



# Phytochemical Analysis Both of Water and Ethanol Extract from Some Herbs Combinations, Nanoemulsion Formulation, and Antioxidant Effects

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## Abstract

**AIM:** The purpose of this study was to analyze the phenolic content and antioxidant activity of both water and ethanol extracts of a combination of several herbs (*Phyllanthus urinaria*, *Andrographis paniculata*, *Curcuma longa*, *Zingiber officinale*, *Citrus limon*, and *Cymbopogon citratus*), as well as product development in the form of nanoemulsions.

**METHODS:** The research was conducted by making three combinations of herbs mixtures with various compositions (Formulas A, B, and C). The combined herbs powder was then made in the form of water and ethanol extracts. The ethanol extract of each herbal combination was then made also in the form of a nanoemulsion by spontaneous emulsion.

**RESULTS:** The ethanol extract of each herbs combination contained higher phenolic compounds and antioxidant activity than the water extract. The nanoemulsion product of the combined herbs ethanol extract had a particle size of 21.1; 34.9; and 50.7 nm and showed high antioxidant activity.

**CONCLUSION:** The combined several herbs (*P. urinaria*, *A. paniculata*, *C. longa*, *Z. officinale*, *C. limon*, and *C. citratus*) ethanol extract and its nanoemulsion products have the potential to be developed as natural antioxidants.

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**Keywords:** Antioxidant; Combination herbs; Nanoemulsion

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## Introduction

The world is currently still struggling with the COVID-19 pandemic. According to a report from the WHO as of July 28, 2021, the number of COVID-19 cases in the world has reached 196 million people, with deaths reaching 4,190,000 people (2.1%). Indonesia ranks 14<sup>th</sup>, with 3,290,000 cases and 88,659 (3.5%) deaths [1]. Various efforts have been made to overcome this, including using masks, maintaining distance, and maintaining cleanliness by frequently washing hands with soap or hand sanitizer. Besides that, it also increases the body's immunity by consuming healthy and balanced foods, and increasing stamina by consuming herbal products. Several studies have shown that many herbal plants have virus-fighting properties by stimulating the body's immunity [2], [3], [4]. Empirical data show that several herbs extracts that are widely used for antiviral treatment show relatively safer side effects. These herbs extracts generally fight viruses not directly, but function as immunomodulators that can improve the

body's immune system [5]. Immunomodulators are compounds or drugs that can modulate the function and activity of the immune system either by stimulating or improving its function.

Several herbs plants that have been extensively studied and used empirically as immunomodulators include *Curcuma longa*, *Phyllanthus urinaria*, *Andrographis paniculata*, *Zingiber officinale*, *Cymbopogon citratus*, and *Citrus limon*. The combination of types of herbs will determine the composition of the active ingredients contained in it. Research on these types of herbs shows that these plants contain active components that can increase the body's immunity. *C. longa* contains the compound curcumin and its derivatives called curcuminoids which exhibit interesting biological activities, such as antioxidant, anti-inflammatory, anti-tumor, and immunomodulatory properties. *P. urinaria* also shows the presence of bioactive compounds that have antiviral, anti-tumor, hepatoprotective, antidiabetic, antioxidant, anti-hypertensive, anti-inflammatory, and antimicrobial activities [6], [7]. Various compounds contained in the leaves of *P. urinaria* include flavonoids, tannins,

coumarins, and lignans. Likewise, *A. paniculata* is an herbs plant that is widely found in medicinal plants with a very bitter taste. This plant has been used for centuries to treat respiratory infections, fever, herpes, sore throat, and various other chronic and infectious diseases [8]. Several compounds found in *A. paniculata* plants include 1,5-dimethyl-1,5-cyclooctadiene, 2-hydroxyethyl benzoate, and squalene. The rhizome of the *Z. officinale* plant has bioactive compounds gingerol, shogaol, and paradol, as well as a number of terpenoid compounds that have been shown to exhibit biological activity that is very beneficial for health [9]. Lemongrass used as a flavoring and refreshing drink contains essential oils consisting of neral, citral, geranyl acetate, geranial, limonene, and camphene, which have antibacterial, antifungal, and antioxidant activities [10]. Likewise, lemons are rich in ascorbic acid which is a natural antioxidant.

