

The Development of Creambath Preparation with Combination of Garlic Extract (*Allium sativum* L.) and Custard Apple Seeds Extract (*Annona squamosa* L.) as Anti-Dandruff and Anti Head Lice (*Pediculus humanus capitis*)

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ABSTRACT

Allicin and ajoene are active compounds in garlic which have proven to be of benefit as antifungal. Meanwhile, the content of oleic acid and triglycerides from the custard apple seeds extract showed anti-fungal activity *in vitro*. This study aimed to obtain a preparation of creambath combination of garlic extract and custard apple seeds extract which have anti-dandruff and anti head lice activity. Garlic extract was obtained by adding phosphate buffer with freeze-dry method, while custard apple seeds extract was obtained from maceration using petroleum ether. Garlic extract provides an anti-dandruff effect with a minimum inhibitory concentration (MIC) of 6.25 mg/mL and the smallest custard apple seeds extract (LD 100%, 3 hours) which is 3.13 mg/mL can provide anti head lice effect. Variations in the concentration of the combination of garlic extract and custard apple seeds extract used in creambath preparations were (6.25 mg/mL and 3.13 mg/mL), (12.5 mg/mL and 6.25 mg/mL) and (25 mg/mL and 12.5 mg/mL). The resulting creambath preparations have characteristics that are light green, green tea flavored, homogeneous, semisolid form, o/w cream type, pH 5.85-6.25, viscosity 34,000-72,000 cps with thixotropic pseudoplastic flow properties, average size particle 34.31-57.66 µm, anti-dandruff activity with diameters of inhibitory 9, 12, and 16 mm in 72 hours incubation time and LD 100% hair lice activity in 157, 133 and 105 minutes.

Keywords: Creambath preparations, garlic extract, custard apple seeds extract, anti-dandruff and anti head lice

INTRODUCTION

Dandruff is an abnormality characterized by excessive scaling, itching of the scalp is accompanied by or without signs of inflammation (Tjahjadi, 1995).

Dandruff can be caused by many factors, one of which is a fungus from the genus *Malassezia* or *Pitosporum* which is known to play an active role in causing dandruff (Saint-Leger *et al.*, 1988; Saltshaker, 2004). Traditionally, many plants have anti-fungal activity, one of them is garlic (*Allium sativum* L.). The results showed that garlic has various benefits including anti-bacterial, antidiabetic and antifungal (London *et al.*, 2011). Garlic has antifungal properties due to the chemical compounds of allicin and ajoene (Negri *et al.*, 2011).

Misdiagnosis of head lice are common, especially in the egg phase is difficult to distinguish from other particles found in the hair such as dandruff, hair spray droplets, and dirt particles.

Head lice (*Pediculus humans capitis*) is a skin or head hair infection which is caused by an infestation of *Pediculus humans var. capitis*

(Djoundi *et al.*, 2007). Various classes of insecticidal drugs are used to treat head lice, such as pyrethrin, permethrin, lindane, carbaryl, but the use of insecticides has adverse effects, such as toxicity and potential resistance (Burns *et al.*, 2004).

One of the plants that is efficacious as an anti-head lice is custard apple seeds (*Annona squamosa* L.). Custard apple seeds have been proven effective in killing head lice. Oleic acid and triglyceride content from custard apple seed extract showed anti head lice activity *in vitro*. Researchers has shown that petroleum ether extract from leaves and seeds killed more than 90% of head lice *in vitro* by 52 and 26 minutes respectively (Gritsanapan *et al.*, 1996). Therefore, this study was conducted to develop creambath preparations using a combination of garlic extract and custard apple seeds to overcome dandruff and head lice.

METHODOLOGY

Garlic Dry Extract

100 grams of fresh garlic is washed with water, then garlic is mixed with 250ml 10mM pH 7.0 phosphate buffer and homogenized, then the mixture is squeezed. The clear layer obtained was

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Table I. The Formula for *Creambath* Preparation of Combination of Garlic Extract and Custard Apple Seeds Extract

