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by Ely Setiawan

Formulation, Characterization and Sunscreen Potential Evaluation of Lemongrass *(Cymbopogon citratus)* Oil Nanoemulsion

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Abstract. Lemongrass (*Cymbopogon citratus*) oil is one of the natural resources with antioxidant activity and has the potential to be applied as a sunscreen to prevent free-radical reactions caused by UV light. This research aimed to formulate lemongrass oil nanoemulsion, characterize, and examine its potency as a sunscreen. Lemongrass oil nanoemulsion was formulated with 1, 3, 5, and 7% of oil concentrations. The characterization included organoleptic tests, pH tests, viscosity tests, transmittance percentage (%T) tests, nanoemulsion type tests, droplet size tests, and thermodynamic stability tests. Based on the organoleptic test, nanoemulsions with oil concentrations of 1, 3, and 5% showed clear visual and good stability, while the nanoemulsion 7% exhibited turbid visual and instability. The pH tests revealed that the prepared formulas were safe to be used on the skin. The viscosity tests displayed the viscosity values were <200 mPas, while the %T tests of the formulas were near 100%. In addition, the nanoemulsion types of the prepared lemongrass oil nanoemulsions were found to be o/w, with a droplet size of <10 nm. Evaluation of the potential of nanoemulsion as sunscreen was conducted using a spectrophotometer UV-Vis (290-400 nm) with ethanol as a blank solution and non-nano-emulsified lemongrass oil as a comparison. Based on the test, sunscreen activities of lemongrass oil nanoemulsions 1, 3, and 5% were higher than sunscreen activities of non-nano-emulsified lemongrass oil with the same oil concentrations. The nanoemulsion formula with the highest sunscreen activity was nanoemulsion with a concentration of 5%. This formulation showed an SPF value of 2.59, a percentage transmission of erythemal (%Te) value of 11.27%, and a percentage transmission of pigmentation (%Tp) value of 45.54%. Furthermore, the sunscreen activity of the lemongrass oil nanoemulsion 5% was categorized as a regular suntan.

INTRODUCTION

Indonesia is a tropical country with a high intensity of sunlight. One of the types of sunlight radiation that reaches the earth's surface is ultraviolet (UV) light. Direct exposure to the skin by UV light can cause negative effects such as photocarcinogenesis, inflammation, hyperplasia, immunosuppression, and premature aging [1]. Hence, the application of sunscreen is very important to prevent the negative effects of UV light exposure. Sunscreen can protect skin by absorbing, scattering, or reflecting UV light [2].

Lemongrass *(Cymbopogon citratus)* is one of the natural products that thrive in Indonesia. Lemongrass oil is known to have the potential as a sunscreen caused by its high antioxidant activity. An antioxidant is one of the important compositions of chemical sunscreen because antioxidants can protect skin from UV light-induced free

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radicals by forming a bond with reactive oxygen species (ROS) [3]. Based on research by Mahmood (2018), lemongrass oil has the potential as a sunscreen with an SPF (Sun Protection Factor) value of 8.5, whereas the creamed lemongrass oil has an SPF value of 22 [4].

The potency of a compound as sunscreen can be known by the sun protection factor (SPF), percentage transmission of erythemal (%Te), and percentage transmission of pigmentation (%Tp) values. The SPF value is a universal indicator that describes the effectiveness of a UV protector product. An SPF value can be defined as the ratio of UV light energy that causes minimum erythema on protected skin compared to unprotected skin in one person. The skin will darken after 10 minutes of sunlight exposure. A sunscreen product that shows an SPF of 2 means the product has 2 x 10 minute protection time, which is 20 minutes. Therefore, the higher SPF value of sunscreen will cause higher protection for the skin [5]. Classification of sunscreen efficacy based on the SPF value is presented in Table 1 [6]. In addition, %Te indicates the number of lights transmitted after contact with sunscreen to cause erythema on the skin. Meanwhile, %Tp indicates the number of lights transmitted after contact with sunscreen to cause pigmentation on the skin [7]. Table 2 presents the classifications of sunscreen based on %Te and %Tp values [8].

TABLE 1. Classifications of sunscreen based on the SPF values.

