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SYNTHESIS OF SILVER NANOPARTICLES USING ETHANOLIC EXTRACT OF *Nelumbo nucifera* Gaertn. LEAF AND ITS CYTOTOXIC ACTIVITY AGAINST T47D AND 4T1 CELL LINES

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ABSTRACT

The cytotoxic activitiy of silver nanoparticles bio-functionalized by *Nelumbo nucifera* leaf extracts were examined in the current work. The nanoparticle (AgNPs) was characterized by scanning electron microscopy, UV–visible spectroscopy, and a particle size analyzer. Cytotoxic activity of silver nanoparticles against T47D and 4T1 cells using the MTT method using concentrations of 100 µg/mL, 50 µg/mL, and 25 µg/mL. For comparison, doxorubicin was used at the same dose. The size of the spherical nanoparticle was 66.4±0.93 nm and the polydispersity index was 0.23±0.05. Silver nanoparticles have anti-cancer properties against T47D and 4T1 cell lines based on inhibitory concentration. The inhibitory concentration (IC_{50}) of AgNPs on T47D and 4T1 cells were obtained at 12.10±0.08 μg/mL and 98.77±1.27 μg/mL, respectively. Meanwhile, the IC_{50} values of doxorubicin in T47D and 4T1 cells were 4.45±0.03 μg/mL and 36.77±1.15 μg/mL, respectively. Overall, the results revealed that the green-synthetized silver nanoparticle had cytotoxic effects on breast cancer cells in comparison to doxorubicin.

Keywords: Silver Nanoparticles, *Nelumbo nucifera*, Cytotoxic, T47D, 4T1

INTRODUCTION

Currently, nanotechnology is the fastest-growing industrial sector in the world, with a never-ending search for new nanomaterials and production methods.[1] Many researchers are interested in metal nanoparticles due to their unique properties and applications in catalysis, photonics, optoelectronics, and biological tagging, as well as therapeutic applications.[2] There are numerous ways to make silver nanoparticles (AgNPs). These mechanisms can be used to reduce silver ions, from chemical and electrochemistry to radiation and photochemistry to Langmuir–Blodgett. Using biological synthesis to make harmless nanostructured materials makes it possible to use or make fewer toxic substances.[3,4] Microorganisms such as bacteria, fungi, and yeast have already been used to biosynthesis silver nanoparticles.[5] However, research into plant systems as prospective nano factories has piqued interest in biological nanoparticle manufacturing. Silver nanoparticles were also synthesized utilizing extracts from plants such as *Aloe vera*, *Cinnamon zeylanicum*, *Stevia rebaudiana*, and *Papaya.*[6-8] The goal of this work was to create silver nanoparticles quickly using ethanolic leaf extract from *Nelumbo nucifera* and test their anticancer effectiveness in T47D and 4T1 cell lines.

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EXPERIMENTAL

Material and Methods

$AgNO_3$ was bought from Sigma-Aldrich for a reliable grade of purity without further purification. And the ethanolic leaf extract of *Nelumbo nucifera* (EENN) which consists of higher medicinal values. These plants are collected from the traditional market, Medan, North Sumatera, Indonesia.

Preparation of Ethanolic Leaf Extract of *Nelumbo nucifera*

The extract was obtained by using the maceration method. A total of 500 g of dry powder was put into a closed container and soaked in 3.75 L of 70% ethanol for 5 days. After that, a filtering process was carried out to obtain the first extract solution. The dry powder was soaked again with the same solvent for 2 days and filtered again to obtain a second extract solution. The first and second solutions were combined in one container and concentrated using a rotary evaporator to obtain the extract. The Extract was stored in the refrigerator to prevent its damage.[9]

Synthesis of Silver Nanoparticles (AgNPs)

The AgNPs were synthesized by mixing 40 mL of 2 mM AgNO3 solution with 1 mL of 0.125% EENN and then stirred using a magnetic stirrer for 15 minutes at a room temperature of 25°C. The results of this reaction were then identified to determine whether the synthesis of AgNPs was successful.[10]

UV-vis Spectra Analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV–vis spectrum of the reaction medium after overnight incubation, after diluting a small aliquot of the sample into distilled water. The stability of the AgNPs formed was analyzed at a wavelength of 200-600 nm at 0, 30, 60 minutes, and 24 hours after incubation.[11]