No.	Ingredients	Concentration (%)			
		Base	F1	F2	F3
1.	Garlic Extract	-	MIC	2 x MIC	4 x MIC
2.	Custard Apple Seed extract	-	LD100%	2 x LD100%	4 x LD100%
3.	Vaseline Album	6,2	6,2	6,2	6,2
4.	Paraffin Liq	13,8	13,8	13,8	13,8
5.	Isopropyl Myristate	1,5	1,5	1,5	1,5
6.	Stearic Acid	7,5	7,5	7,5	7,5
7.	Glyceryl Monostearate	5	5	5	5
8.	Nipagin	0,1	0,1	0,1	0,1
9.	Nipasol	0,05	0,05	0,05	0,05
10.	TEA	0,2	0,2	0,2	0,2
11.	Xanthan Gum	0,2	0,2	0,2	0,2
12.	Green Tea Perfume	0,1	0,1	0,1	0,1
13.	Brilliant Green	0,025	0,025	0,025	0,025
14.	Aqua Distillate	ad 100	ad 100	ad 100	ad 100

filtered by passing a 0.22 μm filter and then stored at -70°C and dried garlic water extract with freeze drying (Shams-Ghahfarokhi *et al.*, 2006).

Rough Extract of Custard apple Seeds

Fresh custard apple seeds are washed and dried in 55°C oven heat for 24 hours. Dry seeds are milled with a blender. ± 1.0 kg dry powder macerated with petroleum ether (10 L) for 2 days at room temperature (25°C) then filtered, the mixture of the filtrate is thickened with the evaporator continued to the water batch until constant weight / obtain crude extract of custard apple seeds (Tiangda *et al.*, 2000; Gritsanapan 1996).

Determination of Anti-Fungal Activities from Garlic Extract

The minimum inhibitory concentration (MIC) value as a minimum concentration value can inhibit the growth of *Pityrosporum ovale* which is characterized by the formation of clear zones after 24-72 hours (Harmita *et al.*, 2005; Sitompul *et al.*, 2016).

Determination of Anti head lice Activities

Testing of anti-head lice, custard apple seed extracts used in vitro techniques for head lice samples taken from school-age girls (9-13 years) and given treatment. 0.05 ml of custard apple seed extract is put into a petri dish and spread thinly over a 2 cm^2 zone. Seven same-sized head lice collected from the hair of a school girl were placed on a petri dish containing variations in concentration (10%; 15%; 20%; 25% and 30%). If

the lice are immobile, it is determined as dead lice, calculated every 30 minutes until all head lice die (McCage *et al.*, 2002).

Creambath Preparations Combination of Garlic Extract and Custard apple Seed Extract

The oil phase consists of Vaseline albums, mineral oil, isopropyl myristate, stearic acid, glyceryl monostearate, and nipasol. The oil phase is heated above the water bath. The water phase consists of TEA, xanthan gum, nipagin, and aquadestilata and is heated over a water bath to a temperature of 70°C . Dried garlic extract was included in aquadestilata at 35°C together with crude extract of custard apple seeds added to base cream that had been formed and added aquadestilata to 100% of the formula weight (Damaranie *et al.*, 2014). The formula for creambath preparation combined with garlic extract and custard apple seed extract (Table I).

Determination of Anti-Fungal Activity in Creambath Preparations Combination of Garlic Extract and Custard apple Seed Extract

Antifungal testing of cream samples by the diffusion method. Inoculated by *Pityrosporum ovale* (equivalent to McFarland standard 0.5 which is equivalent to the number of fungi or yeast as much as 5×10^6 cfu/ml) as much as 200 μL and flattened on the surface of the PDA in Petri using a spreader. Furthermore, the cream samples were weighed 65 ± 5 milligrams each and added 20 ml of sterile aquadest on the 6 mm disc paper waiting to dry on LAF, then placed on the surface of PDA media that had been inoculated with *Pityrosporum*

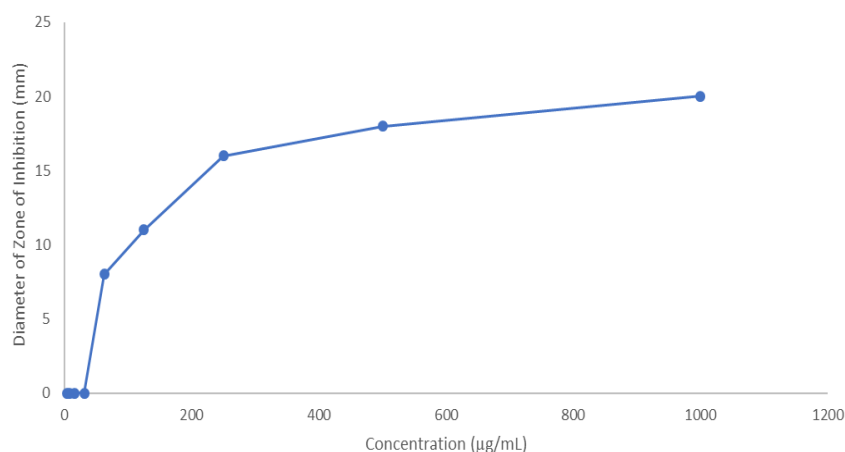


Figure 1. Graph of the results of testing the diameter of the ketoconazole inhibition zone