SPF value	Ability
2-4	Minimum
4-6	Medium
8-15	Good
>15	Maximum

TABLE 2. Classifications of sunscreen based on the %Te and %Tp values

Classification	%Te	%Tp
Total block	<1	3-40
Extra protection	1-6	42-86
Regular suntan	6-12	45-86
Fast tanning	10-18	45-86

Research and development of nanotechnology in the field of cosmetics are growing rapidly to overcome the weaknesses of the products [9]. It is widely known that nanotechnology could increase the effectiveness of a product. Nanoemulsion is a transparent emulsion formed by the dispersion of oil and water and stabilized by a film coating of surfactant. Nanoemulsion has very small droplet sizes between 1 to 100 nm [10]. Nanoemulsion has advantages such as widening particle surfaces [11], increasing kinetic stability, and preventing sedimentation and creaming of emulsion [12].

Many types of research have been carried out in formulating essential oils into nanoemulsions. Clove-lemongrass oil nanoemulsion [13], lemongrass-rosemary oil nanoemulsion [14], and lemongrass-lime oil nanoemulsion formulation [15] have been successfully prepared. These reports have proven that nanoemulsion increases the effectiveness and stability of the products compared to pure oil or emulsified oil. Therefore, in this work, we presented the formulation of lemongrass oil nanoemulsions, characterization, and evaluation of their sunscreen properties.

TOOLS AND MATERIALS

Tools used in this study were beaker glass, Erlenmeyer, graduated pipette, measuring glass, filler, pH meter, oven, viscometer Ostwald, refrigerator, magnetic stirrer, spectrophotometer UV-Vis (Shimadzu), and Particle Size Analyzer (Microtrac, Universitas Negeri Yogyakarta). Materials used in this study were lemongrass oil, tween 80 (Merck, Germany), propylene glycol, methylene blue (Merck, Germany), and ethanol (Merck, Germany).

METHODS

Preparation of Nanoemulsion

Preparation of lemongrass oil nanoemulsions was carried out according to the method by Sharma et al. [13]. Nanoemulsions were prepared in four formulas by varying the concentrations of lemongrass oil at 1, 3, 5, and 7%. Surfactant systems, which consist of 25% of Tween 80 and 10% of propylene glycol were added in the same amounts to each of the formulations. The mixtures of lemongrass oil and surfactant system were then stirred at 750 rpm for 30 minutes. After that, the distilled water was added gently into the mixture under magnetic stirring at 1200 rpm for 30 minutes. The water phase was added until the volume of each formula reached 100%.

Characterization of Nanoemulsions

The prepared nanoemulsions from Lemongrass oil were characterized based on their organoleptic properties [16], a viscosity [17], transmittance percentage, pH, nanoemulsion type, and droplet size [18].

Evaluation of Nanoemulsion Sunscreen Potency

The prepared nanoemulsions were diluted in ethanol until the concentration reached 20.000 ppm [7]. As a comparison, the pure lemongrass oil was also diluted in ethanol until the concentration reached 1, 3, and 5%, and each of the solutions was diluted further until the concentration reached 20.000 ppm.

An SPF value can be determined using spectrophotometry UV-Vis by calculating the area under the curve (AUC) in the wavelength range of 290-400 nm. The AUC value is calculated using Equation 1 [7]:

$$[AUC]^{\lambda p}_{\lambda p\text{-}A} = \frac{A(p-a)+A(p)}{2}\{\lambda(p)-\lambda(p-a)\} \qquad (1)$$

Descriptions:

A(p) = absorbance of the sample at the longer wavelength between two wavelengths
A(p-a) = absorbance of the sample at the shorter wavelength between two wavelengths
λ(p) = the longer wavelength between two wavelengths
λ(p-a) = the shorter wavelength between two wavelengths

Furthermore, the SPF value can be determined using Equation 2 [7]:

$$\text{Log SPF} = \frac{\Sigma AUC}{\lambda_n - \lambda_1} \qquad (2)$$

with λ_n as the shortest wavelength, which is 400 nm, and λ_l as the longest wavelength, which is 290 nm.

Determination of the %Te value can be done by measuring the absorbance of the sample in the wavelength range of 292.5-317.5 nm and then being calculated using Equation 3 [7]:

$$\%Te = \frac{Ee}{\Sigma Fe} = \frac{\Sigma T.Fe}{\Sigma Fe} \qquad (3)$$

Descriptions:

Te = transmission value
Fe = flux of erythema
Ee = amounts of a flux of erythema transmitted by nanoemulsion in the wavelength range of 292.5-317.5 nm.