The use of this plant to maintain health in traditional communities is usually used single or in combination, in the form of dry herbs, or in the form of wet brew or commonly called "Jamu." As a solvent, water is used by brewing or boiling it. Furthermore, the use of medicinal plants in a more modern way is currently made in the form of extracts and made in the form of tablets or capsules. The solvent commonly used for extraction is alcohol, which is then removed by evaporation. The use of various solvents will of course affect the amount of dissolved active components and also their biological activity. Several studies have shown that the stability and absorption of herbs extracts have limitations. Thus, the technology that needs to be developed immediately is a more effective and efficient technique for the formulation and delivery of herbs extracts. One method that is being developed is using nanotechnology. Nano-sized herbs drug delivery systems are likely to have future potential to increase activity and overcome problems associated with plant-based pharmaceuticals [11]. Nanoemulsions use oil and surfactants, are transparent and thermodynamically stable, and will improve biocompatibility [12]. The purpose of this study was to analyze the total phenol content and antioxidant activity both of water and ethanol extracts of a combination of several herbs (*P. urinaria*, *A. paniculata*, *C. longa*, *Z. officinale*, *C. limon*, and *C. citratus*), and their development in the form of nanoemulsion.

## Methods

### Apparatus and reagent

This research uses several equipment, including digital analytical balance, Rotavapor R114, hotplate with magnetic stirrer, refrigerator, vortex, incubator, particle size analysis Horiba SZ-100, pH

meter, and UV-Vis spectrophotometer (UV-Vis 722N). The materials used in this study were *A. paniculata* and *P. urinaria* herbs, *C. longa* and *Z. officinale* rhizomes, *C. limon* fruit, and *C. citratus* stems obtained from traditional markets in Yogyakarta, aquades, 2,2-diphenyl-1-picryl hydrazine (DPPH, Merck), ethanol 96%, chloroform (Merck), H<sub>2</sub>SO<sub>4</sub>, FeCl<sub>3</sub> 0.1%, olive oil, ammonia, Wagner reagent, Na<sub>2</sub>CO<sub>3</sub>, Folin-Ciocalteu, ascorbic acid (Merck), gallic acid (Merck), virgin coconut oil, tween 80, phosphate buffer pH 7.0, filter paper, and aluminum foil.

### Sample preparation

The sample in this study was a combination of several herbs consisting of *A. paniculata* and *P. urinaria* herbs, *C. longa* and *Z. officinale* rhizomes, *C. limon* fruit, and *C. citratus* stems with the composition as shown in Table 1. Water extract from each combination of herbs made by adding aquades in a ratio of 1:5 and heated for 15 min. The filtrate is then separated to a total volume of 500 mL. Ethanol extract from each herbal combination was made by maceration using ethanol for 24 h. The filtrate obtained was separated and dried with a vacuum evaporator to obtain a concentrated extract.

**Table 1: Combination herbs formulations**

Herb	Percentage of ingredients in formula (%)		
	A	B	C
<i>C. longa</i>	30	30	40
<i>P. urinaria</i>	30	40	20
<i>A. paniculata</i>	20	10	20
<i>Z. officinale</i>	10	10	10
<i>C. citratus</i>	5	5	5
<i>C. limon</i>	5	5	5
Total	100	100	100

*C. longa*: *Curcuma longa*, *P. urinaria*: *Phyllanthus urinaria*, *A. paniculata*: *Andrographis paniculata*, *Z. officinale*: *Zingiber officinale*, *C. citratus*: *Cymbopogon citratus*, *C. limon*: *Citrus limon*.

### Phytochemical screening

Phytochemical screening qualitatively from each combination of aqueous extracts and herbal ethanol included tests for sterols and triterpenoids using the Salkowski method, alkaloid testing using the Wagner method, tannin and phenolic tests with the addition of 0.1% FeCl<sub>3</sub> reagent, and saponin testing by observing the formation of foam [13].

