The Efficacy of Atis Leaves Extract as an Alternative Treatment for Pediculosis

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ABSTRACT

In some countries in the tropical area like in the Philippines, lice are one of the problems of its citizens especially school aged children. Considering the importance of Traditional Health specially to those rural areas where infestation of lice are rampant, the researchers conducted an experiment with the Atis leaves as a substitute to commercial prepared shampoo against lice. We used those leaves because it is easy to find, cheap and also to promote efficient use of alternative resources. Financial incapability is one of the reasons why people cannot cure their lice.

School aged children of Brgy San Roque, Sto. Tomas Batangas provided our specimen which is the lice in order to achieve a desirable amount that is utilized in our study. The Atis leaves extract was used unto the lice and it manifested effectively though it is less compared to the commercially prepared lice shampoo. Even though the effectiveness of the said experiment is not that effective, we still had recommended it to the less fortunate people because they can easily utilized it maximizing only the application to achieve the desired effect.

Keywords: Pediculosis, treatment, atis, leaves extract, lice, school children

INTRODUCTION

Pediculosis is also known as a head lice infestation, it is a medical condition caused by the colonization of hair by the parasitic insect *Pediculus Humanus Cap*itis or head louse. Pediculicide is the substance that used to treat head lice (Centers for Disease and Control, 2008).

In the Philippines, head lice are a common problem with children. According to the department of Education, head lice infestation is the third leading health problem of public school students next to dental problems. A survey of Department of Education, 2009 reveals that fifty nine percent of Filipino Children have it. Children between age four to ten, usually girls are most prone. Their survey also showed a significant 34% of elementary school children in public schools are afflicted with this problem. *Pediculus Humanus Capitis* or what we call head lice. These tiny wingless parasitic insects infest mammals and sucks blood from the scalp of the host. They have been living for a thousand of years. The eggs are attached to hair shafts close to the scalp to benefit from a warmer incubation temperature and they hatch in about a week. They can be confused with dandruff but are distinguishable because they stick to the hair and are difficult to remove. Head lice like to feed every three to six hours and more likely to survive on human scalps (Mancini, 2011). If lice get isolated from a human's head, it usually dies within twenty to forty eight hours. Head lice can be transmitted to people through direct hair to hair contact with an infected person. Action such as playing, hugging, sitting close with each other, as well as sharing personal items like combs, hats and pillows can be one of the cause of transmission of lice. Though head lice cannot fly or jump, they have this ability to crawl from one host to the next. They can travel nine inches in just a minute (Centers for disease and Control, 2007). Atis or Anona Squamosa leaves extract is the main variable in this study. It contains substances that can repel insects or more specifically it can irritate and remove lice.

Recently, a phytochemical analysis in the laboratory of Department of Science and Technology was conducted and it was proven that the leaves contain anthraquinones, which is a derivative of anthracene that can be used as an insecticide. This test proves that the Atis leaves extract contains an insecticidal property (Department of Science and Technology, 2010). Herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients (World Health Organization, 2008). Using Atis extract as an

alternative treatment is an example of using herbal medicine. World Health Organization states that the use of herbal medicines is safe and effective.

Statement of the Problem

The Study aimed to determine the efficacy of Atis leaves extract as an alternative treatment for pediculosis

Review of Related Literature

The Philippine abounds with thousands of plant species of medicinal value. For generations of rural to sub-urban fold, the use of herbal medicine has been main stay treatment. This tradition takes its root several centuries back and has been preserved through generations. With the continued escalation of health care, unabated rise of drug prices, it is very clear that there is a need to find alternative ways to treat their conditions (Mosqereda, 2005).

Instead of using synthetic drugs that can be bought in pharmacies, the Department of Health (Department of Health, 2011) is convincing people to use traditional herbal medicine. They attest that it is cheaper, accessible, and have little or no side effects.

In some Asian and African countries, 80% of the population depends on traditional medicine for primary health care. In many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (e.g. acupuncture).

Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international market place. Annual revenues in Western Europe reached US$ Five billion in 2003-2004. In China sales of products totaled US$ 14 billion in 2005. Herbal medicine revenue in Brazil was US$ 160 million in 2007(World Health Organization, 2008).

The socioeconomic status of these cases shows no boundaries, but it stands to reason that the poor, homeless, and children do account for the vast majority of the cases because these groups traditionally find themselves with more opportunity for head-to-head contact as a result of close living conditions, cohabitation, and simply the increased population in a given geographical area. Age is a factor that plays a substantial role in the occurrence of infestations, with children representing an abundantly large percentage of the population with lice. When it comes to gender, studies vary as to whether or not there is a statistical difference in incidence between boys and girls. Although not designed to evaluate gender specifically, one study found a significant disparity, with girls

being 7.4 times more likely to have lice (26.7% versus 3.6%) and 13 times more likely to be infested with nits (23.8% versus 1.8%) than boys (n = 280) (Heukelbach, 2008).

According to Maglaya 2008, Environment plays a direct influence on the health of people. Unsanitary environment is a major factor that causes problems like dengue, diarrheal diseases, and Pediculosis. State of the country's environment is the direct result of the interaction of a number of factors such as industrialization, government policies, poverty and an uncaring attitude towards the environment. Unfortunately, many of us do not care for our environment. Community health nursing should therefore include the environment in its health programs.

Potential for transmission is highest when people are in crowded, institutionalized conditions or in war or prison camps, where sanitation is bad and clothing cannot be changed often. Physical contact and stray hairs, even under sanitary condition (Schmidt & Roberts' Foundations of Hill 2008 Pediculus Humanus), easily transmit them.

Annona squamosa Linn or what we call Atis here in the Philippines, belonging to family Annonaceaeis commonly found in India and cultivated in Thailand and originates from the West Indies and South America. It is mainly grown in gardens for its fruits and ornamental value. It is known as custard apple, sugar apple, sweet après in English, and sharifa in Hindi & sitaphalam in Telugu in India & corossolier & cailleux, pommier cannelle in French (Barve, 2011).

Atis was introduced by the Spaniards at an early date. The plant is a small tree with only a height of three to five meters. The leaves when young are hairy, oblong in shape and eight to fifteen centimeters in length, with a petiole 1 to 1.5 centimeters long. The flowers are pendulous, hairy, three-angled and greenish-white or yellowish. The fruit is large, somewhat heart-shaped and six to nine centimeters in length. When the fruit is already ripe it turns into yellow green (Burkill, 2009).

The Atis tree grows just about anywhere a seed falls. And it grows fast so that you can get to harvest fruits in a little over a year from seedling stage. A tree bears fruits about three times a year. They are in season from March to September, with June being the peak (Department of Agriculture, 2009).

There are many health benefits of Atis. The fruit contains calories, amino acids, vitamins and minerals. Each 100 grams of the edible portion of Atis has about 50 mg of ascorbic acid, 60 mg of lysine and 23-55 mg of phosphorus.

The seed can be pounded into paste that is applied to the head to get rid of lice, an annoying hair irritant (Philippine Council for Health Research and Development, 2010).

Anona Squamosa L. or Atis leaf is a plant belonging to the family *Annonaceaeis.* It is popularly cultivated in all parts of Thailand, especially in the northeast, as sweet fruit. The seed of this plant is well known for killing head lice in many countries.

The human head louse (*Pediculus humanus capitis*) is a small insect causing a public health problem, especially in poor sanitary conditions. In Thailand, research has shown the anti-head lice activity of A. squamosa reported that the extract of custard apple seeds in coconut oil at ratio of 1:2 could kill 98% of head lice within 2 hrs while leaf extract shows less potency (Chavasiri, 2006).