Particle Size Analysis of AgNPs

A total of 15 mL of AgNPs solution was dissolved in 100 mL of distilled water and stirred slowly to form a dispersion. Entered into the particle size analyzer to determine the average size of AgNPs.[12]

Scanning Electron Microscopy (SEM) Analysis of Silver Nanoparticles

A scanning electron microscope (JEOL 6380A; Tokyo, Japan) was used to record the micrograph images of synthesized AgNPs.[13]

Cytotoxic Assay

The cytotoxicity assay of the prepared AgNPs was measured using T47D and 4T1 cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay. The MTT test is a colorimetric, nonradioactive method for determining cell viability by detecting tetrazolium salt metabolization. T47D and 4T1 cells were seeded at a density of 5×10^4 cells/well into 96-well plates. Then, the cells were treated with different concentrations of synthesized AgNPs and doxorubicin as a positive control (100 μg/mL, 50 μg/mL, and 25 μg/mL) and incubated in the presence of 5% CO_2 and 95% humidity at 37°C for 24 h. MTT (5 mg/mL) was added to the incubated cells, then incubated further for another 4 h. The crystals were dissolved in 200 μL of DMSO and the absorbance was measured colorimetrically at 570 nm.[14]

Data Analysis

Each experiment was repeated 3 times. The data obtained were processed with the help of Microsoft Excel and SPSS 23 using the ANOVA test.

RESULTS AND DISCUSSION

In the current investigation, the reduction of silver ions present in the aqueous solution of silver nitrate during the interaction with the constituents of EENN was observed using UV-Vis spectroscopy with wavelengths ranging from 300 to 600 nm in 0, 30, 60 minutes, and 24 hours after reaction. The product obtained 60 minutes after the reaction was the most optimal and stable product. Where this product provides the greatest absorption which is 1.353 at 437 nm (Fig.-1). According to prior research, the synthesis of AgNPs may be monitored using a UV-Vis spectrophotometer with absorbance in the 400-450 nm range.[15,16] Changes in wavelength are not too large from 0 minutes, 30 minutes, 60 minutes, and 24 hours.

