ISSN 0974-3618 (Print)
0974-360X (Online)

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RESEARCH ARTICLE

Synthesis and Optimization of Nanoparticle Chitosan-Tripolyphospate *Centella asiatica* using Ionic Gelation Method with Difference Sonification Time

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ABSTRACT:

Nanoparticles from natural polymer materials chitosan are widely applied in drug delivery systems because of their unique properties, such as biocompatible, biodegradable, mucoadhesive, and increased permeation. This study aimed to synthesize chitosan nanoparticles *Centella asiatica*. The method used is ionic gelation using the crosslinker polyanion tripolyphosphate (TPP) with a difference in sonification time: 90, 120, 150 minutes. The difference in sonification to obtain the preparation conditions that can produce particles under 200nm with a good level of dispersion and stability, a study on the effect of sonification time on chitosan nanoparticles physical characteristics is performed. The PSA analysis result showed that the average particle size of *C.asiatica* extracts at 90, 120, 150 minutes sonication was 286.2nm, 269.2nm, 299.1nm. The results of the examination using FTIR showed that the *C. asiatica* extract had N-H and P = O groups, which meant that the ammonium ion had interaction of chitosan with the polyanion of TPP and *C. asiatica* extract. N-H absorption of pure chitosan shifted from 1597.06cm-1 to 1640.56cm-1, indicating a crosslinking between ammonium ions in chitosan and phosphate ions from TPP and *C. asiatica* extract. The formulation of using XRD showed that the amorf form of nanoparticle *C. asiatica* extracts is supported by particle morphology imaged using SEM. Based on the results obtained, the synthesis of nanoparticles *C. asiatica* extract in this study has been successful.

KEYWORDS: *Centella asiatica*, Nanoparticles, Chitosan, Ionic gelation.

INTRODUCTION:

Most people have used a variety of Indonesian plants as traditional medicine for generations[1]. This is partly because raw materials are easily obtained and affordable and can be obtained without a doctor's prescription. One type of plant used in traditional medicine is *C. asiatica*.

The active compounds of *C. asiatica* are triterpenoid saponins, genins, essential oils, flavonoids, phytosterols, sugars tannins, amino acids, fatty acids, alkaloids, and mineral salts. *C. asiatica* extract in the form of herbal concoctions, caplets, and other preparations requires relatively large amounts, and the active compounds have low solubility in water. The active compounds with low solubility in water will affect the bioavailability of active compounds in the body. To overcome this problem, nanoparticles of chitosan TPP *C. asiatica* extract can be developed to increase active extract compounds' bioavailability[2-12].

Received on 25.05.2022 Modified on 09.08.2022
Accepted on 05.10.2022
Research J. Pharm. and Tech 2023; 16(8):3847-3851.
DOI: 10.52711/0974-360X.2023.00635

Today, the application of nanotechnology is extensive, including applications in the field of health and pharmacy, which include drug delivery, medical implants, and the cosmetics field[13]. Drug delivery efforts using nanoparticles will cause drugs to spread more easily in the blood and quickly give effect[14]. Nanoparticles that are often used as drug delivery and are not harmful to the human body are chitosan nanoparticles[15].

Chitosan is a natural polysaccharide that is non-toxic and quickly biodegradable[16]. The structure of chitosan resembles cellulose and can form a gel in an acidic atmosphere. Chitosan has properties as a matrix in drug delivery systems[17]. The advantages of this characteristic mean chitosan have extensive applications and use, such as the examples previously described. Besides, processing it into nanoparticles enables chitosan to be a more effective pharmaceutical or drug delivery agent. The greatest results can be achieved by its ability to achieve therapeutic action. In most treatments, some in conventional doses, only a small portion of the dose reaches the target. In contrast, most of the drug is distributed to other parts of the body according to its physicochemical and biochemical properties[18].

One method used for the synthesis of chitosan nanoparticles is ionic gelation[18]. Hence ionotropic gelation method can use to prepare these chitosan nanoparticles as it is very simple and having lots of advantages then other methods. In this method, the formulation principle of nanoparticles is electrostatic interactions between amine groups in positively charged chitosan and negatively charged TPP polyanion to form a three-dimensional intramolecular structure[19].

MATERIALS AND METHOD:

Material:

Centella asiatica simplicia, ethanol (Bratachem), chitosan (Sigma Aldrich), Tripolyphosphate (Sigma Aldrich), and acetic acid (Merck).