Atis is native to Tropical America and widely grown in arid regions of India. Its root leaves and fruits are medicinally important. The leaves contain saponin and are suppurative and insecticidal. They are used for treatment of ringworm (Taenia versicolor) ,destroying lice, proctoptitis in children and skin diseases, and as a poultice to produce suppuration. It is also used as an anthelmintic, pediculicide, scabicide, and anti-inflammatory. Person shaving hysterical on fainting fits are made to inhale crushed leaves or their juice. Crushed leaves are applied to the nose area in cases of fainting spells. Poultice of fresh leaves is useful in dyspepsia (Kumar, 2012).

In Indonesia, leaves of Atis are used as pediculicide. While In the Philippines the leaves and bark of Atis are used as astringent. The ripe fruit is recommended in cases of anemia. The seeds provoke vomiting. The powder of the seed, mixed with alcohol, applied externally, is good for dandruff. The poultice from the fresh leaves is recommended for dyspepsia. Crushed leaves can be applied to the nose to relieve fainting spells. The unripe fruit and seeds have vermicidal and insecticidal properties. The decoction of the root is a drastic purgative. Seed is used in the Philippines as a pediculicide by crushing, mixing with coconut, and applying topically. In Thailand seeds and leaves are used as a pediculicide. Crush 10-20 seeds (or 7-8 leaves, if seeds are not available), remove seed coat, mix with animal or vegetable oil (1:2), strain and press to obtain most of the oil. Apply the oil to the hair, cover with a thin cloth and wash thoroughly after 1-2 hrs (Best reported combination is ground seed mixed with coconut oil (1:2); it kills 98% of lice in 2 hours; it is used externally to relieve scabies, ringworm and Tinea versicoloe. A clinical trial showed that the

seed and the leaf can treat hair lice with minor side effects or irritation. The seed contains a caustic resin that is a toxic irritant principle and is used to get rid of hair lice (Ecoport, 2002).

The leaves, fruit and the seeds contain acrid principle that possess vermicidal and insecticidal properties. The crushed seeds is mixed with water as a paste and applied to the hair scalp to destroy head lice. The seeds are also used as an abortifacient if applied to the utero in pregnant women. The leaves with salt make a good cataplasm to induce suppuration. The fresh crush leaves of atis can be applied to the nostrils to help relieve fainting (Sanyal and Ghose, 2009).

Atis or sugar-apple (*Annona Squamosa*) leaves extract is the main variable in this study. It contains substances that can repel insects or more specifically it can irritate and remove lice. Recently, a pythochemical analysis in the laboratory of DOST was conducted and it was proven that the leaves contain anthraquinones, which is a derivative of anthracene that can be used as an insecticide. This test proves that the atis leaves extract contains an insecticidal property. Such processes like preparation, filtration, squeezing, and pounding was undertaken for the possibility if making a lice remover out of Atis (*Annona squamosa*) leaves extract. As the researchers used the finished product to some infected subjects, it was concluded, and that it was effective. Many lice were found in the towel used to dry the hair of the infected subjects. The result of the study shows that lice were removed on the infected subjects. Therefore, the researchers concluded that atis leaves extract is useful and can be use as a lice remover to threat those who are infected. Aside from the fact, it is cheaper compared to commercial products (Montoya, 2008).

According to Heong 2011, the insects should be homogenous, exclude all variations such as age, sex, condition. Some insects such as beetle can be reared without difficulty in large numbers; others such as human lice may be troublesome to produce in quantity. With precise experimental conditions and insects difficult to rear batches as small as 5 to 10 may be used. In allocating, it is best to apportion them in such a way that the insects are randomized. Otherwise, the insects chosen first may all occur in the same batch, and if their susceptibility is slightly abnormal; it will bias the mortality.

Parasite is specie that feeds on the body of another without killing it and generally attacks only a single host. The eggs are usually laid upon the host. Among the insects, there are two general groups of parasites; those that attack vertebrates and those that attack insects. The former include (Anoplura) the

bird lice (Mallophaga) some of the louse flies (hippoboscidae) and the fleas (Siphonaftera) (Gullan 2010).