### Analysis of phenolic compound levels

The water and ethanolic extracts of each combined herb were then determined for the levels of phenolic compounds using a spectrophotometric method with Folin-Ciocalteu reagent [14]. The combined herbal extract sample was dissolved with ethanol at a concentration of 1%. A total of 0.1 mL of the solution was put into a test tube, added 0.2 mL of Folin-Ciocalteu, 2 mL of distilled water, and 1 mL of Na<sub>2</sub>CO<sub>3</sub> solution. The solution was homogenized and incubated in a water bath at 50°C for 10 min and cooled. The absorbance of each solution was measured at a

wavelength of 760 nm. Phenolic levels are expressed as mg/mL gallic acid.

### Combination herb nanoemulsion preparation

The nanoemulsion product from the combined herbal ethanol extract was prepared by the spontaneous emulsion method [15]. The ethanol extract of each combination mixture was mixed with oil and tween 80 (comparison of oil and tween 80 [1:4]) while stirring and heated at 70°C for 20 min to make the mixture homogeneous. Phosphate buffer pH 7.0 was added drop-wise while stirring constantly, continuously for 1 h at 70°C. The characteristics of herbal nanoemulsion products include particle size, polydispersity index (PI), zeta potential, and % transmittance.

### Antioxidant activity test

Each sample of the combination of herbal extracts and their nanoemulsion products was further tested for their antioxidant activity using the DPPH method [16]. Each extract was prepared in several concentration series, reacted with DPPH solution in ethanol; then stirred until homogeneous, and incubated at room temperature for 30 min. The absorbance of each sample was measured using spectroscopy at 516 nm. The percentage of inhibition was obtained from the calculation of the percentage decrease in the absorbance of the sample solution compared to the control. Antioxidant activity is expressed as IC<sub>50</sub>.

## Results and Discussion

Combination of six types of herbs, which include *A. paniculata*, *P. urinaria*, *C. longa*, *Z. officinale*, *C. limon*, and *C. citratus*. The three variations of composition have been made as shown in Table 1, and then the extract is made using water and ethanol. The water extract was concentrated, but it still could not dry and be still in the form of a slightly concentrated solution. Meanwhile, the ethanol extract was concentrated using a vacuum evaporator, to obtain a thick extract. Phytochemical screening of each sample obtained data is shown in Table 2.

**Table 2: Phytochemical screening results of each extract**

S. No.	Formula	Steroid and terpenoid		Alkaloid		Phenolic		Tanin		Saponin	
		WE	EE	WE	EE	WE	EE	WE	EE	WE	EE
1.	Combination herbs A	-	+	-	+	+	+	+	+	-	+
2.	Combination herbs B	-	+	-	-	+	+	+	+	+	+
3.	Combination herbs C	-	+	-	-	+	+	+	+	-	-

(+) exists, (-) not detected, WE: Water extract, EE: Ethanol extract

The data in Table 2 show that all samples of water and ethanol extracts of three types of herbal combinations showed the presence of phenolic compounds and tannins. Meanwhile, steroids and terpenoids were only detected in the ethanol extract. This is consistent with the results of the previous studies that phenolic compounds can dissolve in polar solvents, including water made by infusion or decoctions [17]. Some components were not detected with the color reagent because the concentration of these compounds was very small.

Based on the phytochemical screening data which showed that all the combined herbal extracts showed the presence of phenolic and tannin, and then the total phenolic content was determined by spectrophotometer using Folin-Ciocalteu reagent. The principle of the reaction to determine the levels of phenolic compounds with Folin-Ciocalteu reagent is the reduction of phenolic groups with Folin's reagent which is characterized by the formation of a blue complex compound. The addition of distilled water serves to dilute the Folin solution while Na<sub>2</sub>CO<sub>3</sub> is added to form an alkaline solution. The absorbance of the solution formed was measured at a wavelength of 760 nm. In this determination, standard phenolic compounds are used, namely, gallic acid which is a simple phenolic acid derived from stable hydroxybenzoic acid. Gallic acid was made in solution at various concentrations, reacted with Folin-Ciocalteu reagent, and measured its absorbance at a wavelength of 760 nm. The regression equation obtained from the standard gallic acid curve is  $y = 0.0046x - 0.0329$  and  $R^2 = 0.9851$ . Furthermore, a regression equation was used to calculate the levels of phenolic compounds of each extract. The levels of phenolic compounds of each extract were expressed as the number of milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). The results of the calculation of the levels of phenolic compounds of each extract are shown in Table 3.