The %Tp value can be determined by measuring the absorbance of the sample in the wavelength range of 322.5-372.5 nm and then being analyzed using Equation 4 [8]:

$$\%Tp = \frac{Ep}{\Sigma Fp} = \frac{\Sigma T.Fp}{\Sigma Fp} \qquad (4)$$

Descriptions:

Tp = transmission value

Fp = flux of pigmentation

Ee = amount of flux of pigmentation transmitted by nanoemulsion in the wavelength range of 322.5-372.5 nm.

RESULTS AND DISCUSSIONS

Preparation of Nanoemulsions

In this work, the preparation of the lemongrass oil nanoemulsion is categorized as spontaneous emulsification [19]. This procedure is also referred to as the low-energy method. The nanoemulsions were prepared by mixing oil phases (lemongrass oil in concentrations of 1, 3, 5, and 7%), tween 80 (25% of total concentration) as a surfactant, and propylene glycol (10% of total concentration) as a cosurfactant at room temperature. The mixture was then stirred for 30 minutes at 750 rpm. After 30 minutes, distilled water was added gently until the volume of the formula reached 100%.

In the spontaneous emulsification method, the stirring process should not be too fast or too slow. Too fast stirring would cause foaming that would also cause interference during particle size analysis. Too slow stirring would cause the formula to be hard to homogenize. The prepared nanoemulsion samples were then characterized to determine whether these formulations followed the standards.

Characterization of Nanoemulsion

Organoleptic tests

The organoleptic tests were carried out to study the physical appearance of prepared nanoemulsion samples. The parameters of color, odor, phase separation, and clarity of samples were evaluated. The results of the organoleptic tests are shown in Table 3.

TABLE 3. Organoleptic test of the prepared nanoemulsion samples

Parameters	**Samples**			
	1 %	3%	5%	7%
Color	Slightly yellow	Yellow	Yellow	Yellow
Odor	Fragrant	Fragrant	Very fragrant	Very fragrant
Phases Separation	Very homogeneous	Very homogeneous	Very homogeneous	Homogenous
Clarity	Very clear	Very clear	Very clear	Very not clear/very turbid

Lemongrass nanoemulsions 1, 3, and 5% did not undergo phase separation; however, nanoemulsion 7% showed a turbid appearance. The addition of surfactant concentration will decrease interface tension. When the surfactant reaches a certain concentration, the surfactant will aggregate and form micelles [20]. The turbid appearance of nanoemulsion 7% was caused by the lack of surfactant concentration so that the micelle was not formed properly. This appearance also indicates that the nanoemulsion 7% has a large droplet size and instability. Based on the organoleptic test, nanoemulsion 7% was not further characterized and tested.

Transmittance Tests Percentage

This test was conducted using a spectrophotometer UV-Vis using a wavelength of 650 nm and using distilled water as a blank. The results of the transmittance test are shown in Table 4. The results showed that the transmittance percentage of nanoemulsions 1, 3, and 5% were close to 100%. A transmittance percentage close to 100% implies that the formula is transparent [21]. This finding also indicates that the prepared nanoemulsions were clear and had small-sized droplets [22].

TABLE 4 The results of Transmittance percentage, viscosity, and pH tests

Formula	%T	Viscosity (mPas)	pH
1%	99.985	38.153	6.5
3%	99.862	103.272	6.2
5%	99.808	154.282	5.8

Viscosity tests

This test was conducted using a viscometer by Ostwald at room temperature. Theoretically, the viscosity of nanoemulsion should be in the range of 1-200 mPas [17]. Based on the viscosity test, nanoemulsions 1, 3, and 5% have good viscosity corresponding to the parameter (Table 4). It can be noticed that a higher viscosity value of the nanoemulsion will be obtained as the oil concentration increases because lemongrass oil is more viscous than water.

pH tests

In this work, the prepared nanoemulsion was aimed at being used on the human skin. Therefore, the pH value of the prepared nanoemulsion should be in the range of 4.5-7.5 for the safety of the skin [24]. Based on pH test results, nanoemulsion has a pH value in the range of 5.8-6.5 (Table 4). Consequently, that the prepared nanoemulsions can be categorized to be safe to be used on the skin. The results also revealed that the pH value decreased as the oil concentration increased. Lemongrass oil contains fatty acids such as linoleic acid, oleic acid, and myristic acid that cause lemongrass oil to be more acidic than water [24]. Thus, nanoemulsions with a higher oil concentration would be more acidic or have a lower pH value.