Extraction of *C. asiatica:*

Simplicia was macerated using 70% ethanol solvent, soaked for 24hours, then filtered. The maceration process was repeated three times until a clear colored filtrate was obtained. The filtrate obtained was concentrated with a rotary evaporator at 50ºC.

The nanoparticle of Chitosan TPP *C. asiatica* extract:

The method used is ionic gelation with a difference in sonification time: 90, 120, 150 minutes. 0.1g of *C.asiatica* extract was dissolved in 70% ethanol by 5 mL. Each of the 0.5%, 0.75%, and 1% chitosan solutions was put into 100ml of acetic acid and stirred until dissolved. Tripolyphosphate (TPP) as much as 0.1 g, 0.15g, and 0.2g were then dissolved, each with distilled water up to 20mL, 30mL, and 40mL. Then, at each concentration of the solution, 1mL of tween 80 was added and stirred using a homogenizer at 1,000rpm for 10minutes. The mixture of chitosan, TPP, and *C. asiatica* extract was homogenized using a disperser with a speed of 3,000rpm for 30minutes. The ultrasonication results were dried using freeze-drying to obtain powder samples.

Examination of Particle Size and Potential Zeta:

Particle size measurements and particle size distributions were performed using the Microtec Nanowave II Particle Size Analyzer. Samples of 1.0g nanoparticles were then added with distilled water up to a volume of 10mL and put in a cuvette. The cuvette filled with the sample was inserted into the sample holder, the tool turned on, and the particle size menu selected. The instrument measured the sample for 10 minutes. The data generated are a particle size calculated from fluctuations in the average intensity of light scattering.

Test of Functional Group Nanoparticles Chitosan-*C. asiatica* extract with FTIR:

The test was carried out using an Infrared Fourier Transform (FTIR FT1000). A total of 2.0mg of chitosan nanoparticles of extract C. asiatica was homogenized with 100mg KBr. The mixture was dried with a vacuum freeze dryer for one day. Furthermore, the powder mixture ticks were irradiated with infrared light.

Test of Crystality Nanoparticles Chitosan-*C. asiatica* extract with XRD:

The examination was carried out using X-Ray Diffraction (XRD). The 200mg sample was printed on a 2 x 2.5cm mold made of aluminum. The level of crystallinity was determined using XRD with a wavelength source of 1.5406°A[20].

Test of Particle Morphology:

Particle morphology examination was performed by Scanning Electron Microscope (SEM). This examination was carried out to find out the particle morphology. The 1g of sample was placed into a copper lattice coated with carbon, which was dried beforehand at room temperature, carried out at a voltage of 120KVA. The magnification used was 500x[21].

RESULT:

Test of Particle Size and Potential Zeta of Chitosan *C. asiatica* Nanoparticles:

The success of a sample into nanoparticles is characterized by measured sample size distribution. From the analysis of PSA chitosan nanoparticles, it is known that the average particle size and the addition of

TPP show the nanometer particle size. The examination results obtained an average particle size of chitosan nanoparticles of *C. asiatica* extract at sonication 90, 120, and 150 minutes, respectively of 286.2nm, 420nm, and 416 nm. In this study, TPP stabilizers aim to stabilize the particles by inhibiting the formulation of aggregates so that the expected average size of particles in chitosan-extract *C.asiatica* nanoparticles is smaller than the particle size of chitosan-extract. The chitosan particle size is 780-800 nm. The data showed that the synthesis of chitosan nanoparticles could reduce particle size, increasing the bioavailability of active compounds in the body. Details of particle measurement results are presented in Table 1.

Table 1: Particle size and zeta potential of chitosan extract *C. Asiatica* nanoparticles.

Time sonification (minute)	Particle size (nm)	Polydispersity Index	Zeta Potential value (mV)
90	293.20	0.195	20
120	420.00	0.145	20
150	416.00	0.060	20

The particle size obtained in this study meets the requirements for the formulation of nanoparticles, 10-10,000 nm. Many studies have shown that nanoparticles have several advantages over microparticles as a drug delivery system. Nanoparticles with size 100nm have an absorption capacity 2.5 times greater than microparticles, which are 1µm in size and have absorption times six times greater than microparticles that are 10µm in size. Based on the particle size and the size distribution of chitosan, *C. asiatica* extract can be the best drug delivery system.