**Table 3: The levels of phenolic compounds of the herbs combination extract**

S. No.	Herbs	TPC ± SD (mg GAE/g water extract)	TCP ± SD (mg GAE/g ethanol extract)
1.	Combination herbs A	45.60 ± 2.83	1131.67 ± 95.76
2.	Combination herbs B	68.92 ± 12.50	1265 ± 52.38
3.	Combination herbs C	21.34 ± 1.38	1149.78 ± 132.25

TPC: Total phenolic content, GAE: Gallic acid equivalent

The levels of phenolic compounds in each herbs combination are shown in Table 3. These data indicate that the phenolic compounds contained in each combination of herbs extracts are more soluble in ethanol. This is consistent with the results of the previous studies which showed that phenolic compounds were more soluble in ethanol than other solvents [18]. However, phenolic compounds from each herbs combination are also soluble in boiling water. Phenolic compounds such as hydroxycinnamic acids and flavonoids can be dissolved in water by the infusions and decoctions method [17].

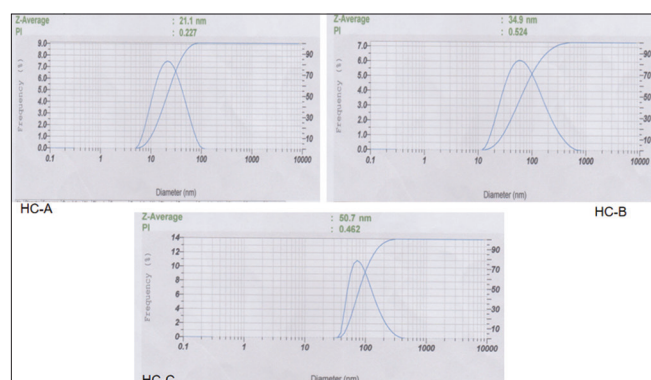


Figure 1: Graph of distribution particle size nanoemulsion combination herbs (HC-A, HC-B, and HC-C)

The ethanol extract of each combination herbs showed significantly higher content of phenolic compounds than the aqueous extract. However, its use in medicine has drawbacks, because ethanol extract is difficult to dissolve in water and has low bioavailability. Therefore, in this study also developed in the form of a nanoemulsion product. The nanoemulsion formulation used the spontaneous emulsion method with the addition of oil and tween 80 as surfactant. The ratio of oil and surfactant used in this study was 1:4 in accordance with the results of the previous studies [15]. The nanoemulsion product of each combination herb obtained has a particle size distribution pattern as shown in Figure 1. The results of the characterization obtained data are shown in Table 4. The characteristics of the combined herbs extract nanoemulsion each showed a particle size of <100 nm, with a PI value between  $0.2 < PI < 0.6$ , so it is relatively stable and not easy to form a precipitate [19]. The nanoemulsion product from the combined herbs ethanol extract was also clear and transparency as measured using a spectrophotometer at a wavelength of 625 nm showing % transmittance above 80%.

