

Effectiveness of Custard Apple (*Annona squamosa*) Seed Extract in Treating Pediculosis Capitis

Jellemer M. Benitez, Chelsea V. Cimafranca, Louise Phillina Paz V. Gimenez and Jezyl C. Cutamora

Abstract

*Pediculosis capitis infestation, commonly known as head lice, a worldwide public health concern affecting individuals of all age groups, and prevalence in the general population can be as high as 40 percent. The main task attained in the study was the determination of the pediculicidal effects of Annona squamosa seed extract of different concentrations. Methodology utilized a true experimental design done in 3 trials; 10 live head lice were observed under each concentration of 25%, 50%, 75% atis seeds extract for the experimentation and 75% permethrin concentration as controlled group. In vitro testing was used and the subjects were placed in prepared petri dishes for experimentation. Data results found out that there is p-value of 0.067 at $\alpha = 0.05$ for mean time elapsed (secs) for 100% head lice mortality. Significant p-level of 0.002 for pediculicidal activity for the first 5 minutes has been noted. The researchers derived up with a conclusion that Custard Apple (*Annona squamosa*) seed extract in 75%, 50% and 25% concentration is as effective as Commercial Pediculicide (Permethrin) in killing pediculosis Capitis. 50% concentration has comparable results to Permethrin and is the most effective due to its earlier onset of pediculicidal activity.*

Keywords: Pediculosis capitis, Annona squamosa, permethrin, pediculicide, extract

1.0 Introduction

Pediculosis capitis infestation, commonly known as head lice, is the manifestation of the obligate ectoparasite, *Pediculus humanus capitis*, which only affects the human scalp. Head lice infestation is a worldwide public health concern affecting individuals of all age groups, and prevalence in the general population can be as high as 40 percent. Head lice are also a common bane in the Philippines. According to the Department of Education (2008) (as cited by Yapchiongco, 2010) head lice infestation is the third leading health problem of public school students.

Children show the highest prevalence and bear the highest burden of disease. The children

may suffer from discomfort and illness in severe cases of infestation. It is rampant mostly in primary-school children in both developed and developing countries and large sums are spent on the purchase of medications to treat infections (AlBashtawy & Hasna, 2012). This study mainly focuses on comparison between pediculicidal activity of chemical and natural treatment with the use of commercialized pediculicide (permethrin) and custard apple (*Annona squamosa*) seed extract.

In response to this worldwide problem, several measures have been employed and treatments have been developed to aid in the prevention of head lice. Such treatments include local chemical treatments, mechanical treatments, systemic

treatments, natural treatments, suffocating agents and "household remedies". Development of head lice resistance to current therapies, including lindane, permethrin, and malathion, has also become a worldwide problem which is on the rise. The extent of this resistance is uncertain (Meinking, Entzel, Villar, Vicaria, Lemard and Porcelain, 2000). On the other hand, the seed of *Annona squamosa* is well known for killing head lice in many countries. It is a plant belonging to the family Annonaceae (Intaranongpai, Chavasiri & Gritsanapan, 2006).

The growing discoveries of different plant alternatives along with the increasing interest in research studies will together help find better solutions to existing problems. The high prevalence of pediculosis capitis and the many different studied treatments brings wider opportunities for research to take place.

The study aims at helping the members of the community; most commonly families of children afflicted with pediculosis assess an effective and better treatment that creates a big impact on different aspects of the members' lives.

2.0 Literature Review

Several studies were conducted regarding treatments for pediculosis capitis. Among these treatments include commercial pediculicide, such as Permethrin and a natural pediculicide which is with the use of custard apple (*Annona squamosa*) seed extract.