Nanoemulsion type tests

This test was carried out by using methylene blue. A few drops of methylene blue were added to the nanoemulsion. After observation, the result showed that methylene blue was dispersed evenly in nanoemulsions 1, 3, and 5%.

Methylene blue is a water-soluble coloring substance. On the other hand, the prepared nanoemulsions 1, 3, and 5% are expected to have more water content compared to their oil composition. Therefore, methylene blue can be completely dispersed in the nanoemulsion. Based on this test, it can be concluded that the prepared nanoemulsion types were oil in water (o/w).

Droplet sizes measurement

Fig. 1 and Fig. 3 show that nanoemulsions 1 and 5% have more than 1 peak, which indicates there are a few droplets that have different sizes. The figures showed that a few droplets were measured to be greater than 100 nm with a low percentage of volume distribution (Table 5). This result might be caused by the formation of foam during the stirring process and later it might disturb the droplet size measurement.

Nanoemulsions 1, 3, and 5% have a dominant size below 10 nm, indicates that the nanoemulsions have nano-scale size. Based on research by Shakeel et al. (2008), nanoemulsion should have a droplet size in the range of 1-100 nm. The test result also showed that the droplet size increased with the increase in oil concentration. This is caused by the fact that, as the concentration of lemongrass oil gets higher, the surfactant ability to aggregate oil will decrease [20].

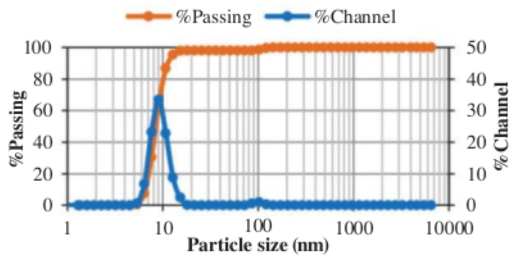

FIGURE 1. Particle size distribution of nanoemulsion 1%

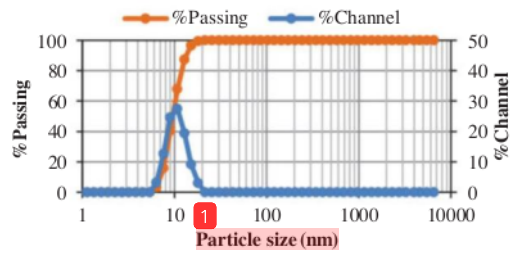

FIGURE 2. Particle size distribution of nanoemulsion 3%

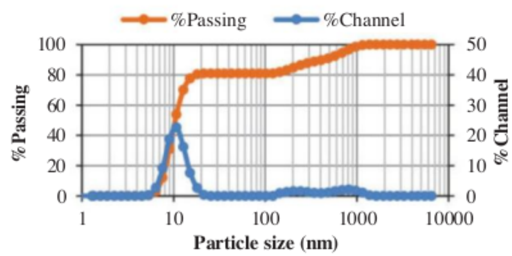

FIGURE 3. Particle size distribution of nanoemulsion 5%

TABLE 5. Particle size distribution of nanoemulsions

Sample	Particle size (nm)	Volume (%)
1%	8.35	98.1
	92.1	1.9
3%	9.57	100
5%	9.69	80.9
	193.8	6.8
	651	12.3

Nanoemulsion sunscreen potency

The diluted lemongrass oil and lemongrass oil nanoemulsion were measured by spectrophotometer UV-Vis to determine the SPF, %Te, and %Tp values. The results of SPF, %Te, and %Tp values are shown in Fig. 4, Fig. 5, and Fig. 6 respectively.