Table 4: Characteristics of the combined herbs ethanol extract nanoemulsion

S. No.	Sample	Particle size (nm)	Zeta potential (mV)	Polydispersity index (PI)	%T
1.	Combination herbs A	21.1	-16.3	0.227	87
2.	Combination herbs B	34.9	-14.1	0.524	85
3.	Combination herbs C	50.7	-12.5	0.462	82

In this study, the antioxidant activity of each extract of water, ethanol, and nanoemulsion product of combination herbs with the DPPH method was tested. The DPPH reagent functions as a scavenger radical that will react with phenolic compounds from the sample. The presence of compounds that act as antioxidants causes a decrease in the color of the DPPH solution which was originally blue-purple to yellow. In this study, the antioxidant activity of each sample was measured at various concentrations. The data on the relationship between % inhibition and the concentration of each sample were made a linear regression graph as shown in Figure 2. Furthermore, the obtained regression equation was

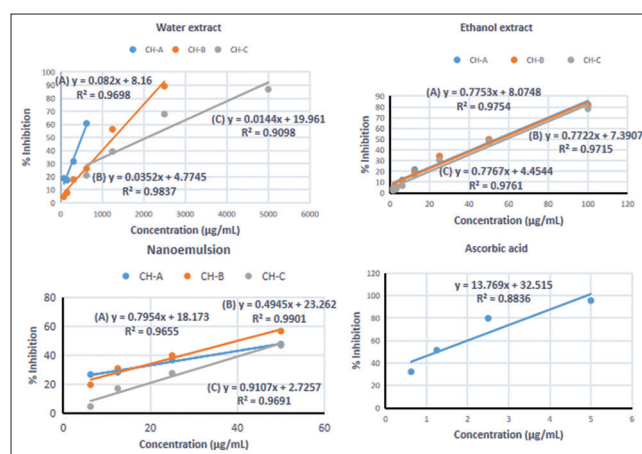


Figure 2: Graph of free-radical inhibition of water extract, ethanol extract, nanoemulsion of herb combination, and ascorbic acid

used to determine the  $IC_{50}$  of each sample. The  $IC_{50}$  mean data for each sample in three measurements are shown in Table 5.

Table 5: Antioxidant activity of each combination herbal extract

S. No.	Sample	Antioxidant activity $IC_{50} \pm SD$ $\mu\text{g/mL}$		
		Water extract	Ethanol extract	Nanoemulsion Product
1.	Combination Herbs A	$507.13 \pm 15.16$	$52.25 \pm 2.19$	$54.07 \pm 1.15$
2.	Combination Herbs B	$1283 \pm 19.46$	$54.16 \pm 1.55$	$40.01 \pm 2.36$
3.	Combination Herbs C	$2073 \pm 173.93$	$56.63 \pm 2.03$	$51.91 \pm 2.87$
4.	Ascorbic acid (positive control)	$1.27 \pm 0.12$		

$IC_{50}$  is the effective concentration of the extract which causes the inhibition of the DPPH concentration to decrease by 50%. The lower the  $IC_{50}$  value, the higher the antioxidant activity. On the other hand, a high  $IC_{50}$  value indicates a low antioxidant activity. Ascorbic acid is a natural antioxidant that is widely used as a positive control in determining antioxidant activity using the DPPH method. The  $IC_{50}$  calculation results for each herbal extract are shown in Table 5. The antioxidant potential of all extracts was in correlation with total phenol content [20]. Therefore, extracts with high phenolic content showed high antioxidant activity. The total phenolic compound content of the combined herbs ethanol extract of the three formulas was almost similar, so that the antioxidant activity was also almost the same. Antioxidant activity with  $IC_{50}$  values below  $100 \mu\text{g/mL}$  showed good activity.

The antioxidant activity of the nanoemulsion products of each combination herbs ethanol extract also showed good activity. The advantage of this herbs nanoemulsion product is that it is easily soluble in water, so it is easily absorbed. The disadvantages of using herbs extracts in medicine are their low solubility and bioavailability. One of the technologies currently being developed is herbs formulations in the form of Self-nanoemulsifying drug delivery system which is a system in the form of an isotropic mixture of oil, surfactant, and cosurfactant phases which when mixed with water will form an O/W nanoemulsion

(oil in water) spontaneously. The nanoemulsion formed was thermodynamically stable with droplet size <100 nm, thereby increasing the absorption and bioavailability of herbs compounds [21].