Annona squamosa Linn is a small green tree with its different parts used in folkloric medicine for the treatment of various disease (Suresh, Mamoharan and Panjamurthy, 2006). Custard apple (*Annona squamosa*) is a fruit that has proven itself useful in several ways including its anti-lice effects. According to Parvin, Islam, Rahman and

Haque (2003), the seeds are acrid & poisonous and powdered form serve as fish poison and insecticides. The seeds of custard contain anonaine as a constituent, which has an insecticidal property. The aqueous extract of the seeds of custard apple can be easily prepared and used against various insect pests. If seeds are not available the leaves can also be utilized. A home scale production technique of seed extract includes the following steps: (1) crushing the seed and tying the powdered seeds in muslin cloth, (2) placing this muslin cloth with pulp in water, (3) let it stand for 24 hours, and (4) squeezing the muslin cloth with pulp, and (5) collection and storage of the extract (*Example of production technique of extract*, n.d.).

In the study conducted by Arabit, Palqueza, De Leon, Balicat, and Salvania (2011) entitled "Organic Pesticide from Atis (*Annona squamosa*) Leaves Extract as an Alternative to Commercial Synthetic Pesticide", Atis leaves extract with different concentrations were used and effectiveness was tested in terms of the mortality rate of the pests. The results of their study showed that there was no significant difference between the experimental product and the commercial product in terms of the pest's mortality rate, at 0.05 level of significance, and further stated that the experimental product is just as effective as the commercial pesticide in eradicating pests. They concluded that the organic pesticide from *Annona squamosa* leaves extract could be an alternative to commercial synthetic pesticide.

In the study conducted by Kosalge and Fursule (2009) wherein they utilized filter paper diffusion bioassay method in testing the licial activity of *Azadirachta indica* A. and *Annona squamosa* seed extracts. The results of their study demonstrated the possibility of using *Azadirachta indica* A. and

Annona squamosa L. products for controlling head lice by tribals in their area, which are difficult to control because of the resistance to the currently used anti-lice agents.

In another study conducted by Intaranongpai, Chavasiri, and Gritsanapan (2006) in the effectivity of *A. Squamosa* as an anti-lice treatment, *Annona squamosa* (Atis) seed extract which contains compounds is proven in vitro to have anti-head lice activity. Their study is focused on the separation and identification of the active compounds against head lice from the hexane extract of *A. squamosa* L seed. Oleic acid and triglyceride with one oleate ester were revealed as the two major compounds. These compounds were found to kill all tested head lice in 49, 11 and 30 minutes, respectively. According to Intaranongpai *et.al* (2006), this first finding will be useful for quality assessment and the chemical stability of the anti-head lice preparation from this plant.

In another in vitro study conducted by Yagumyum (2002) with the efficacy of custard apple (*Annona squamosa*) as a pediculicide, the results showed a statistically significant pediculicidal effect produced among the various seed oil extracts in emulsion. The emulsion with 100% concentration produced a higher kill rate compared with the other concentrations. He came up with a conclusion that *Annona squamosa* seed oil extract in emulsion produced pediculicidal activity and further added that there was no significant difference between the 75 percent and 100 percent concentrations when pediculicidal effect was considered. However, the 100 percent concentration produced the highest number of lice killed at the one-hour time interval.

The studies about the pediculicidal activity of different custard apple seed extracts that were

stated above were much further supported with the study conducted by Tiangda, Gritsanapan, Sookvanichsilp, and Limchalearn (2000) wherein their study was focused on the efficacy and stability of *Annona squamosa* seed extract prepared as a cream. Tiangda *et al.* (2000) concluded that "the petroleum ether extract of *Annona squamosa* seeds prepared as a cream is stable for at least 12 months" and "the custard apple cream may be, therefore, suitable for use as an alternative therapy against headlice.

On the other hand, commercial pediculicides were still considered as treatment to pediculosis capitis. One of which is Permethrin. According to Toynton *et. al* (2009), permethrin is an insecticide in the pyrethroid chemical family. Permethrin as an insecticide works as it affects the nervous system in insects, causing muscle spasms, paralysis and death.

According to Public Health Medicine Environmental (2008), chemical treatment is the only treatment, which has been scientifically demonstrated to be effective. PHMEG identified four main groups of chemical treatments which are the Pyrethroids (Phenothrin, Permethrin), Organophosphates (Malathion), Carbamates (Carbaryl) and Dimeticone. PHMEG reported that these are all still effective, even though there is some degree of resistance reported which will require a change in therapy after an initial confirmed failure.

