under stirring time variations of: a. 60 min; b. 90 min; and c. 120 min.

Protein denaturation inhibition test is one of the screening methods in determining anti-inflammatory drug candidates. Determination of inhibition of protein denaturation was conducted by measuring absorption by UV-Vis spectrophotometry. Compounds that inhibit protein denaturation greater than 20% can be used as a reference value in the development of anti-inflammatory drugs [18).

Based on Table 3, the activity as protein denaturation inhibitor from the combination of *N. sativa* and *C. caudatus* K. extracts (non-microencapsulated) was higher than that in microencapsulated of the extracts combination of *N. sativa* and *C. caudatus* K. Also, the diclofenac sodium had the lowest IC_{50} values, indicating the highest activity among three samples.

These results indicate that the active compounds in the *N. sativa* and *C. caudatus* K. extracts can inhibit protein denaturation process. Inhibition by the combination of *N. sativa* and *C. caudatus* K. extracts in this process can be influenced by one of the bioactive compounds contained; therein, such as timoquinone and alpha hederin from *N. sativa* or quercetin and kaempferol from *C. caudatus* K [19].

Table 3. Results of the protein denaturation inhibition assay

Sample	IC_{50} (µg/mL)*
Microencapsulated *N. sativa* and *C. caudatus* K. extracts	491.67^{c}
N. sativa and *C. caudatus* K. extracts (non-microencapsulated	113.37^{b}
Diclofenac sodium	90.96^{a}

*Different notations indicate significant differences between condition using the One-Way ANOVA test with a level of confidence α = 5%.

The presence of these compounds in *N. sativa* and *C. caudatus* K. extracts can protect the protein in the denaturation test by stabilizing the protein bonds. Therefore, there is no continuous denaturation. Another thing is based on the structure of the active compound extract in *N. sativa* and *C. caudatus* K. extracts which are mostly flavonoid compounds, with the -OH group in the structure of the active compounds, that are able to stabilize the protein structure with its lone pair of electrons [20).

There are several compounds that inhibit a protein from the process of protein aggregation or denaturation, namely by stabilizing its structure, including polymer compounds and small molecules in the category polyphenolic compounds [21].

Microencapsulated of extracts combination resulted in the lower biological activity than that non-microencapsulated of extract combination. These can be caused by encapsulated materials that protects the active compound. The microencapsulation has the role of protecting the drug until it reaches the target place; therefore, it is necessary to release the bioactive compounds first, until they producesperformance on the target, or it can also be called a controlled release [22].

CONCLUSION

This study has effectively carried out the microencapsulation of *N. sativa* and *C. caudatus* K. extracts using the freeze-drying technique with chitosan and Na-TPP as wall materials. The optimum concentration Na-TPP in the manufacture of microencapsulation of *N. sativa* and *C. caudatus* K. extracts were 0.2% with a stirring time of 90 min, resulted in mean particle sizes of 37.68 μm. The microencapsulated of extracts combination had the IC_{50} value of 491.67 μg/mL, when tested as protein denaturation inhibitor.

ACKNOWLEDGEMENT

The Indonesian Endowment Fund for Education/Lembaga Pengelola Dana Pendidikan (LPDP) from the Indonesian Ministry of Finance has provided the financial support for the study.

REFERENCES

[1] Bordoni, L., Fedeli, D., Nasuti, C., Maggi, F., Papa, F., Wabitsch, M., et al. 2019. Antioxidant and Anti-Inflammatory Properties of Nigella sativa Oil in Human Pre-Adipocytes. *Antioxidants*. 8(2):51 (12 pages).

[2] Daud, D., Moh Fekery, N.F., Hashim, N. 2017. Reproductive Health of Rats with Benign Prostatic Hyperplasia Following Cosmos caudatus Ethanolic Extract Consumption. *J Appl Pharm Sci*. 7(6):202–205.

[3] Parveen, A., Farooq, M.A., Kyunn, W.W. 2020. A New Oleanane Type Saponin from the Aerial Parts of Nigella sativa with Anti-Oxidant and Anti-Diabetic Potential. *Molecules*. 25(9):2171 (13 pages).

[4] Kooti, W., Hasanzadeh-Noohi, Z., Sharafi-Ahvazi, N., Asadi-Samani, M., Ashtary-Larky, D. 2016. Phytochemistry, pharmacology, and therapeutic uses of black seed (Nigella sativa). *Chin J Nat Med*. 14(10):732–745.

[5] Bunawan, H., Baharum, S.N., Bunawan, S.N., Amin, N.M., Noor, N.M. 2014. Cosmos Caudatus Kunth: A Traditional Medicinal Herb. *Glob J Pharmacol*. 8(3):420–426.