Lice are parasitic insects that infest mammals. Infestation with lice is called Pediculosis. Hundreds of varieties of lice infest humans. Three common kinds are *Pediculus Capitis* (head lice) *Pediculus Corporis* (body lice) *Pediculus Puris* (crab lice). *Pediculus Capitis* is found on the scalp and tends to stay hidden in the hair; the nurse can suspect their presence in the clothing if (a) the person habitually scratches (b) there are scratches on the skin and (c) there are hemorrhagic spot on the skin where the lice have suck blood (Kozier and Erb's, 2008).

Lice require blood from the host and feed several times a day. The victim is often unaware of these silent passengers until itching occurs, which is a result of sensitization to louse saliva, develops several weeks later. Scratching can result in secondary bacterial infections. The head louse has legs especially adapted to grasp scalp hair. During a lifespan of a little over a month, the female louse produces several eggs (nits) a day. The eggs are attached to hair shafts close to the scalp. The very young stages of louse are also called nits. Empty egg cases are whitish and more visible. They do not necessarily indicate the presence of live lice. As the hair grows (at the rate of about 1cm a month), the attached nit moves away from the scalp.A point of interest is that the incidence of Pediculosis among blacks in the United States, lice have become adapted to the cylindrical hair shafts found in whites. In Africa, lice have adapted to the not cylindrical hair shafts of blacks (Tortora, 2010).

The adult louse is 2 to 3 mm long and usually light gray, although color may differ. The female lives up to 3 to 4 weeks. After mating, an adult female louse lays one to six eggs a day for up to one month until death. The eggs incubated by body heat, hatch in 10 to 14 days. Once the eggs hatch, nymphs leave the shell casing, breed for about 9 to 12 days, and develop into an adult lice and mate, and then females lay eggs. If not treated, this cycle may repeat itself every 3 weeks. While living on the head, the louse feeds by injecting small amounts of saliva and taking tiny amounts of blood from the scalp every few hours (Fursule, 2009).

The term or span of life is limited for each species. Females usually live longer than males and unmated females live longer than mated females. Food is an important factor in determining the length of lice. Insects that have well developed mouth will not live long if they do not receive food (Fundaments of Entomology, 2010).

Lice cannot survive for more than 48 hours away from a human scalp. Nits (lice eggs) can survive for up to 10 days but need a blood meal from a human one-hour after hatching. For that reason human head lice cannot survive for long periods of time on animals, but animals can be the carrier of the lice. For instance, if two children are playing with a dog, the dog can 'transport' the louse from one child to another (Insight Pharmaceuticals, 2013).

The nurse can suspect their presence in the clothing if (a) the person habitually scratches (b) there are scratches on the skin and (c) there are hemorrhagic spot on the skin where the lice have suck blood (Kozier and Erb's, 2008).

If someone in your household or other close contact has lice or scabies, it is possible for you to get them too. To prevent this from happening, the person that has lice or scabies needs to be treated as soon as possible. It is also important to treat the whole household. All clothing and bed linens that the person wore or came in contact with in the two days before treatment should be washed in HOT water and dried in high heat. The person's combs and brushes should be soaked in rubbing alcohol or a disinfectant for one hour. Finally, floors and furniture should be vacuumed (Organization of Teratology Information Specialist, 2008).

A preparation containing 20% custard apple (*Annona squamosa*) seed extract was shown to be highly effective against head lice infestations in a small controlled trial. More than 90% of lice were dead three hours after application of the product to infested schoolchildren, as compared to 60% dead lice in the control group, treated with 25% benzyl benzoate emulsion. It has been used in traditional Thai medicine against pediculosis since long ago (Spear, 2007).

A filter paper diffusion bioassay method was carried out in order to determine the licicidal activity of extracts. Petroleum ether extract of each plant tested individually, showed high levels of mortality on adult lice, both plant extract showed significant decrease in the mean time required to kill lice with 1% and 10% concentrations whereas increase in the meantime was observed with 0.1% concentration when compared to 1% lindane. *Annona squamosa L.* extract showed more potent activity than *Azadirachta indica A.* extract at all concentrations 0.1, 1 and 10%. These results demonstrate the possibility of using *Azadirachta indica A*. and *Annona squamosa L.* products for controlling head lice by tribals in this area, which are difficult to control because of their resistance to the currently used anti-louse agents. The assay proved simple,

effective and gave reproducible results (Investigation of licidal activity of some plants from Satpuda hills; Kosalge, 2009).