Furthermore, two systematic reviews have been published, focusing mainly on the effectiveness of these commercial pediculicides. The first by Vander, Stichele, Dezeure, and Bogaert (1995) who concluded that sufficient evidence proved the effectiveness of permethrin alone and that more evidence was needed for treatments such as

malathion and carbaryl. Pyrethrines were assessed as not sufficiently effective. A second systematic review by the Cochrane collaboration (as cited by Dodd, 2000), concluded that effectiveness was proven for permethrin, malathion and synergized pyrethrins. However, this review included only 3 clinical trials on pediculicides and several methodological concerns were raised regarding this review.

Anyhow, with the above related studies and literatures, the researchers have come up in mind and chose to conduct this study of comparing the effectiveness of *Annona squamosa* seed extracts with commercial pediculicide as treatment to pediculosis capitis. The previous studies will serve as the support in the conceptualization and justification of the study bearing in mind its objectives.

3.0 Methodology

True experimental research design- complete randomized is used in this study. The researchers used the head lice gathered from the respondents aging 4-11. There were four treatments, one control group (Permethrin 75%) and three experimental groups with different concentration of 25%, 50% and 75% in custard apple seed extract. It was done in three trials and conducted in Alumnos, Basak San Nicolas Health Center and utilized in vitro testing. An improvised laboratory setting was made within the health center.

The research study utilized the posttest-only randomized experimental design in order to compare the results of the two treatments: commercial pediculicide (permethrin) and custard apple seed extract. The researchers would then be able to analyze whether the two treatments exhibit differences in pediculicidal activity, evaluate which

of the two treatments has less killing time of pediculosis capitis and differentiate which of the three concentrations of *Annona squamosa* has a more desirable effect.

The atis seed extract was produced by manual removal from the fruit's flesh and pulverizing after. 30g of the pulverized seeds were then mixed with 60ml of distilled water and was filtered using cheese cloth. The concentrations were produced by adding 7.5 ml water to a 2.5 ml atis extract to create a 25% atis seed extract concentration, adding 5 ml water to a 5ml atis extract to create a 50% atis seed extract concentration and adding 2.5 ml water to a 7.5 ml atis extract to create a 75% atis seed extract concentration. The control treatment was made by adding 2.5 ml water to a 7.5 ml permethrin solution. Each concentration is a 10 ml solution.

With the approval of the children and parents consent, the researchers collected one hundred twenty adult head lice. Approximately one hour is allotted in the collection of the samples which were placed directly in petri dishes- each containing 10 adult head lice and short hair strands. After giving the stabilizing period of one hour, the in vitro testing followed.

The ten head lice placed in each petri dishes were observed under the magnifying glass. Activities and body movements of the head lice were observed prior to application of the solutions. The method of determining the potency of pediculicide is based on its property to interfere with the normal process and development of the lice resulting subsequently to their death. Death of a head louse is considered when there is a complete absence of movements, including gut movement, movement of antennae or legs, with or without stimulation using forceps. The subjects will be

observed over a period of time. First, observation will be gathered for the first 15 minutes, as to how many head lice are officially considered dead. The observations will be continuously observed for the next 30 minutes and 45 minutes. The final observation to be gathered will be the official killing time of one half of the total number of head lice in each treatment. This will be done in three trials.

This research study utilizes one-way between groups Analysis of Variance as its statistical treatment. It is usually used to test the equality of three or more means at one time by using variances. The study focuses on comparing commercial pediculicide and *Annona squamosa* seed extract. In this analysis, the results of three treatments of *Annona squamosa* in different concentrations along with one treatment of commercial pediculicide can be compared. This tool is also usually appropriate

for post-test only randomized experimental design. The results of the study will be further analyzed to find out whether there is a significant difference between the use of commercial pediculicide and *Annona squamosa* seed extract in its different concentrations in killing head lice. The researchers will use an in vitro experimentation for the study. The data would be gathered, recorded and to be further analyzed with the use of analysis of variance through the Statistical Product of Social Sciences (SPSS) version 16.