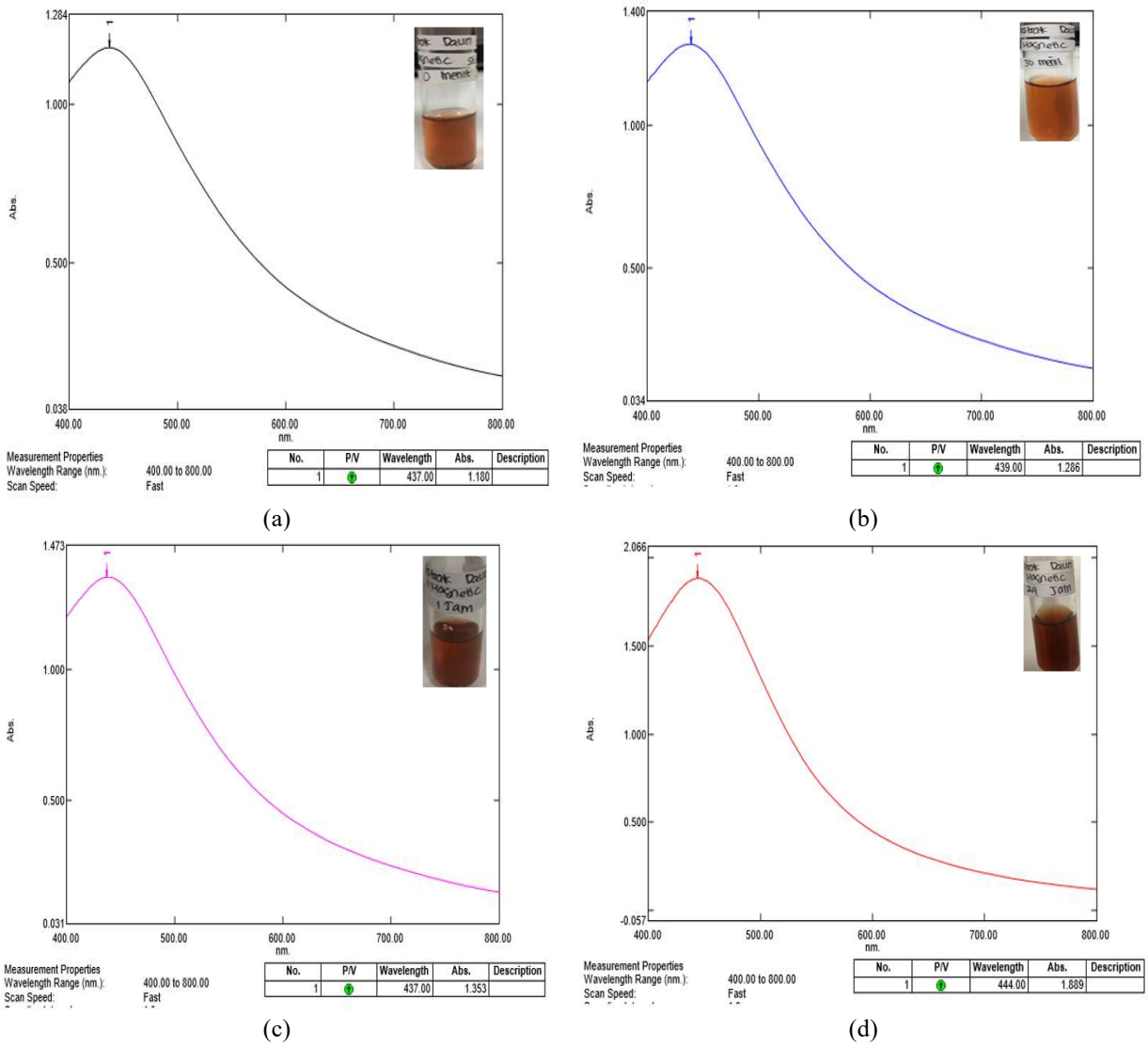


Fig.-1: UV-Vis Absorption Spectrum of AgNPs. (a) Detection after 0 Minutes Reaction, (b) Detection After 30 Minutes Reaction, (c) Detection After 60 Minutes Reaction, (d) Detection After 24 hours

This shows that the resulting AgNPs are relatively stable. The AgNPs obtained were then measured for their particle size using a particle size analyzer (PSA). Based on Fig.-2 shows the size distribution of AgNPs with AgNO3 concentration of 2mM at 60 minutes with the size data obtained in the form of an intensity of 3 repetitions with an average diameter size of AgNPs obtained which is 66.4±0.93 nm and an average PI (Polydispersity Index) is 0.23±0.05. The polydispersity index is a parameter to determine the particle size distribution of AgNPs[16,17]. The range of polydispersity index values is 1-0, if the polydispersity index value is < 0.7 then it is monodispersed, but if it is polydispersed it has a polydispersity index > 0.7[18-20]. The results of the PI in this study showed that the PI nanoparticles were polydispersed with a value obtained above 0.7. SEM analysis shows high-density AgNPs synthesized by EENN (Fig.-3). In Fig.-3, it can be seen the results of observations with SEM with image magnification of 7,500×, 15,000×, and 25,000× that AgNPs are slightly spherical in shape, some are not uniform, some are small and large, with varying sizes due to aggregation. nanoparticles. The hydrogen bond and electrostatic interactions between the bioorganic capping molecules linked to the AgNPs caused the SEM picture of silver nanoparticles[21-23]. Even inside the aggregates, the nanoparticles were not in direct contact, indicating that a capping agent had stabilized them. Because of the SEM observations, the bigger silver particles may have aggregated from the smaller ones[24,25].