A lotion that you will apply to the hair and scalp. It is approved to treat people ages 6 years and older. It works by paralyzing and killing the lice and eggs. When used as directed, it is safe but it can irritate the skin a bit as it works. Some people get dry hair or their skin can burn or sting (U.S Food and Drug Administration, 2009).

Pyrethrins are derived from daisy like flowers of pyrethrum, Chrysanthemum cinerariaefolium. These are now obtained chiefly from East Africa, though they came originally from Dalmatia and for many years prior to 1940 from Japan. The pyrethrins are extracted from flowers and dissolved in oil such as deodorized kerosene for control of household pest such as flies and cockroaches. The pyrethrins are available in mixtures with DDT, upon insects. Insects frequently recover from the effects of pyrethrin poisoning, so it is advisable to combine pyrethrins with some such as DDT to assure good kill. DDT or dichloro-dipheny-trichloro-ethane is a stable, white, crystalline chemical which may be prepared in form of a dust or oil. It is a definitely a poison and typical symptoms of tremors may result from consumption of rather large quantities (Applied Entomology, 2013).

Treatment for pediculosis relies on conventional household cleaning practices and the use of some neurotoxic pediculicide, which have been found to produce resistance in some strains of head lice. These household practices may not be effective in decreasing the Prevalence of pediculosis due to the problem of reinfection. Also, they are too cumbersome to perform. Pediculicide chemicals, such as Permethrin and Malathion, were also found to Induce resistance and allergic reactions among children (Philippine Science Letters, 2011).

The number of new commercially available natural products for head lice has expanded over the last decade to a much greater extent than products containing defined chemical insecticides. However, the evidence on the efficacy of these new products based on published results of in vitro and clinical trials is markedly deficient. Evidence on safety is also deficient. All over, the counter natural products should be supported by in vitro data and well-designed comparative therapeutic trials using head lice derived from the populations for whom the product is intended. Since the prevalence and degree of insecticide resistance of head lice to pyrethrin, permethrin, and malathion is expected to increase, alternative topical therapies for pediculosis are needed. It is possible

that, in the long run, plant extracts, or their constituent compounds, will replace chemical insecticides on the market (Canyon, 2007).

MATERIALS AND METHODS

Materials

The leaves of Atis were collected from Barangay San Roque, Sto. Tomas, Batangas. Atis leaf was authenticated from University of the Philippines Los Banos – Laguna by Professor Annalee S. Hadsall. This certifies that the plant we had used was really the Atis leaf which is cultured and can be found in the Philippines.

The spray containers were bought from a supermarket. It has the same size but with different color of caps to be easily distinguished from one another. Before, the experiment we had soaked and washed it with warm water to maintain clean technique.

Participants

School age children girls are chosen to provide us the head lice with the approved informed consent from their parents. Participants on this research are present only in regards in collecting head lice, and that all the collected head lice will be the pure subject of the study. Since the participants are minors then we had obtained Informed consent from the parents.

Locale

The specimen was collected in the vicinity of Barangay, San Roque, Sto. Tomas, Batangas City, where school age children girls will be ask for us to have the head lice as the subject of the study. This area is also chosen to be the research locale of the study for the reason of availability of the Atis leaves throughout the year.

Preparation of leaf extract

The researchers have picked fresh young Atis leaves; and weigh it according to the desired grams. Then they blended it on with correct amount of distilled water that will serve as their extract, they made it with two different dosages. The first dosage is 50 mg of atis leaves per 25 ml of distilled water (50 g/25 ml), the more concentrated atis extract while the other one is 50 mg of atis

leaves per 50 ml of distilled water (50 g/50 ml), which is less concentrated. After they have blended it, they filtered it in a clean cloth, poured it in their spray bottle and labeled it accordingly.