Based on Fig. 4, the SPF values of lemongrass oil nanoemulsions 1, 3, and 5% respectively were 1.32, 1.55, and 2.59. The SPF values of non-nano-emulsified lemongrass oil with the concentration of 1, 3, and 5% were 1.10, 1.33, and 1.70, respectively. It can be observed that lemongrass oil nanoemulsion has a higher SPF value than non-nano-emulsified lemongrass oil. This outcome is caused by the smaller globule of nanoemulsion that creates a wider surface area to absorb UV light radiation [25].

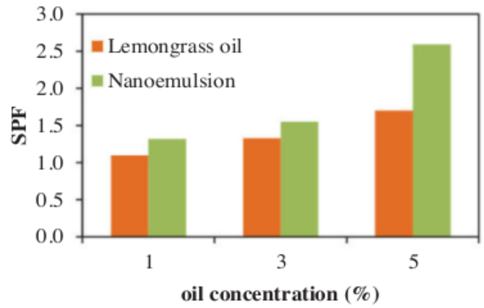

FIGURE 4. Results of SPF value determination

Lemongrass oil nanoemulsion 5% has the highest SPF value, which was 2.59. It implies that lemongrass oil nanoemulsion 5% is able to protect skin from UV light for 25.9 minutes. Sunscreen with a 2.59 SPF value is categorized as sunscreen with the minimum ability [6]. The SPF values are proposed could be increased by increasing lemongrass oil concentration.

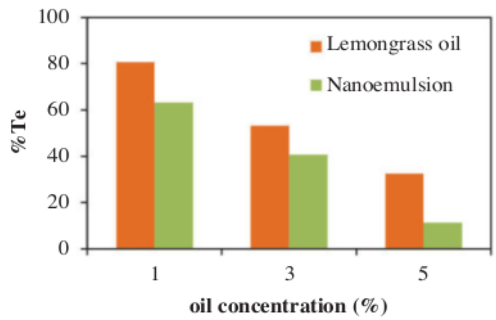

FIGURE 5. Results of %Te value determination

Based on Fig. 5, the %Te values of lemongrass oil nanoemulsions 1, 3, and 5%, respectively, were 63.24, 40.61, and 11.27%. The test was also done towards non-nano-emulsified lemongrass oil 1, 3, and 5%, and the results were 80.76; 53.14; and 32.45% respectively. The %Te value of lemongrass oil nanoemulsion is always lower than the %Te value of non-nano-emulsified lemongrass oil. This result implies that lemongrass nanoemulsion transmitted less UV light radiation. Based on %Te value determination, it was recognized that lemongrass oil in a nanoemulsion formula could increase its UV light protection effectivity.

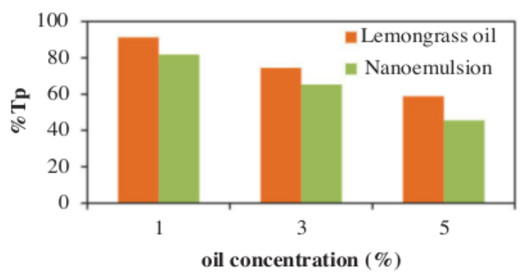

FIGURE 6. Results of %Tp value determination

Based on Fig. 6, %Tp values of lemongrass oil nanoemulsions 1, 3, and 5% respectively were 81.98, 65.18, and 45.54%. The test was also done on non-nano-emulsified lemongrass oil 1, 3, and 5%, and the results were respectively 91.26, 74.35, and 58.97%. Based on %Tp value determination, with the same oil concentration, the %Tp value of lemongrass oil nanoemulsion is always higher. From this test, it is also confirmed that lemongrass nanoemulsion has higher efficacy in protecting skin from UV light radiation compared to non-nano-emulsified lemongrass oil.

According to the %Te and %Tp values, lemongrass nanoemulsions 1 and 3% are not effective for protecting skin from UV-A and UV-B light because their %Te and %Tp values are beyond the maximum range values (18% for %Te and 86% for %Tp). On the other hand, lemongrass nanoemulsion 5% has the effectivity protection of UV light with %Te value of 11.27% and %Tp value of 45.54%. Lemongrass nanoemulsion 5% is categorized as a regular suntan.

CONCLUSION

All of the prepared lemongrass oil nanoemulsions showed better activity as sunscreen than non-nanoemulsions. Lemongrass oil nanoemulsion 5% has an SPF value of 2.59; %Te value of 11.27% and %Tp value of 45.54%. Lemongrass nanoemulsion 5% is the formula that has sunscreen activity with the minimum ability and is categorized as a regular suntan.