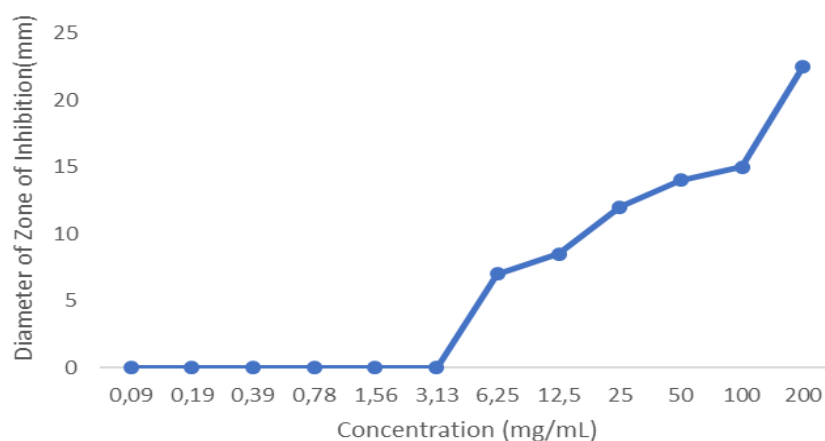


Figure 2. Graph of inhibition zone diameter of garlic extract

ovale, then incubated at 37° C, for 24 hours. The clear zone that arises around the cylinder is measured in diameter. The minimum inhibitory concentration (MIC) value as a minimum concentration value can inhibit the growth of *Pityrosporum ovale* which is characterized by the formation of clear zones after 24 to 72 hours (Tiangda *et al.*, 2000; Gritsanapan 1996).

Testing of anti-head lice activity of custard apple seed extracts used in vitro techniques for head lice samples taken from school-age girls (9-13 years) and given treatment. Approximately 50±5 milligrams each of the creambath preparations were put into a petri dish and spread thinly over a 2 cm² zone. Seven same-sized head lice collected from the hair of the school girl were placed on a petri dish containing creambath preparations. If the lice are immobilized then it is determined as dead head lice, counted

the time until all head lice die (McCage *et al.*, 2002).

RESULTS AND DISCUSSION

MIC Results and Measurement of Inhibited Zone Diameter

The concentration series of garlic extract was used from 0.0977-200 mg / mL, and the series of positive standard concentrations (ketoconazole) from 3.9 -1000 µg / mL. The negative control used was methanol and sterile aquadest.

From the results of the observations it can be concluded that at concentrations of 3.91 to 31.25 µg / mL showed results (-) with no inhibition zone (clear) which means there is no inhibition of the growth of *Pityrosporum ovale*. So that the minimum inhibitory concentration (MIC) of ketoconazole against *Pityrosporum ovale* was

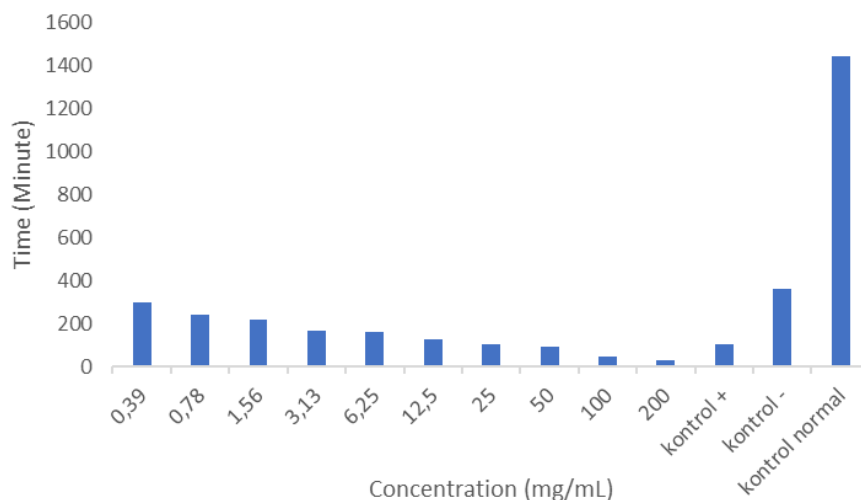


Figure 3. Diagram of the effect of concentrations of custard apple seed extract on time of death of head lice