Particle size and size distribution characteristics are essential in nanoparticle systems. Particle size and size distribution are determined by in vivo distribution, toxicity, and targeting ability in nanoparticle systems. Besides, particle size and size distribution can also influence drug delivery, drug release, and nanoparticle stability[22].

The polydispersity index (PI) value is measured by the particle size distribution width with a value smaller than 0.3, indicating that the sample has a narrow distribution and a homogeneous nanoparticle formula[23]. The smaller the polydispersity index value shows that the size distribution of nanoparticles is getting narrower, which means that the nanoparticles' diameter is getting more homogeneous[24]. All three sonification treatments showed values below 0.3 so that these treatments were uniform and homogeneous.

The stability of a system is known with the potential zeta value. A particle is declared stable if it has a potential zeta value outside the range of -30mV to 30mV. The 90-minute sonification treatment showed smaller particle size values than the other treatments. Positive values on zeta potential indicate that many nanoparticles are around the surface; the formed nanoparticles are much influenced by free amino groups, which will increase the charge on the surface and the potential zeta value of the nanoparticles.

Test of Functional Group Nanoparticles Chitosan-*C. asiatica* extract with FTIR:

The FTIR instrument is used to identify multiple groups in compounds but cannot determine their constituent elements. At FTIR, infrared radiation is passed through the sample. The sample absorbs some of the infrared radiation, and some are transmitted. The frequency of a specific vibration is equal to the frequency of infrared radiation going directly to the molecule and will absorb the radiation. The test results of the chitosan nanoparticle group of *C. asiatica* extract can be seen in Figure 2.

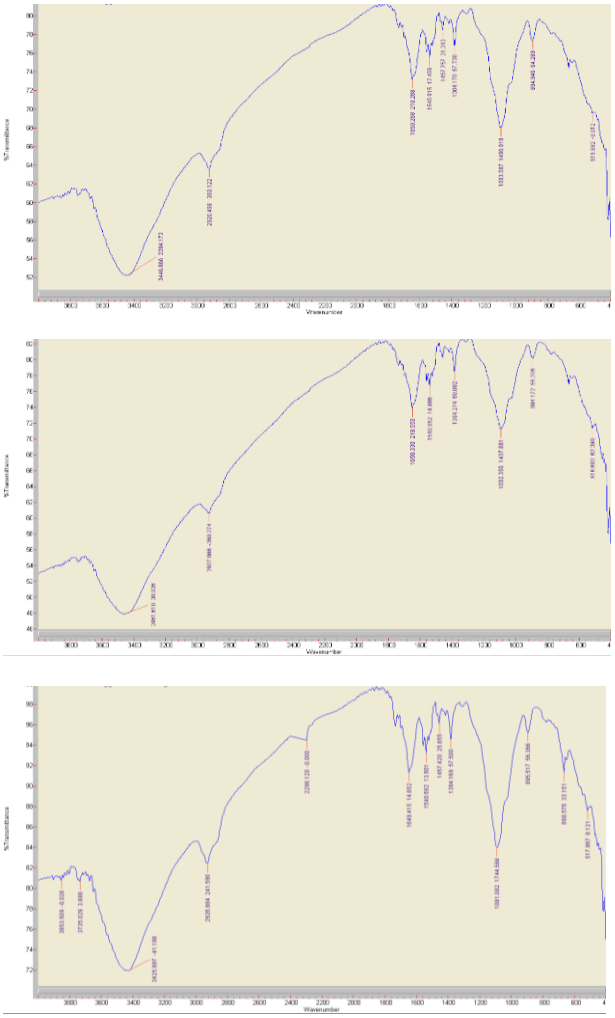

Fig 2. The results of the examination of the functional groups of chitosan nanoparticles were *C. asiatica* extract sonication 90 (A), sonication 120 (B), and sonication 150 (C) using FTIR.

Figure 2 shows the chemical profile in a different spectrum pattern and has an essential characteristic. Chitosan has specific groups, namely -NH2 and –OH. Determination of the existence of *C.asiatica* extract in chitosan is needed to determine its coating ability. One method that can be used to determine the presence of *C. asiatica* extract is FTIR.

The infrared spectrum can detect functional groups that are used to identify compounds in a polymer sample. FTIR in this study uses intermediate level wave numbers, namely 4000-400cm^{-1}. The determination of the wavelength number is due to the determination of functional groups of organic compounds.