Research on the types of herbs *C. longa*, *P. urinaria*, *A. paniculata*, *Z. officinale*, *C. citratus*, and *C. limon* showed that these plants contain active components that can increase immunity [22], [23]. Therefore, this study continues to examine the activities of each herbal extract and its nanoemulsion product as an immunomodulator that can improve the body's immune system in overcoming the COVID-19 virus. Nanoparticles are a drug delivery system suitable for use in antiviral drugs, including those to treat SARS-CoV-2, so that effective antiviral drugs can be produced and without side effects [24]. Formulation of herbal extracts in the form of nanoemulsions will increase bioavailability, such as the results of research on curcumin nanoparticles formulated using beta-carotene and Poly-Lactic-co-Glycolic Acid [25].

## Conclusion

Combined herbs ethanolic extracts consisting of *C. longa*, *P. urinaria*, *A. paniculata*, *Z. officinale*, *C. citratus*, and *C. limon* in various compositions contain phenolic compounds and high antioxidant activity. The total phenolic content and antioxidant activity of the combined herbs ethanol extract were higher than that of the water extract. The nanoemulsion formulation of combined herbs ethanol extract showed a particle size smaller than 100 nm and showed good stability. The combination of these herbs ethanol extracts and their nanoemulsion products has the potential to be developed as a natural antioxidant.

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## Author Contributions

All authors have contributed equally.

## References

- World Health Organization. Coronavirus Disease (COVID-19) Pandemi. Geneva: World Health Organization; 2021. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>. [Last accessed on 2021 Jun 25].
- Block KI, Mead MN. Immune system effects of echinacea, ginseng, and astragalus: A review. *Integr Cancer Ther.* 2003;2(30):247-67. <https://doi.org/10.1177/1534735403256419> PMID:15035888
- Putri DU, Rintiswati N, Soestyo M, Haryono SM. Immune modulation properties of herbal plant leaves: *Phyllanthus niruri* aqueous extract on immune cells of tuberculosis patient *in vitro* study. *Nat Prod Res* 2018;32(4):463-7. <https://doi.org/10.1080/14786419.2017.1311888> PMID:28391709
- Amirghofran Z, Hashemzadeh R, Javidnia K, Golmoghaddam H, Esmaeilbeig A. *In-vitro* immunomodulatory effects of extracts from three plants of the Labiatae family and isolation of the active compound(s). *J Immunotoxicol.* 2011;8(4):265-73. <https://doi.org/10.3109/1547691X.2011.590828> PMID:21711089
- Ogbole OO, Akinleye TE, Segun PA, Faleye TC, Adeniji AJ. *In vitro* antiviral activity of twenty-seven medicinal plant extracts from Southwest Nigeria against three serotypes of echoviruses. *Virology.* 2018;15(1):110. <https://doi.org/10.1186/s12985-018-1022-74> PMID:30021589
- Amalraj A, Pius A, Gopi S, Gopi S. Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives a review. *J Tradit Complement Med.* 2017;7(2):205-33. <https://doi.org/10.1016/j.jtcme.2016.05.005> PMID:28417091
- Du G, Xiao M, Yu S, Wang M, Xie Y, Sang S. *Phyllanthus urinaria*: A potential phytopharmacological source of natural medicine. *Int J Clin Exp Med.* 2018;11(7):6509-20.
- Tan MC, Oyong GG, Shen CC, Consolacion Y, Ragasa CY. Chemical composition of *Andrographis paniculata* (Burm.f.) Nees. *Res J Pharm Biol Chem Sci.* 2016;7(4):2405-8.
- Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, et al. Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). *Foods.* 2019;8(6):185. <https://doi.org/10.3390/foods8060185> PMID:31151279
- Bassolé IH, Lamien-Meda AL, Bayala B, Obame LC, Ilboudo AJ, Franz C, et al. Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils alone and in combination. *Phytomedicine.* 2011;18(12):1070-4. <https://doi.org/10.1016/j.phymed.2011.05.009> PMID:21665450
- Ansari SH, Islam F, Sameem M. Influence of nanotechnology on herbal drugs: A review. *J Adv Pharm Technol Res.* 2012;3(3):142-6. <https://doi.org/10.4103/2231-4040.101006> PMID:23057000
- Ajazuddin SS. Applications of novel drug delivery system for herbal formulations. *Fitoterapia.* 2010;81(7):680-9. <https://doi.org/10.1016/j.fitote.2010.05.001> PMID:20471457
- Harborne AJ. *Phytochemical Methods a Guide to Modern Techniques of Plant Analysis.* 3<sup>rd</sup> ed. Netherlands: Springer; 1998.
- Hagerman A, Harvey-Mueller I, Makkar AH, Mueller IH, Makar HP. Quantification of Tannins in Tree Foliage Laboratory Manual. Vienna: FAO/IAEA; 2000.

