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 well 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 taken to 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 on weight uniformity, friability, and hardness in each 5%, 10%, and 15% *Nigella sativa* Linn. crude extract lozenges. 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 is under family of Ranunculaceae, has 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 additional drug therapy on patients of metabolic syndrome (Najmi et.al., 2012). The anti bacteria effects 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 was 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 procedure

The *Nigella sativa* Linn. (NS) seeds was 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 the modified broth dilution technique of 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 were designed in 3 formulas with different NS crude extract concentration as shown in Table 1.

Table 1. The composition of ingredient in NS lozenges

Ingredient	Formula		
	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 measured with hardness tester (Stokes Mosanto-Prima).

Taste perception test : 30 lozenges for each formula were given to 30 volunteers. Each volunteer was asked to give his evaluation about the taste of NS lozenges: sweetness, bitter and pungent taste. Their responces were scored and calculated to detemine 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 in Table 2. The minimum concentration of NS extract which has inhibited the visible *Streptococcus pyogenes* bacteria growth is 3.92 mg/ml. Flavonoids and saponin compounded in the NS extract can inhibit *Streptococcus pyogenes* growth (Wulansari et.al., 2011).

A negative ΔOD value is meant that there is no bacteria growth. While a positive ΔOD value meant that there is bacteria growth, becaused of its enlarged absorbance. The bacteria can still grow in it NS crude extract concentration. MIC of NS extract against other bacteria 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 in Table 3.

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	+

Table 3. The evaluations of NS lozenges

Lozenges test	Formula		
	I	II	III
Weight (g)	507.58±5.8933	509.12±4.8995	502.86±3.4035
Variant coefficient (VC) 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 weight uniformity's variant coefficient of less than 5% (Sulaiman, 2007). The result of this research, all formula had a uniform tablet weight. Uniformity of weight could be influenced by the size and shape of granules. The round and uniform granules would have good flow rate and could perfectly fill the tablet machine, thereby reducing the weight variance of tablet.

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

Lozenges as tablet is considered to be good, if it 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 is more required than in general tablet or chewable tablet. It is hoped that lozenges will have a longer time to dissolution in the mouth.

Formula I lozenges had the greatest hardness. This was becaused the used of lower NS crude extract concentration. The ability of granular particles to bond together would be even bigger and stronger. And it should be 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. It is usually, but not always, a hard tablet has a long disintegration and dissolution time. The 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 had the smallest friability. Formula I contained a small quantity of extract. So that the tie between the particles produced the large tablet hardness. Binding capacity and hardness of formula I caused 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). There were no significanly different friability betwen Formula I and

Formula II, and betwen Formula II and Formula III. The friability values of three formulas met the specified of no more than 1% (Sulaiman, 2007).

The result of taste preference test is shown in Table 4. From the 30 volunteers, 20 volunteers choosed formula I. Formula II was chosen by 3 volunteers, and formula III was chosen by 7 volunteers. NS extract lozenges with 5% extract concentration was then considered as the most preferred taste lozenges.

Table 4. The Score of Taste Evaluation Test

Formula	Average Score (Total score/30)		
	Sweetness*	Bitter**	Pungent*
I	2.77	4.06	2.83
II	2.47	3.70	2.77
III	2.77	3.50	2.74

Notes : * : Score range 1 (least) – 5 (most)
** : Score range 1 (most) – 5 (least)

Conclusion

This study has concluded that a *Nigella sativa* Linn. extract has a potential antibacterial activity against *Streptococcus pyogenes* with MIC of 3.92mg/ml. The 5% concentrated *Nigella sativa* Linn. extract can be used as an active ingredient of lozenges that in the best physical characteristics and the most preferred taste.

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