4.0 Results and Discussion

On Table 2 is the result of the observation process done which showed that within the first 5 minutes, treatment permethrin has shown 100% mortality as compared to *Annona squamosa* treatments with different concentrations.

Table 2. Pediculicidal Activity among the Four Treatment Periods

TREATMENTS		Trial	Number of Dead Head Lice in different time observations				Exact time of death for the last Head Lice in each sample (in minutes)
			5min	15min	30min	45min	End time
Treatment 1 (+) control	75% Permethrin	1	10	10	10	10	3:35
		2	10	10	10	10	1:21
		3	10	10	10	10	4:54
Treatment 2	75% <i>Annona squamosa</i>	1	4	7	10	10	25:30
		2	2	10	10	10	15:15
		3	7	8	10	10	16:30
Treatment 3	50% <i>Annona squamosa</i>	1	7	8	10	10	26:41
		2	10	10	10	10	5:44
		3	7	10	10	10	11:16
Treatment 4	25% <i>Annona squamosa</i>	1	2	7	8	10	44:19
		2	3	9	10	10	15:46
		3	3	8	10	10	24:49

The table presents the four treatments (75% Permethrin as control group and *Annona squamosa* in three concentrations: 75%, 50%, and 25%). The treatments' actual number of dead head lice as observed in different time durations in three trials are shown. It also reflects the exact time of death for the last live head lice in each sample recorded in seconds.

Based on the result of the experiment, Permethrin has become the first to achieve 100% mortality among the treatments within the

shortest span of time. Within the first 5 minutes of observation, next to Permethrin, is 50% *Annona squamosa* concentration which has also attained high percentage of mortality in the three trials. Following this, is 75% *Annona squamosa* concentration and the treatment with the least percentage of mortality is 25% *Annona squamosa* concentration.

For the first 5 minutes of observation, the subjects were identified according to the mortality in each of the treatments.

Table 3.1 Pediculidal Activity for the First 5 minutes with One-way Analysis of Variance

Treatment	N	Mean	Std. Deviation	F-Value	Sig.
Permethrin	3	10.00	.000	13.920	0.002*
75% <i>Annona squamosa</i>	3	4.33	2.517		
50% <i>Annona squamosa</i>	3	8.00	1.732		
25% <i>Annona squamosa</i>	3	2.67	.577		
Total	12	6.25	3.306		

Note: * - significant at $\alpha = 0.05$

On Table 3.1, statistically, Permethrin is the only treatment that has achieved 100% mortality of the adult head lice for the first 5 minutes followed by 50% *Annona squamosa*, 75% *Annona squamosa*, and 25% *Annona squamosa*. It further shows that there is a statistically significant difference between the group means as determined by one-way ANOVA using a critical alpha of 0.05 (F: 13.920, P= 0.002). This indicates that pediculidal activity of *Annona squamosa* seed extract vary and differs from the pediculidal activity of commercialized pediculicide in treating pediculosis capitis (head lice) for the first 5 minutes and that the treatments were not as equally effective within the first 5 minutes of exposure. According to Toynton et. al (2009), permethrin is an insecticide that contains chemical components of 3-phenoxybenzyl

(1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate which works by affecting directly the nervous system in insects, causing muscle spasms, paralysis and death. Presence of these chemical components explains the faster pediculidal effects of permethrin as compared to the *Annona squamosa*, which contains oleic acid and triglyceride with one oleate ester (as cited by Intranongpai, et.al ,2006), which suggest slower pediculidal effects. To verify the differences between the group means of the treatments, Post Hoc Analysis, statistical test Tukey was used.

Using Tukey statistic, the mean of every treatment to the means of every other treatment were compared.