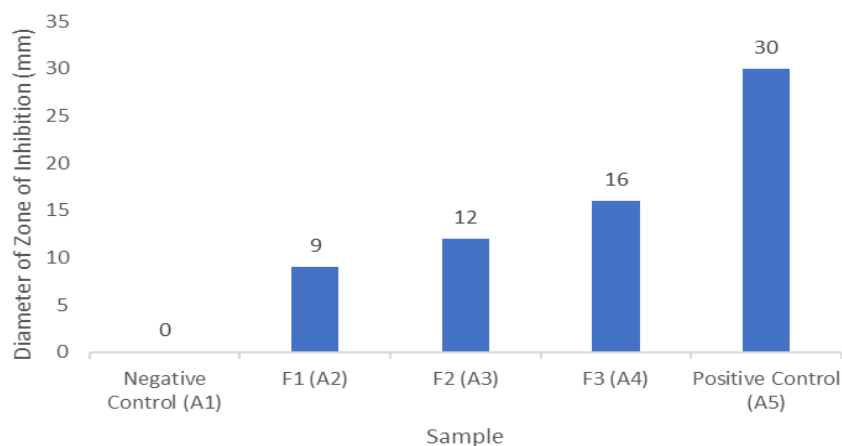


Figure 4. Diagram of inhibition of creambath combination of garlic extract and custard apple seed extract

determined to be 62.5 µg / mL with the inhibition zone formed at 8 mm with an incubation period of 3 days.

From the results of the observations it can be concluded that at concentrations of 0.09 to 3.13 mg / mL showed the results (-) with no inhibition zone diameter formed meaning that there was no inhibition of the growth of *Pityrosporum ovale* So that the minimum inhibitory concentration (MIC) of garlic extract against *Pityrosporum ovale* is 6.25 mg/mL.

Results for Anti-Hair Lice Activities for Custard apple Seed Extract

The results of the examination of head lice death were based on 100% mortality of head lice within 180 minutes^{21,59} in figure 3. The

concentration series of custard apple seed extract was used from 0.39 - 200 mg / mL.

From the results of the observations it can be concluded that at concentrations of 0.39 mg/mL up to 1.56 mg / mL showed results that did not meet the requirements where all head lice showed mortality above the required time (above 180 minutes) (Tiangda *et al.*, 2000; Intaranongpai *et al.*, 2006) means that at 180 minutes there has not been a death of 100% of head lice.

At the concentration of Custard apple seed extract 3.13 mg / mL to 200 mg / mL showed mortality in all head lice with less than 180 minutes, and with an increase in the concentration of custard apple seed extract inversely proportional to the time needed to kill all lice.

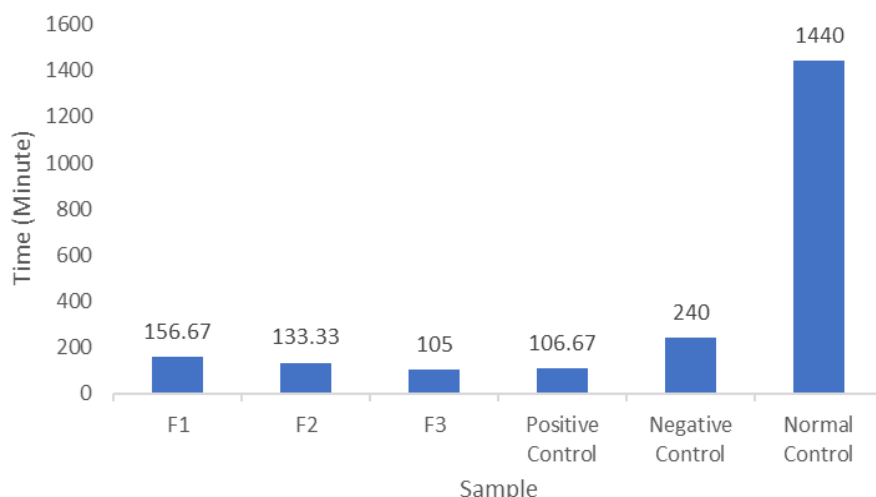


Figure 5. Diagram of The Effect of Creambath on The Combination of Garlic Extract