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REFERENCES

1. M. Yaseen, T. Mahmood, A. M. Yousaf, Y. Shahzad, G. Bjorklund, and R. Lysiuk, Life and Environmental Sciences. **55** (2), 11–20 (2018).
2. M. A. Suva, Journal of PharmaSciTech, **3**(2), 95-7 (2014).
3. M. M. Dolingkar, and S. L. Deore, Sunscreens: A Review, Pharmacogn. J., **8**(3), 171-179 (2016).
4. T. Mahmood, A. M. Yousaf, Y. Shahzad, and G. Bjørklund, B. Life and Environmental Sciences, **55**(2), 11–20 (2018).
5. E. Dutra, and D. Olivera, Brazilian Journal of Pharmaceutical Sciences, **40**(3), 381-385 (2004).
6. V. Damogalad, H. J. Edy, and H. S. Supriati, Pharmacon: Jurnal Ilmiah Farmasi, **2**(1), 39-43 (2013).
7. M. Mutmainah, Y.D. Franyoto, I. Puspitaningrum, and L. Kusmita, Indian Journal of Science and Technology, **13**(45), 4506-4512 (2020).
8. I. Ahmad, M. Arifuddin, and L. Rijai, International Journal of PharmTech Research, **9**(9), 428-436 (2016).
9. S. Kaul, N. Gulati, D. Verma, S. Mukherjee, and U. Nagaich, Journal of Pharmaceutics (Cairo), **1**, 1-9 (2018).
10. F. Shakeel, S. Baboota, A. Ahuja, J. Ali, M.S. Faisal, and S. Shafiq, Thai J. Pharm. Sci, **32**(1), 4-9 (2008).
11. L. Salvia-trujillo, M. A. Rojas-graü, R. Soliva-fortuny, and O. Martín-belloso, Postharvest Biology and Technology, **105**(1), 8–16 (2015).
12. C. Solans, P. Izquidero, J. Nolla, N. Azemar, and M. J. Gracia-celma, Current Opinion in Colloid and Interface Science, **10**(3) 102-110 (2005).
13. A. Sharma, N. K. Sharma, A. Srivasta, A. Kataria, S. Dubey, S. Sharma, and B. Kundu, Industrial Crops & Products, **123**(6), 353–362 (2018).
14. D. Rojas, O. R. Garcia, and A. E. Restrepo, Food Science and Biotechnology, 1–8 (2018).
15. M. Juniatik, K. Hidayati, F.P. Wulandari, and N. Pangestuti, Traditional Medicine Journal, **22**(1), 7-15 (2017).
16. A. Ana, S. Subekti, S. Hamidah, and K. Komariah, Conference Series: Materials Science and Engineering-2017, IOP Conference Proceedings 180, (AOP Publishing, Bandung, 2017), 1-7.
17. P. K. Gupta, J. K. Pandit, A. Kumar, P. Swaroop, and S. Gupta, The Pharma Research, **3**(1), 117-138 (2010).
18. K. Gurpeet and K. Singh, Indian J. Pharm. Sci, **80**(5), 781-789 (2018).
19. K. Bouchemal, S. Briançon, E. Perrier, and H. Fessi, International Journal of Pharmaceutics,**280**(1), 241-251 (2004).

20. D. J. McClements, Soft Matter, **8**(1), 1719-1729 (2012).
21. H. H. Ali and A. A. Hussein, AAPS Open, **3**(4), 1-16 (2017).
22. M. Laxmi, M. Bhardwaj, S. Mehta, and A. Mehta, Artificial Cell, Nanomedicine and Biotechnology, **43**(1), 334-344 (2014).
23. S. M. Ali and G. Yosipovitch, Acta Dermato-Venereologica, **93**(3), 261-267 (2013).
24. M.A. Belewu, K.M. Okukpe, F.O. Oladipo, I. Kareem, F.I. Kolawole, A.M. Lawal, O. Ahmed and A.H.A. Badmos, Medicinal Plants, **3**(2), 169-171 (2014).
25. A. Arianto and C. Cindy, Open Access Maced J Med Sci, **7**(22), 3757-3761 (2019).

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