(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.
Permethrin	75% <i>Annona squamosa</i>	5.667*	1.269	.009*
	50% <i>Annona squamosa</i>	2.000	1.269	.442
	25% <i>Annona squamosa</i>	7.333*	1.269	.002*
75% <i>Annona squamosa</i>	Permethrin	-5.667*	1.269	.009*
	50% <i>Annona squamosa</i>	-3.667	1.269	.078
	25% <i>Annona squamosa</i>	1.667	1.269	.580
50% <i>Annona squamosa</i>	Permethrin	-2.000	1.269	.442
	75% <i>Annona squamosa</i>	3.667	1.269	.078
	25% <i>Annona squamosa</i>	5.333*	1.269	.013*
25% <i>Annona squamosa</i>	Permethrin	-7.333*	1.269	.002*
	75% <i>Annona squamosa</i>	-1.667	1.269	.580
	50% <i>Annona squamosa</i>	-5.333*	1.269	.013*

*. The mean difference is significant at the 0.05 level.

The table expresses the statistically specific difference in the means between the treatment variables for the results of the first 5 minutes. Permethrin showed significant differences with the *Annona squamosa* treatments except the 50% *Annona squamosa* concentration. This implies that the effect of 50% concentration is comparable to that of Permethrin. It further shows that (1) between permethrin and 75% *Annona squamosa* concentration, there is 0.009 significance and compared to the 0.05 significant level, the value is less. This means that 75% *Annona squamosa* concentration is not at par with Permethrin with regards to head lice mortality only for the first 5 minutes. (2) Between Permethrin and 25% *Annona squamosa* concentration, there is 0.002 significance. This indicates that 25% *Annona squamosa* concentration is highly not comparable to the pediculicidal activity of permethrin. (3) However, the 50% *Annona squamosa* concentration shows 0.013 difference from the 25% *Annona squamosa* concentration which means that 25% *Annona*

squamosa concentration is not comparable to both permethrin and 50% *Annona squamosa* concentration.

According to Chieh (n.d.), rates of chemical reactions depend on the nature of the reactants, the temperature, the presence of a catalyst, and concentration. Concentration effect is important because chemical reactions are usually carried out in solutions. When the study was conducted unexpected findings were observed. The 50% *Annona squamosa* seed extract concentration showed earlier onset of pediculicidal activity among the three *Annona squamosa* seed extract concentrations as shown on Table 4.2.

For 15 minutes of continuous observation, the subjects were again identified according to the mortality in each of the treatments.

Table 4. Pediculicidal Activity for the 15 minutes (One-way ANOVA)

	N	Mean	Std. Deviation	Std. Error	F-Value	Sig.
Permethrin	3	10.00	.000	.000	2.167	0.170
75% <i>Annona squamosa</i>	3	8.33	1.528	.882		
50% <i>Annona squamosa</i>	3	9.33	1.155	.667		
25% <i>Annona squamosa</i>	3	8.00	1.000	.577		
Total	12	8.92	1.240	.358		

The table shows that there was no statistically significant differences in the group means as determined by one-way ANOVA using a critical alpha of .05 (F: 2.167, P= .170). This indicated that pediculicidal activity of *Annona squamosa* seed extract is the same as the pediculicidal activity of commercial pediculicide in treating pediculosis capitis (head lice) for 15 minutes and that the

treatments were equally effective. This implies that given a duration of even 15 minutes, the *Annona squamosa* treatments could already achieve approximately 80-100% mortality of the subjects under the treatments.

For 30 minutes of continuous observation, the subjects were again identified according to the mortality in each of the treatments.

Table 5. Pediculicidal Activity for 30 minutes (One-way ANOVA)

	N	Mean	Std. Deviation	Std. Error	F	Sig.
Permethrin	3	10.00	.000	.000	1.000	0.441
75% <i>Annona squamosa</i>	3	10.00	.000	.000		
50% <i>Annona squamosa</i>	3	10.00	.000	.000		
25% <i>Annona squamosa</i>	3	9.33	1.155	.667		
Total	12	9.83	.577	.167		

The table shows that there was no statistically significant differences in the group means as determined by one-way ANOVA using a critical alpha of .05 (F: 1.000, P= .441). This indicated that pediculicidal activity of *Annona squamosa* seed extract is the same as the pediculicidal activity of commercialized pediculicide in treating pediculosis capitis (head lice) for 30 minutes. This implies that *Annona squamosa* seed extract could attain a hundred percent mortality of head lice with a duration of 30 minutes with the exception of the 25% *Annona squamosa* seed extract. It is slow, however

still as effective as the commercial pediculicide.