Table II. Characteristics of Creambath Preparations in Combination with Garlic Extract and Custard Apple Seed Extract

No.	Characteristic	Base	F1	F2	F3
1.	Organoleptic				
	Color	Light Green	Light Green	Light Green	Light Green
	Odor	Green tea	Green tea	Green tea	Green tea
	Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
	Consistency	Semisolid	Semisolid	Semisolid	Semisolid
2.	pH	6.25	6.03	5.94	5.85
3.	Type of cream	W/O	W/O	W/O	W/O
4.	Viscosity (10 rpm & spindle no. 6)	72,000 cp	49,500 cp	41,000 cp	34,000 cp
5.	Flow Properties	Pseudoplastic	Pseudoplastic	Pseudoplastic	Pseudoplastic
		Thixotropic	Thixotropic	Thixotropic	Thixotropic
6.	Average Size Particle	34,31 μm	39,16 μm	41,50 μm	57,66 μm

Negative and normal controls showed negative results with no head lice deaths during the treatment time (180 minutes) so it can be concluded that negative controls did not influence the mortality rate of head lice.

The expected death of head lice is 100% within 180 minutes. The smallest concentration that can kill head lice within 180 minutes is 3.13 mg/mL, higher concentrations can kill 100% of hair lice at a faster time and smaller concentrations kill 100% of head lice in a longer time (> 180 minutes).

Characteristics of Creambath Preparations Combination of Garlic Extract and Custard Apple Seed Extract

Creambath preparations are formulated using base creambath and 3 variations of combinations of garlic extract and custard apple seed extract. The characteristics of the

formula for creambath preparation were a combination of garlic extract and custard apple (Table II).

Measurement Results of Inhibitory Zones of Creambath Preparations Combination of Garlic Extract and Custard apple Seed Extract

Creambath combination of garlic extract and custard apple seed extract which tested its activity against fungi *Pityrosporum ovale* was formula 1, 2, and 3, negative control (base cream) and positive control (ketomed® shampoo). The results of the examination of anti-dandruff activity and measurement result of the average inhibition zone produced (Figure 4).

From the results of the observations, it can be concluded that on the creambath base showed results (-) with no inhibition zone diameter formed which means that there is no inhibition of the growth of *Pityrosporum ovale*. So, it was

determined that the base had no anti-dandruff activity in the absence of inhibition of the *Pityrosporum ovale*.

The biggest inhibition zone is produced by formula 3 which is 15.5 mm with an incubation time of 3 days. In formula 1 to formula 3, it forms an inhibitory region characterized by an increase in inhibition zone diameter at each increase in the concentration of the combination of garlic extract and custard apple seed extract. In the positive control, an inhibition zone of 30 mm was formed with an incubation time of 3 days.

Results of Creambath Preparations Combination of Garlic Extract and Custard apple Seed Extract on Head Lice Death

Creambath preparations used were negative control (base creambath), normal control (without treatment), creambath 1 - 3 formula and positive control (peditox® shampoo).

From the observation in Figure 5, it can be concluded that negative controls and normal controls do not cause death in all head lice up to 180 minutes as required so that it can be determined that negative controls do not have acted as an anti head lice.

Formula 1 to formula 3 shows the death of all head lice with less than 180 minutes, and with an increased concentration of combinations of garlic extract and custard apple seeds, the time needed to kill all hair lice is getting shorter.

CONCLUSION

Garlic extract with minimum inhibitory concentration (MIC) of 6.25 mg/mL can provide anti-dandruff effects and custard apple seed extract with the smallest concentration (100% LD, 3 hours) which is 3.13 mg/mL can provide anti head lice effect. The creambath combination concentration contains garlic extract and custard apple seed extract (6.25 mg/mL and 3.13 mg/mL), (12.5 mg/mL and 6.25 mg/mL) and (25 mg/mL and 12.5 mg/mL) and has met physio-chemical requirements, have anti-dandruff activity with 9, 12 and 16 mm inhibitory power (DDH) at 72 hours incubation time and 100% LD head lice activity within 157, 133 and 105 minutes.

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