Minimum Inhibitory Concentration Test Of Crude Extract Of Nigella Sativa Linn. Seeds And Its Formulation In Lozenges

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MINIMUM INHIBITORY CONCENTRATION TEST OF CRUDE EXTRACT OF *Nigella sativa* Linn. SEEDS AND ITS FORMULATION IN LOZENGES

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Abstract

Nigella sativa Linn. is known in many biological activities. The aim of this study were to fine antibacterial activities of *Nigella sativa* Linn. crude extract against *Streptococcus pyogenes* as Minimum Inhibitory Concentration (MIC) with dilution method and its formulation in lozenges dosage form. Taste perception test of the lozenges, was then done by 30 volunteers. The result showed that MIC of *Nigella sativa* Linn. crude extract was 3,92 mg/ml. From the formulation used of 5%, 10%,and 15% *Nigella sativa* Linn. crude extract; performed a different physical characteristics in each 5%, 10%, and 15% *Nigella sativa* Linn. crude extract lozenges on weight uniformity, friability, and hardness. The 20 volunteers agreed that a 5% *Nigella sativa* Linn. crude extract lozenges was the most preferred taste.

Key words: *Nigella sativa* Linn., lozenges, MIC.

Introduction

Nigella sativa Linn. which belong to botanical family of Ranunculaceae, have been claimed to have many pharmacological effects including anti inflammatory (Elbandy, *et.al*, 2009), anti oxidant in the treatment of rhinosinusitis (Yoruk, *et.al*, 2010), and can be used as add on drug therapy on patients of metabolic syndrom (Najmi, *et.al*, 2012). The anti bacteria effect of *Nigella sativa* Linn. extract against *Streptococcus pyogenes* have been reported (Wulansari, *et.al*, 2011). Since *Streptococcus pyogenes* is one of *pharyngitis* (strep throat) caused bacteria, it makes *Nigella sativa* Linn. becoming the alternative medicine of strep throat. The aim of this study are to determine the Minimum Inhibitory Concentration (MIC) value of *Nigella sativa* Linn. extract against *Streptococcus pyogenes* and formulate it into lozenges.

Methodology

Plant material and extraction prosedure

The *Nigella sativa* Linn. (NS) seeds were purchased from local herbal shop in Semarang, Indonesia. A modified extraction procedure by Zaman, *et.al* (2004) was used. About 1 kg NS seeds powder were macerated with 2 L of ethanol for 48 hours. NS crude extract was evaporated using vacuum rotary evaporator.

Broth Dilution Assay

The MIC values were determined by using a modified broth dilution technique by Kamal, *et.al* (2010). Overnight culture of *Streptococcus pyogenes* bacteria growth in nutrient broth (NB) cultures ($1.5x10^8$ cfu/ml) were diluted in NB 10^3 times ($1.5x10^5$ cfu/ml). NS crude extract diluted in DMSO and made in to several concentration. 100µL of each concentration NS extract and 100µL of bacteria culture were added to test tube containing 10 mL of NB culture. The tubes were incubated at 37°C for 24 hours. The tubes were examined for visible turbidity and optical density at 620nm using NB as control. The lowest concentration inhibited the visible growth of the test organism was recorded as MIC.

Formulation of Lozenges

The formulation of NS crude extract lozenges were using NS extract granule with wet granulation method. Lozenges was designed in 3 formulae with different NS crude extract concentration as shown in Table 1.

Table 1. The composition of ingredient in NS lozenges.

Ingredient	Formulae		
	I	II	III
NS extract (g)	12.5	25	50
PVP (g)	50	50	50
Menthol (g)	1	1	1
Mg stearate (g)	1	1	1
Corr.coloris(g)	0.125	0.125	0.125
Aspartam (g)	1.25	1.25	1.25
Mannitol (g)	50	50	50
Avicel to (g)	500	500	500

Evaluation of Lozenges

Weight uniformity test : at amount of 20 lozenges were taken out randomly, and each lozenges weight was measured. The average weight of lozenges was calculated.

Friability test : at amount of 20 lozenges taken out randomly, weighted, cleaned up of dust and put it on the friability tester (Roche-Erweka). After running for 4 minutes (speed 25 rpm), the lozenges were cleaned and weighted.

Hardness test : at amount of 20 lozenges were taken out randomly and its hardness were measure with hardness tester (Stokes Mosanto-Prima).