[6] Abdullah, A., Dhaliwal, K.K., Roslan, N.N., Lee, C.H., Kalaiselvam, M., Radman, H.M. et al. 2015. The Effects of Cosmos caudatus (Ulam Raja) on Detoxifying Enzymes in Extrahepatic Organs in Mice. *J Appl Pharm S*ci. 5(1):82–88.

[7] Murugesu, S., Perumal, V., Balan, T., Fatinathan, S., Selvarajoo, P.D., Rozali, M.A.B., et al. 2020. A Review of Cosmos caudatus as A Promising Antidiabetic Plant. *Malays J Med Health Sci*. 16(4):333–3443.

[8] Cui, J., Jia, J. 2020. Natural COX-2 Inhibitors as Promising Anti-inflammatory Agents: An Update. *Curr Med Chem.* 27(1):1–25.

[9] Fatchiyah, F., Setiawan, B., Suharjono, S., Noor, Z. 2015. The anti-osteoporosis effects of CSN1S2 protein of goat milk and yoghurt on a complete Freund's adjuvant-induced rheumatoid arthritis model in rats. *Biomark Genomic Med*. 7(4):139–146.

[10] Corrêa-Filho, L.C., Lourenço, M.M., Moldão-Martins, M., Alves, V.D. 2019. Microencapsulation of *β* -Carotene by Spray Drying: Effect of Wall Material Concentration and Drying Inlet Temperature. I*nt J Food Sci.* 2019:1–12.

[11] Ćujić-Nikolić, N., Stanisavljević, N., Šavikin, K., Kalušević, A., Nedović, V., Samardžić, J., et al. 2019. Chokeberry polyphenols preservation using spray drying: effect of encapsulation using maltodextrin and skimmed milk on their recovery following in vitro digestion. *J Microencapsul.* 36(8):693–703.

[12] Ang, L.F., Darwis, Y., Por, L.Y., Yam, M.F. 2019. Microencapsulation Curcuminoids for Effective Delivery in Pharmaceutical Application. *Pharmaceutics*. 11(451):1–23.

[13] Safitri, A., Roosdiana, A., Kurnianingsih, N., Fatchiyah, F., Mayasari, E., Rachmawati, R. 2022 Microencapsulation of Ruellia tuberosa L. Aqueous Root Extracts Using Chitosan-Sodium Tripolyphosphate and Their In Vitro Biological Activities. *Scientifica*. 2022:1–10.

[14] Ariadi Lusiana, R., Putri Protoningtyas, W., Ricky Wijaya, A., Siswanta, D., Mudasir, M., Juari Santosa, S. 2017. Chitosan-Tripoly Phosphate (CS-TPP) Synthesis Through Cross-linking Process: the Effect of Concentration Towards Membrane Mechanical Characteristic and Urea Permeation. *Orient J Chem.* 33(6):2913–2919.

[15] Truong, D.H., Nguyen, D.H., Ta, N.T.A, Bui, A.V., Do, T.H, Nguyen, H.C. 2019. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and *In Vitro* Anti-Inflammatory Activities of *Severinia buxifolia*. *J Food Qual*. 2019:1–9.

[16] Safitri, A., Roosdiana, A., Hitdatania, E., Damayanti, S.A. 2022. In Vitro Alpha-Amylase Inhibitory Activity of Microencapsulated *Cosmos caudatus* Kunth Extracts. *Indones J Chem*. 22(1):212-222.

[17] Burhan, A.M., Abdel-Hamid, S.M., Soliman, M.E., Sammour, O.A. 2019. Optimisation of the microencapsulation of lavender oil by spray drying. *J Microencapsul.* 36(3):250–266.

[18] Adarsh Verma, M., Ajay Kumar, P., Kavitha, D, Anurag, K.B. 2011. Anti denaturation and antioxidant activities of Annona cherimola in-vitro. *Int J Pharma Bio Sci.* 2(2):1–6.

[19] Zuhroh, N., Ningsih, Z., Safitri, A. 2021. An Insight of Co-Encapsulation Nigella sativa and Cosmos caudatus Kunth Extracts as Anti-

Inflammatory Agent Through In Silico Study. *J Kim Sains Dan Apl*. 24(5):152–160.
[20] David, A., Arulmoli, R., Parasuraman, S. 2016. Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. *Pharmacogn Rev*. 2016;10(20):84–9.
[21] Rajan, R., Ahmed, S., Sharma, N., Kumar, N., Debas, A., Matsumura, K. 2021. of the current state of protein aggregation inhibition from a materials chemistry perspective: special focus on polymeric materials. *Mater Adv*. 2(4):1139–1176.
[22] Cahyaningrum, S.E., Herdyastuti, N., Qomariah, N. 2015. Synthesis and Characterization of Chitosan- Alginate for Controlled Release of Isoniazid Drug. *Indones J Chem*. 15(1):16–21.