15. Atun S, Arianingrum R, Cahyaningsih L, Pratiwi FA, Kusumaningrum R, Khairuddean M. Formulation and characterization of quercitrin nanoemulsion isolated from *Dendrothoe falcata* and its antioxidant activity test. *Rasayan J Chem* 2020;13(3):1347-56. <https://doi.org/10.31788/RJC.2020.1335868>
16. Atun S, Dewi Y, Aznam N. Characterization of nanocurcuminoid from ethanol extract of *Curcuma xanthorrhiza* rhizome loaded by chitosan and alginic and its antioxidant activity test. *Rasayan J Chem*. 2020;13(2):817-825. <https://doi.org/10.31788/RJC.2020.1325680>
17. Triantaphyllou K, Blekas G, Boskou D. Antioxidative properties of water extracts obtained from herbs of the species *Lamiaceae*. *Int J Food Sci Nutr*. 2001;52(4):313-7. <https://doi.org/10.1080/09637480120057512>  
PMid:11474895
18. Azman NF, Azlan A, Khoo HE, Razman MR. Antioxidant properties of fresh and frozen peels of *Citrus* species. *Curr Res in Nutr Food Sci*. 2019;7(2):331-9. <https://doi.org/10.12944/CRNFSJ.7.2.03>
19. Smrity SZ, Saifuddin AH, Sultana S. Stability analysis of formulated emulsion containing black cumin (*Nigella sativa*) oil. *Am J Biomed Life Sci*. 2016;4(3):49-53. <https://doi.org/10.11648/j.ajbls.20160403.15>
20. Vlaisavljević S, Rašeta M, Berežni S, Passamonti S, Tramer F. Four selected commercial seaweeds: Biologically active compounds, antioxidant and cytotoxic properties. *Int J Food Sci Nutr*. 2021;72(6):757-766. <https://doi.org/10.1080/09637486.2020.1866503>  
PMid:33386060
21. Joshi RP, Negi G, Kumar A, Pawar YB, Munjal B, Bansal AK, et al. SNEDDS curcumin formulation leads to enhanced protection from pain and functional deficits associated with diabetic neuropathy: An insight into its mechanism for neuroprotection. *Nanomed Nanotech Biol Med*. 2013;9(6):776-85. <https://doi.org/10.1016/j.nano.2013.01.001>  
PMid:23347896
22. Shabat SB, Yarmolinsky L, Porat D, Dahan A. Antiviral effect of phytochemicals from medicinal plants: Applications and drug delivery strategies. *Drug Deliv Translat Res*. 2020;10(2):354-367. <https://doi.org/10.1007/s13346-019-00691-6>  
PMid:31788762
23. Mukhtar M, Arshad M, Ahmad M, Pomerantz RJ, Wigdahl B, Parveen Z. Antiviral potentials of medicinal plants. *Virus Res*. 2008;131(2):111-20. <https://doi.org/10.1016/j.virusres.2007.09.008>  
PMid:17981353
24. Yuliani SH. Nanoparticle as the strategy for the development of sar-cov-2 antiviral. *Int J Appl Pharm*. 2021;13(5):33-43. <https://doi.org/10.22159/ijap.2021v13i5.42604>
25. Chinthala R, Sing SS. Development and pharmacokinetics of curcumin loaded beta caroten nanoparticles in rats. *Int J Appl Pharm*. 2021;13(5):172-6. <https://dx.doi.org/10.22159/ijap.2021v13i5.42339>