Preparation of other treatments

After preparing the Atis leaves extract the researchers also prepared the other treatment that they used in conducting the experiment. Those other treatments are the commercially prepared anti head lice shampoo, the "licealiz" and the other one is the distilled water. In preparing Licealiz shampoo, they just pour the 20 mg:10 ml (one sachet) dosage of it to their prepared spray bottle, while with the distilled water, they just also poured it in the bottle with sufficient amount. After we made the two other treatments, one again, specimens were labeled accordingly.

Conduct of Experiment

The researchers have collected the specimen from random individuals in Barangay San Roque Village at around 07:00 – 08:00 am, which is approved by the Barangay Chairman of the said vicinity and with approved Informed consent from the parents and guardian of that selected children. After they collected our specimen, researchers started conducting the experiment. First, they have prepared five spray bottles containing our five treatments. The first spray bottle contained nothing because the first would be controlled group. The second spray bottle contained licealiz. The third one contained 50 g/25 ml dosage of Atis leaves extract. The fourth contained 50 g/50 ml dosage of Atis leaves extract and for the last treatment, the last spray bottle contained with distilled water.

The researchers have also prepared three clean containers each treatment and for the controlled or the no treatment, with 15 containers. The former put cotton inside each containers, this is where they placed the specimen or the lice. They have placed the specimen in each container by means of their bare hands. This is also, where they sprayed all of the treatments accordingly until the cotton becomes moist. They started to spray at least three consecutive times in all of the treatment at the same time at exactly 8:30 am. Then they monitored the changes especially the time of death of lice with each treatment.

Data Collection

After spraying, all of the treatment on the different containers with the specimen inside, they started to monitor for the effectiveness of all treatment by observing dead lice. For good viewing of lice, from the container they have placed it on a clean sheet of paper then every after observation they put it back in the container. The first monitoring happened in just a span of 30 minutes because they have seen already an effect with the treatment one or treatment with licealiz as seen in Table 1.1. After that the proponents monitored it hourly starting from 09:00 am until all the lice became dead in a span of 4 hours as seen in Table 1.5. They have determined that the lice are already dead by observing its movements and by the use of their bare hands. According to a study of Huchelbach, 2008, death of a head louse was the complete absence of any vital signs such as gut movements, movement of antennae, legs. Lice were defined as active if there are no changes in their activity or their behavior. Level of Ph was also obtained in order to determine the acidity and alkalinity of the different concentrations. Results are 50 g/25 ml: 6.5(alkaline), 50 g/50 ml: 6.1 (alkaline), commercially prepared pediculicide 20 mg/10 ml: 6.8, distilled water: 7.0.

Statistical Treatment

The researchers utilized the following statistical techniques to ensure valid and reliable analysis and interpretation of data.

Comparing Variances

Usually present the comparison of variances in a source table. The F statistic is the comparison of the MS for each effect to the MSE. This allows you to check how likely it is to get an effect that size given chance (the p level). You can then decide if you think the effect is just due to chance (not significant) or so unlikely that you assume it is caused by something.

Two-way ANOVA, interested in Main Effect of Time, Main Effect of Different concentrations, Interaction of A and B. Thus we will partition variance into parts caused by IV_A, IV_B, Int_{AxB}, and Error. Then we will compare the variance associated with each thing of interest to error variance to see if each effect is meaningful.

RESULTS AND DISCUSSION

They researchers had five groups included in our methods starting with Controlled group or with no treatment, Treatment B with licealiz with a dose of 20 mg:10 ml, Treatment C with Atis extract with a ratio of 50gms of Atis leaves to 25 ml of water or 2:1, Treatment D with Atis extract with a ratio of 50gms to 50 ml of water or 1:1 and Treatment E with distilled water only. Trial 1 means container 1, trial 2; container 2 and trial 3; container 3. They have collected the specimen starting from 07:00 am until 08:00 am. They had started placing the specimen or lice in the clean container around 08:30 am.