FTIR's working principle is based on the absorption or transmitting of infrared light by the molecules making up a compound in the sample. If the frequency of a functional group's vibration is the same as the frequency of infrared ray radiation, the molecule absorbs the light. This causes not all infrared rays to be absorbed by the molecule, some of which are transmitted[20]. The results obtained from FTIR are transmittance graphs. Chitosan FTIR results can show the presence of hydroxyl groups at wave number 3425.56. The hydroxyl group in chitosan will appear on wave number 3425.56 cm^{-1} because of the vibration strain interaction between the hydroxyl group and the amide group. In contrast, the chitosan amide function group is at wave number 1640.56 cm^{-1}. FTIR results showed that chitosan-specific functional groups in the nanoparticle extract sonication 90, 120, 150.

Test of Crystality Nanoparticles Chitosan-*C. asiatica* extract with XRD:

The XRD method is based on the X-ray diffraction patterns for each crystalline material. The results of the examination of crystal formulation can be seen in Figure 3. The XRD analysis is used to determine the physical structure of materials. Data obtained from XRD analysis were graphs of the relationship of X-ray diffraction angles in the sample with the intensity of the light reflected by the material. The results of the characterization of nanoparticle samples by XRD showed an amorphous form. The amorphous character shows that the constituent particles of a molecule are arranged irregularly and are less compact. The irregularity in the arrangement of these particles makes it easy for other molecules to be inserted. The more amorphous the nature of a molecule, the more easily inserted by other molecules[20]. A valley peak marks the amorphous shape of a particle at a diffraction angle of 20°.

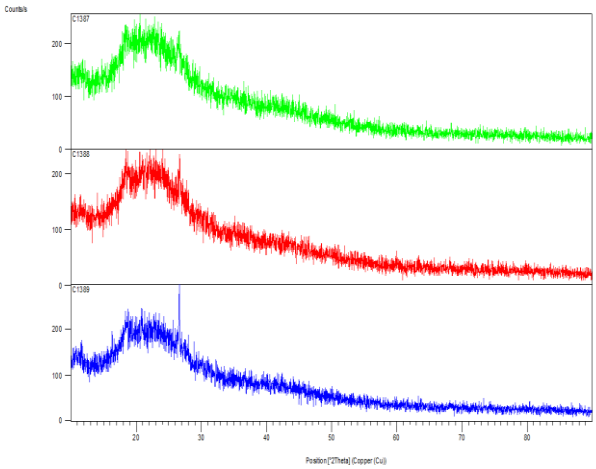

Fig 3: The formulation of chitosan-*C.asiatica* extract nanoparticles crystals with sonication 90 minutes (A), sonication 120 minutes (B), and sonication 150 minutes (C) using XRD.

Test of Morphology Nanoparticles:

Based on the particle morphological analysis results, it was shown that chitosan-*C.asiatica* extract nanoparticles in the treatment sonification 90, 120, 150 minutes were mostly spherical. Functional characteristics of nanoparticles include smaller molecular weight, moderate viscosity, and a high degree of deacetylation. The nanoparticles produced have better characteristics, including having spherical particle shape, smaller and uniform particle size, higher zeta potential. Morphological tests of chitosan nanoparticles are shown in Figure 4.The shape and surface condition of nanoparticles is important because they can be used to determine the nature of drug release[21].

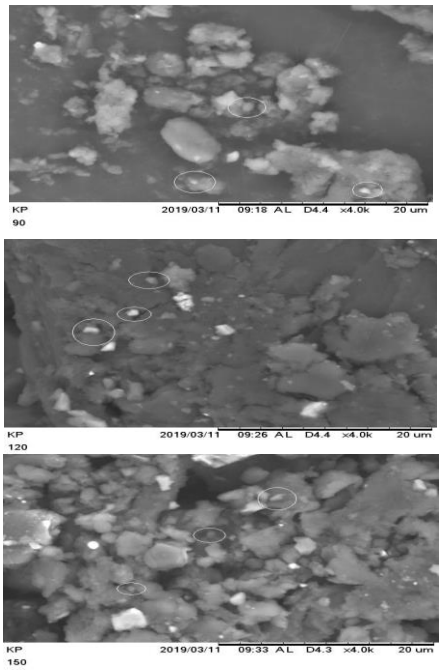

Fig 4. Morphology of chitosan - *C. asiatica* extract with sonication 90 minutes (A), sonication 120 minutes (B), and sonication 150 minutes (C) using SEM.