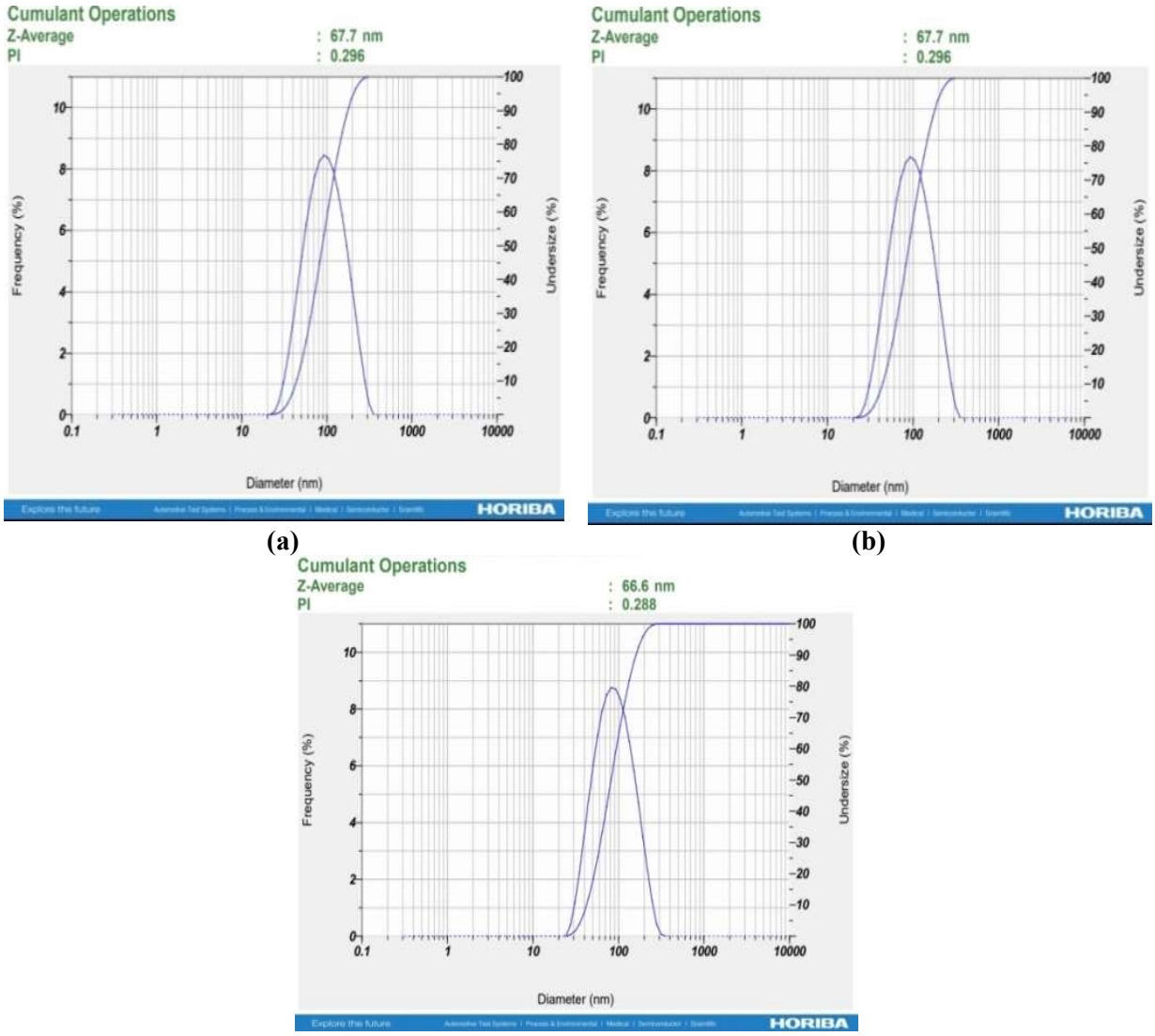
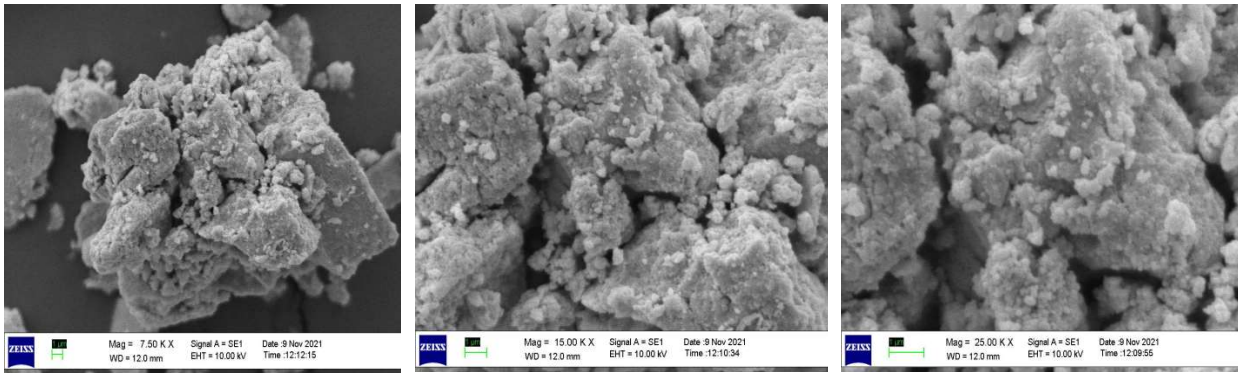