Taste perception test : 30 lozenges for each formulae were gift to 30 volunteers. The volunteers were asked to give their valuation about the taste of NS lozenges as sweetness, bitter and pungent taste. The valuations were scored and calculate the most preferred taste lozenges.

Results and Discussions

The result of MIC test NS crude extract against *Streptococcus pyogenes* bacteria by dilution method is shown as Table 2. The minimum concentration of NS extract which has inhibited the visible *Streptococcus pyogenes* bacteria growth is 3.92 mg/ml.

Table 2. The MIC test of NS crude extract.

NS extract concentration (mg/ml)	*Optical density (OD)*		ΔOD	Capacity inhibition
	before incubation	after incubation		
0.98	0.871	1.2	0.329	-
1.98	0.973	1.204	0.231	-
2.94	1.161	1.353	0.192	-
3.92	1.333	1.225	-0.108	+
4.90	1.448	1.343	-0.105	+
5.88	1.699	1.644	-0.055	+
6.86	1.712	1.589	-0.123	+
7.84	1.739	1.723	-0.059	+
8.91	2.014	2.026	-0.012	+

The negative ΔOD value showed that there is no bacteria growth. The positive ΔOD value means that there is bacteria growth, because there is an increasing absorbance. The bacteria can still growth in it NS crude extract concentration. MIC of NS extract against other bacteria has been reported are *Bacillus subtilis* 375 µg/ml, *Staphylococcus aureus* 1125 µg/ml and *Escherichia coli* 3000 µg/ml (Alam, *et.al*, 2010).

The evaluations of NS crude extract lozenges are shown as Table 3.

Table 3. The evaluation result of NS lozenges

Lozenges test	Formula		
	I	II	III
Weight (g)	507.58±5.8933	509.12±4.8995	502.86±3.4035
Coefficient of variance (CV) of weight uniformity (%)	1.16±0.1497	0.96±0.2291	0.81±0.1962
Hardness (kg/cm^2)	4.11±0.3437	3.68±0.5290	2.44±0.3784
Friability (%)	0.19±0.0266	0.21±0.0207	0.27±0.0637

Uniformity of lozenges is good if it has a coefficient of variance of weight uniformity of less than 5% (Sulaiman, 2007). The result of this research, all formulae have a uniform tablet weight. Uniformity of weight can be influenced by the size and shape of granules. The round and uniform granules will have good flow rate and can perfectly fills the tablet machine, thereby reducing the weight variant of tablet.

The size and CV also depends on the ability of granules flows to the machine. High and constantly flow rate making the tablet more uniform with lower deviation weight. Formula III has the best CV of uniformity weight (0.81±0.1962 %). The statistical analysis result of CV of weight uniformity showed that formula I had significantly difference CV with formula III (sig.≤ 0.05). Formula I had no significantly different CV with formula II, and formula II with formula III had so.

Lozenges as tablet said to be good if has the hardness at least 7 kg/cm^2 (Cooper *and* Gunn, 1975), but the hardness is not the absolute requirement. The hardness of lozenges required higher than general tablet or chewable tablet, in the hope that lozenges will have a longer time to dissolution in the mouth.

Formula I lozenges has the greatest hardness. This is because the lower NS crude extract concentration are used, the ability of granular particles to bond together will be even bigger and stronger, and it looks more compact after compressed into tablet. Tablet hardness can be affected by the compression pressure. Tablet hardness is directly related to dissolution and disintegration time. Generally but not always, a hard tablet has a long disintegration and dissolution time. Hardness of tablet also related with density and porosity. The statistical analysis result of hardness showed that all formula had significantly difference hardness (sig.≤ 0.05).

Friability is a parameter that describes the strength of tablet surface against the variety of treatments that cause abrasion on the surface of tablet. Formula I lozenges has the smallest friability. Formula I containing extract in small quantity so that the tie between the particles produces the large tablet hardness. Binding capacity and hardness of formula I cause the smallest friability compared with other formulas.

The statistical analysis result of friability showed that formula I had significantly different friability with formula III (sig.≤ 0.05). Formula I had no significantly difference friability with formula II, and formula II with formula III had so.

The friability values of three formulas meet the specified of no more than 1% (Sulaiman, 2007).

Conclusion

Paragraph

Acknowledgement

Paragraph

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