CONCLUSION:

In the manufacturing of chitosan nanoparticles-*C. asiatica* extract was carried out by the ionic gelation method. This method's mechanism of formation of chitosan nanoparticles is based on the electrostatic interaction between the amine group in chitosan with the negative charge group from the NaTPP polyanion. Testing chitosan nanoparticles-*C. asiatica* extract using FTIR showed the interaction between *C. asiatica* extract and chitosan solution and TPP stabilizer, marked by shifting wave numbers. PSA analysis showed that the average particle size of *C. asiatica* extracts at 90, 120,150 minutes sonication was 286.2nm, 269.2nm, 299.1nm, respectively. The formulation using XRD showed that the amorphous nanoparticle *C. asiatica* extract was supported by particle morphology imaged using SEM.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest regarding the publication of this article.

ACKNOWLEDGMENTS:

We thank EJA Team, Indonesia for editing the manuscript.

REFERENCES:

1. Andriani Y, Mohamad H, Kassim M, Rosnan N, Syamsumir D, Saidin J, Muhammad T, Amir H. Evaluation on Hydnophytum formicarumtuber from Setiu Wetland (Malaysia) and MuaraRupit (Indonesia) for antibacterial and antioxidant activities, and anticancer potency against MCF-7 and HeLa Cells. J App Pharm Sci. 2017; 7 (09): 30-37.DOI: 10.7324/JAPS.2017.70904
2. Jagtap NS, SS Khadabadi, DS Ghorpade, NB Banarase, SS Naphade. Antimicrobial and Antifungal Activity of Centella asiatica (L.) Urban, Umbeliferae. Res J Pharm Technol. 2(2): 328-330.
3. Suresh M, Rath PK, Panneerselvam A, Dhanasekaran D, Thajuddin. Anti-Mycobacterial Effect of Leaf Extract of Centella asiatica (Mackinlayaceae). Res J Pharm Technol. 3(3): 872-876.
4. Mangala L, Pradumna P. A Study on the Effect of Centella asiatica on Experimentally Induced Inflammatory Bowel Disease in Albino Rats. Res J Pharm Technol. 4(10): 1618-1620.
5. Trivedi MN, Archana Khemani, Urmila D. Vachhani, Charmi P. Shah, D.D. Santani. Pharmacognostic, Phytochemical and Microbiological Studies of the Plants Centella asiatica (Linn.) Urban and Withania somnifera (Linn.) Dunal Treasured as Intelligence Boost. Res J Pharm Technol. 4(11): 1707-1713.
6. Kangralkar VA, Kulkarni AR. Evaluation of effect of Piper betel, Centella asiatica and Aristolochia indica extracts on bacterial enzymes in 1, 2-dimethyl hydrazine induced colon cancer in wistar rats. Res J Pharm Technol. 7(2): 151-154.
7. Raju DC, T Diana Victoria, Nancy Biji, Gandra Nikitha. Evaluation of Antioxidant Potential of Ethanolic Extract of Centella asiatica L. Res J Pharm Technol. 8(9): 1289-1293. DOI: 10.5958/0974-360X.2015.00234.6
8. Raju DC, Reji Joseph Varghese, Nancy Biji. In vitro Production of Neurologically active Phytoconstituents from an elite Chemovariant of Centella asiatica (L.), identified from south Indian Populations. Res J Pharm Technol. 2017; 10(7): 2095-2100.
9. Mudjihartini N, Reni Paramita, Ani Retno Prijanti, Pungguri Ayu Nega Sarsanti, Fadilah Fadilah, Erni Hernawati Purwaningsih. The Effects of Centella asiatica and Acalypha indica L. extracts on aging process. Res J Pharm Technol. 2020; 13(2):787-790. DOI: 10.5958/0974-360X.2020.00148.1
10. Swastini DA, I Nyoman Krisna Udayana, Cokorda Istri Sri Arisanti. Cold cream combination of Garcinia mangostana L. Anredera cordifolia (Ten.) and Centella asiatica extracts on Burn Healing Activity Test. Res J Pharm Technol. 2021; 14(5): 2483-6. DOI: 10.52711/0974-360X.2021.00437