Fig.-2: Particle Size of AgNPs, (a) First Detection, (b) Second Detection, (c) Third Detection

After confirming that the synthesis of AgNPs was successful, the cytotoxic activity was tested on T47D and 4T1 cells using the MTT assay method (Fig.-4).

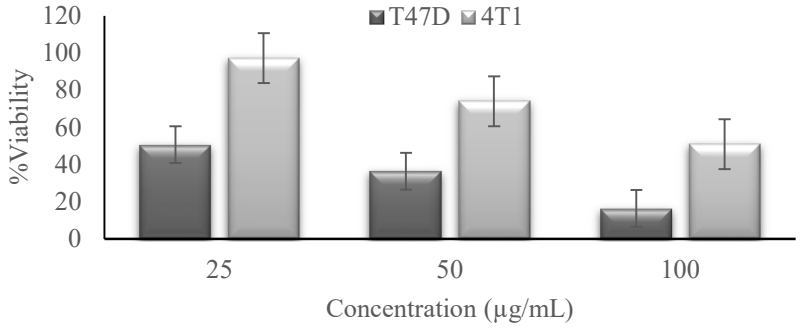


(a) (b) (c)

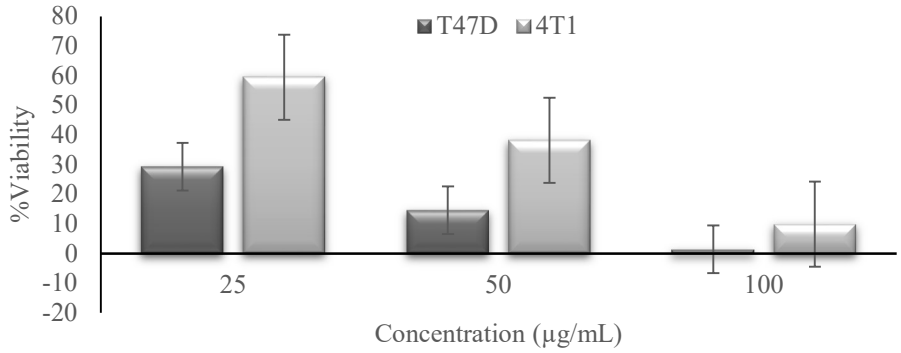
Fig.-3: Morphological Analysis of AgNPs using SEM. (a) 7500x, (b) 15000x, (c) 25000x

In this test, doxorubicin was used as a comparison. The test concentrations used of AgNPs and doxorubicin were 25 μg/mL, 50 μg/mL, and 100 μg/mL. From Figure 4 it can be seen that the increase in the

concentration of AgNPs and doxorubicin causes a decrease in live cells. Exposure to increasing concentrations of AgNPs shows dose-dependent cytotoxicity on T47D and 4T1 cells[26]. At a concentration of 100 µg/mL has high toxicity with a viability of 16.43% then at a concentration of 50 µg/mL has a viability of 36.43% and at a concentration of 25 µg/mL has a viability of 50.72% in T47D cells. Meanwhile, in 4T1 cells, cell viability was 50.94%, 74.05%, and 97.29% at concentrations of 100 µg/mL, 50 µg/mL, and 25 µg/mL, respectively. The same thing was seen in the administration of doxorubicin.