Within 45 minutes of observation, there was no statistically significant difference in the group means indicating that the treatments were equally effective. At this point, all the treatments- 75%, 50%, 25% *Annona squamosa* seed extract, have achieved a hundred percent (100%) mortality rate of the head lice.

In order to determine if the mortality of the adult lice in the treatments is significant, one-way Analysis of Variance was used. The values are presented in Table 3.

Treatment	N	Mean	Std. Deviation	F-Value	Sig.
Permethrin	3	196.67	107.677	3.552	0.067 ^{ns}
75% <i>Annona squamosa</i>	3	1145.00	335.522		
50% <i>Annona squamosa</i>	3	873.67	651.396		
25% <i>Annona squamosa</i>	3	1698.00	875.416		
Total	12	978.33	746.668		

Note: ns – not significant; ** - highly significant at $\alpha = 0.05$

Based on the values shown in Table 3, statistically, Permethrin has the shortest mean time elapsed for 100% mortality of head lice followed by 50% *Annona squamosa*, 75% *Annona squamosa*, and 25% *Annona squamosa*. It further shows that there was no statistically significant differences between group means as determined by one-way ANOVA using a critical alpha of .05 (F: 3.552, P= .067). However, this indicated that the exact time of death for the last head lice in one treatment does not significantly vary from the other. This further implies that the treatments were equally effective.

The results gathered were supported by the study conducted by Arabit, et. al (2011). According to Arabit, et. al (2011), there was no significant difference between the experimental product and the commercial product in terms of the pest's mortality rate, at 0.05 level of significance using T-test statistical treatment.

5.0 Conclusion and Recommendations

With the data gathered, the researchers derived up with a conclusion that custard apple (*Annona squamosa*) seed extract in 75%, 50% and 25% concentration is as effective as commercial pediculicide (Permethrin) in killing pediculosis capitis. However there is a significant difference in their pediculicidal activity for the first 5 minutes. It would take more time for all the concentrations to achieve a hundred percent mortality. And out of the

three custard apple seed extract concentrations, 50% custard apple (*Annona squamosa*) seed extract concentration is the most effective.

The use of custard apple (*Annona squamosa*) seed extracts yielded significant results on the treatment of pediculosis capitis. With this basis, the researchers recommend that the use of custard apple be further studied with regards to actual application and use to the affected population.

Also, with regards to the preparation and possible manufacturing of the solution as a commercial product, it needs to be explored further so that the highest possible pediculicidal effect of Custard Apple Seed extract will be fully utilized.

5.1 Conclusions

With the data gathered, the researchers derived up with a conclusion that *Annona squamosa* seed extract in 75%, 50% and 25% concentration is as effective as commercial pediculicide (Permethrin) in killing pediculosis capitis though there is a significant difference in their pediculicidal activity for the first five minutes. It would take more time for all the concentrations to achieve a hundred percent mortality. And out of the three *Annona squamosa* seed extract concentrations, 50% *Annona squamosa* seed extract concentration is the most effective due to its earlier onset of pediculicidal activity. However, the study is

experimental in nature and actual application on human subjects has not been explored. In relevance to the nursing profession specifically in the field of community health nursing, this study will serve as a springboard for further research studies with regards to actual application on the affected population since it is economical and is easily available in the community.

5.2 Recommendations

The use of *Annona squamosa* seed extracts yielded significant results on the treatment of pediculosis capitis. With this basis, the researchers recommend that the use of *Annona squamosa* be further studied with regards to actual application and use to the affected population.

Also, with regards to the preparation and possible manufacturing of the solution as a commercial product, it needs to be explored further so that the highest possible pediculicidal effect of *Annona squamosa* seed extract will be fully utilized. It is recommended to further explore the cost benefit analysis comparing Permethrin and 50% concentration of *annona squamosa* to determine if this can be an alternative for commercial pediculicide as it is more cost effective and economical.

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