11. Mitha KV, Saraswati Jaiswal Yadav, Rashmi K S, Ganaraja Bolumbu. Neuroprotective effects of feeding Centella asiatica (L) Urb. – Study of hippocampal neurons in pups born to alcohol-fed female Wistar rats. Res J Pharm Technol. 2022; 15(3):1047-2. DOI: 10.52711/0974-360X.2022.00175
12. Yadav AV, Chandrashekhar Devidas Upasani. Protective effect of the combination of Hydroalcoholic extracts of Asparagus Racemosus, Centella Asiatica, Plumeria rubra with Glibenclamide in Experimentally Induced Diabetic Nephropathy in rats. Res J Pharm Technol. 2022; 15(4): 1614-0. DOI: 10.52711/0974-360X.2022.00270
13. Gutierrez Cisneros C, Bloemen V, Mignon A. Synthetic, Natural, and Semisynthetic Polymer Carriers for Controlled Nitric Oxide Release in Dermal Applications: A Review. Polymers. 2021 Feb 28;13(5):760. DOI: 10.3390/polym13050760.
14. Zahin N, Anwar R, Tewari D, Kabir MT, Sajid A, Mathew B, Uddin MS, Aleya L, Abdel-Daim MM. Nanoparticles and its biomedical applications in health and diseases: special focus on drug delivery. Environ Sci Pollut Res Int. 2020;27(16):19151-19168. DOI: 10.1007/s11356-019-05211-0.
15. Ferdous Z, Nemmar A. Health Impact of Silver Nanoparticles: A Review of the Biodistribution and Toxicity Following Various Routes of Exposure. Int J Mol Sci. 2020; 21(7):2375. DOI: 10.3390/ijms21072375.
16. Moran HBT, Turley JL, Andersson M, Lavelle EC. Immunomodulatory properties of chitosan polymers. Biomaterials. 2018; 184:1-9. DOI: 10.1016/j. biomaterials. 2018.08.054.
17. Zhang XY, Zhang PY. Polymersomes in Nanomedicine - A Review. Curr Med Chem. 2017; 13(2): 124-129. DOI: 10.2174/1573413712666161018144519.
18. Hoang NH, Le Thanh T, Sangpueak R, Treekoon J, Saengchan C, Thepbandit W, Papathoti NK, Kamkaew A, Buensanteai N. Chitosan Nanoparticles-Based Ionic Gelation Method: A Promising Candidate for Plant Disease Management. Polymers. 2022; 14(4): 662. DOI: 10.3390/polym14040662.
19. Sibero MT, Trianto A, Frederick EH, Wijaya AP, Ansori ANM, Igarashi Y. Biological Activities and Metabolite Profiling of Polycarpa aurata (Tunicate, Ascidian) from Barrang Caddi, Spermonde Archipelago, Indonesia. Jordan J Biol Sci. 2022; 15(1): 15-20. DOI: 10.54319/jjbs/150103.
20. BudiS, Asih SuliasihB, RahmawatiI, Erdawati. Size-controlled chitosan nanoparticles prepared using ionotropic gelation. Science Asia. 2020; 46(4): 457-461. DOI: 10.2306/scienceasia1513-1874.2020.059
21. Muchtaromah B, Wahyudi D, Ahmad M, Ansori AN, Annisa R, Hanifah L. Chitosan-Tripolyphosphate Nanoparticles of Mango Ginger (Curcuma mangga) Extract: Phytochemical Screening, Formulation, Characterization, and Antioxidant Activity. Pharmacogn J. 2021; 13(5): 1065-1071. DOI: 10.5530/pj.2021.13.138.
22. Fadholly A, Ansori ANM, Proboningrat A, Nugraha AP, Iskandar RPD, Rantam FA, Sudjarwo SA. Apoptosis of hela cells via caspase-3 expression induced by chitosan-based nanoparticles of Annona squamosa leaf extract: In vitro study. Indian J Pharm Educ Res. 2020; 54(2): 416-421. DOI: 10.5530/ijper.54.2.47
23. Fadholly A, Ansori ANM, Utomo B. Anticancer Effect of Naringin on Human Colon Cancer (WiDr Cells): In Vitro Study. Res J Pharm Technol. 2022; 15(2): 885-888. DOI: 10.52711/0974-360X.2022.00148.
24. Winarni D, Husna FN, Syadzha MF, Susilo RJK, Hayaza S, Ansori ANM, Alamsjah MA, Amin MNG, Wulandari PAC, Pudjiastuti P, Awang K. Topical Administration Effect of Sargassum duplicatum and Garcinia mangostana Extracts Combination on Open Wound Healing Process in Diabetic Mice. Scientifica. 2022; 2022: 9700794.