(a)



(b)

Fig.-4: %Viability of T47D and 4T1 Cells, Mean ± SE, n = 3. (a) AgNPs Activity at 25 μg/mL, 50 μg/mL, and 100 μg/mL, (b) Doxorubicin Activity at 25 μg/mL, 50 μg/mL, and 100 μg/Ml

The reaction of MTT with living cells will form purple formazan crystals[27]. This is because the mitochondria of dead cells are not able to respire so the tetrazolium succinate enzyme that can reduce MTT to formazan is not produced[28]. As a result, the dead cells do not form purple formazan. The MTT reaction with the mitochondrial reductase enzyme found in the cells was stopped with a DMSO stopper as an inhibition of crystal formation and then analyzed for absorbance using an ELISA reader. In the present study, the minimum inhibitory concentration (IC_{50}) of AgNPs on T47D and 4T1 cells were obtained at 12.10±0.08 μg/mL and 98.77±1.27 μg/mL, respectively. Meanwhile, the IC_{50} values of doxorubicin in T47D and 4T1 cells were 4.45±0.03 μg/mL and 36.77±1.15 μg/mL, respectively. In fact, AgNPs may induce reactive oxygen species and cause damage to cellular components leading to cell death[29]. In general, green-produced silver nanoparticles have strong anticancer action with negligible or low toxicity, depending on the agents used to reduce and cap silver nitrate into silver[30].

CONCLUSION

Nature has created the most efficient miniature useful materials in lovely and innovative ways. The synthesis of AgNPs using EENN has various advantages, including being cost-effective, efficient, and eco-

friendly, providing better workplaces and communities; and preserving human health and the environment, resulting in less waste and safer goods. Silver nanoparticles made in a green way have a lot of potential in biological applications.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

M. F. Lubis, K. Fitri, and T.N. Khairani carried out research activities, data acquisition, data interpretation, and manuscript preparation. M. Andry, H. S. Winata, A. Violenta, and N. Lubis prepared the script. All authors read and agree to the published version of the manuscript. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. S. Iravani., H. Korbekandi., S. V. Mirmohammadi., and B. Zolfaghari, *Research in Pharmaceutical Sciences*, **9**, 6(2014)
2. H. D. Beyene., A. A. Werkneh., H. K. Bezabh., T. G. Ambaye, *Sustainable Materials and Technologies*, **13**, 18(2017), https://doi.org/10.1016/j.susmat.2017.08.001
3. F. Mares-Briones., M. S. Aguilar., N. Fletes., S. E. Borjas-Garcia., and G. Rosas, *Microscopy and Microanalysis*, **24**, 1728(2018), https://doi.org/10.1017/S1431927618009121
4. M. Nasrollahzadeh, *New Journal of Chemistry*, **38**, 5544(2014), https://doi.org/10.1039/C4NJ01440E
5. M. Nasrollahzadeh., and S. M. Sajadi, *RSC Advances*, **5**, 46240(2015), https://doi.org/10.1039/C5RA08927A
6. S. P. Chandran., M. Chaudhary., R. Pasricha., A. Ahmad., and M. Sastry, *Biotechnology Progress*, **22**, 2(2008), https://doi.org/10.1021/bp0501423
7. S. Anjum., G. Jacob., and B. Gupta, *Emergent Materials*, **2**, (2019), https://doi.org/10.1007/s42247-019-00023-x
8. D. Jain., H. K. Daima., S. Kachhwaha., and S. L. Kothari, *Digest Journal of Nanomaterials and Biostructures*, **4**, 3(2009).
9. M. F. Lubis., H. Syahputra., D. N. Illian., and V. E. Kaban, *Journal of Research in Pharmacy*, **26**, 3(2022), http://dx.doi.org/10.29228/jrp.154
10. R. Damayanti., T. Tamrin., Z. Alfian., and E. Eddyanto, *AIP Confrence Proceeding*, **2267**, 020065(2020), https://doi.org/10.1063/5.0017822

11. M. A. Ahmad., S. Salmiati., M. Marpongahtun., M. R. Salim., J. A. Lolo., and A. Syafiuddin, *Biointerface Research in Applied Chemistry*, **10**, 5(2020), https://doi.org/10.33263/BRIAC105.62536261
12. I. Fatimah., and Z. H. V. I. Aftrid, *Green Chemistry Letters and Reviews*, **12**, 1(2019), https://doi.org/10.1080/17518253.2019.1569729
13. O. Ahmed., N. R. S. Sibuyi., A. O. Fadaka., M. A. Madiehe., E. Maboza., M. Meyer., and G. Geerts, *Pharmaceutics*, **14**, 2(2022), https://doi.org/10.3390/pharmaceutics14020380
14. P. A. Z. Hasibuan., D. Munir., D. Pertiwi., D. Satria., and M. F. Lubis, *Rasayan Journal of Chemistry*, **13(4)**, 4(2020), https://dx.doi.org/10.31788/RJC.2020.1345625
15. A. Manosroi., K. Kumguan., C. Chankhampan., W. Manosroi., and J. Manosroi, *Journal of Nanoscience and Nanotechnology*, **12**, 9(2012), https://doi.org/10.1166/jnn.2012.6500
16. Rosidah., Yuandani., S. S. Widjaja., N. Auliafendri., M. F. Lubis., M. Muhammad., and D. Satria, *Rasayan Journal of Chemistry*, **14(2)**, 1378(2021), http://dx.doi.org/10.31788/RJC.2021.1426075
17. P. A. Z. Hasibuan., U. Harahap., P. Sitorus., and D. Satria, *Heliyon*, **6**, 7(2020), https://doi.org/10.1016/j.heliyon.2020.e04449
18. P. A. Z. Hasibuan., D. Munir., D. Pertiwi., D. Satria., and M. F. Lubis, *Rasayan Journal of Chemistry*, **13**, 4(2020), http://dx.doi.org/10.31788/RJC.2020.1345625
19. W. A. W. Omar., N. A. Azhar., N. H. Fadzilah., N. N. S. N. M. Kamal, *Asian Pacific Journal of Tropical Biomedicine*, **6**, 3(2016), https://doi.org/10.1016/j.apjtb.2015.12.011
20. A. Tsuchiya., Y. Makita., T. Koyano., T. Kowithayakorn., M. Ishibashi., and M. A. Arai, *Journal of Natural Medicines*, **74**, 758(2020), https://doi.org/10.1007/s11418-020-01432-7
21. T. Matsumoto., T. Kitagawa., T. Ohta., T. Yoshida., D. Imahori., S. Teo., H. S. B. Ahmad., and T. Watanabe, *Journal of Natural Medicines*, **73**, 4(2019), https://doi.org/10.1007/s11418-019-01319-2
22. Rudiyansyah., A. H. Alimuddin., Masriani., R. Muharini., and P. Proksch, *Phytochemistry Letters*, **23**, 90(2018), https://doi.org/10.1016/j.phytol.2017.11.019
23. Zulfikar, N. K. Putri., S. Fajriah., M. Yusuf., R. Maharani., J. Al-Anshori, et al. *Molbank*, **2020(4)**, M1157(2020), https://doi.org/10.3390/M1157
24. M. Azizi., H. Ghourchian., F. Yazdian., F. Dashtestani., and H. AlizadehZeinabad, *Plos One*, **12**, 11(2017), https://doi.org/10.1371/journal.pone.0188639
25. M. Pelin., L. Fusco., V. Leon., C. Martin., A. Criado., S. Sosa., E. Vasquez., A. Tubaro., and M. Prato, *Scientific Reports*, **7**, 40572(2017), https://doi.org/10.1038/srep40572
26. A. Manosroi., P. Jantrawut., M. Sainakham., W. Manosroi., and J. Manosroi, *Pharmaceutical Biology*, **50**, 11(2012)**,** https://doi.org/10.3109/13880209.2012.682116
27. T. Chou, *Synergy*, **7**, 49(2018), https://doi.org/10.1016/j.synres.2018.04.001
28. J. Fu., N. Zhang., J. H. Chou., H. Dong., S. Lin., G. S. Ulrich-Merzenich., and T. Chou, *Synergy*, **3**, 3(2016), https://doi.org/10.1016/j.synres.2016.06.001
29. I. M. Mangoato., C. P. Mahadevappa., and M. G. Matsabisa, *Synergy*, **9**, 100056(2019), https://doi.org/10.1016/j.synres.2019.100056
30. N. Bhagya., A. Prabhu., P. D. Rekha., and K. R. Chandrashekar, *Synergy*, **10**, 100063(2020), https://doi.org/10.1016/j.synres.2020.100063

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