TABLE 1.1 – Mortality rate of lice after 30 minutes

After 30 minutes	Trial 1	Trial 2	Trial 3
Treatment A (no treatment)	0/4	0/4	0/4
Treatment B (with licealiz)	3/4	4/4	0/4
Treatment C (with Atis extract 50g/25ml)	0/4	0/4	0/4
Treatment D (with Atis extract 50g/50ml)	1/4	0/4	0/4
Treatment E (with distilled water)	0/4	1/4	0/4

The researchers started observing after 30 minutes of placing the specimen to the clean container. As you can see they have three trials or three containers each treatment group. With Control group, all of the lice were still alive or zero dead lice over 4 total lice. In treatment B or with licealiz, container 1 had already 3 dead lice over 4 lice, the 2nd container had 4 dead over 4 lice, while the third container with licealiz still have no effect. Treatment C or with 50 g/25 ml Atis extract after observing for 30 minutes still have no effect on either one of the container. Treatment D with only 50 g/50 ml Atis extract have an effect on the first petri dish 1 dead over 4 alive, while in the other 2 container it still has no effect. Last but not the least, treatment 4 with distilled water, they have observed in the second container that there is one dead lice over 4 alive while in the 1st and 3rd container still no effect.

TABLE 1.2 – Mortality rate of lice after 1 hour

After 1 hour	Trial 1	Trial 2	Trial 3
Treatment A (no treatment)	0/4	0/4	0/4
Treatment B (with licealiz)	4/4	4/4	0/4
Treatment C (with Atis extract 50g/25ml)	1/4	1/4	1/4
Treatment D (with Atis extract 50g/50ml	2/4	1/4	0/4
Treatment E (with distilled water)	1/4	1/4	0/4

After observing for the first hour. With Controlled group, all of the lice were still alive. In treatment B or with licealiz, container 1 had already 3 dead lice over 4 lice, the 2nd container and also the 3rd one had 4 dead over 4 lice. Treatment C or with 50 g/25 ml Atis extract after observing for one hour, Each of the container had 1 dead over 4 alive lice. Treatment D with 50g/50 ml Atis extract have an effect on the first container 2 dead over 4 alive while in the 2nd one, the effect is only 1 dead lice over 4 alive while the third with extract still remains to have no effect. Last but not the least, treatment E with distilled water, they have observed in the first container that there is one dead lice over 4 alive while in the second container there is 3 dead over 4 alive lice while in the third one still remains with no effect.

TABLE 1.3 – Mortality rate of lice after 2 hours

After 2 hours	Trial 1	Trial 2	Trial 3
Treatment A (no treatment)	1/4	2/4	1/4
Treatment B (with licealiz)	4/4	4/4	4/4
Treatment C (with Atis extract 50g/25ml)	2/4	1/4	3/4
Treatment D (with Atis extract50g/50ml)	2/4	1/4	1/4
Treatment E (with distilled water)	1/4	3/4	1/4

After observation of two hours, With Controlled group, and the first container had 1 dead over 4 alive lice, the second container had 2 dead over 4 alive lice, while the third container had only 1 dead over 4 alive. In treatment 1 or with licealiz, container one, two and three had the same mortality of 4 dead

over 4 alive lice. Treatment C or with 50 g/25 ml Atis extract after observing for two hours, In the 1st container, it showed 2 dead over 4 alive lice, in the 2nd container, it showed only 1 dead over 4 alive, while in the third container, it showed 3 dead over 4 alive lice. Treatment D with 50 g/50 ml Atis extract have an effect on the first container 2 dead over 4 alive while in the 2nd container, the effect is only 1 dead lice over 4 alive while the third container is also the same. Last but not the least, treatment 4 with distilled water, they have observed in the first container that there is one dead lice over 4 alive while in the second container there is 3 dead over 4 alive lice while the third container only showed 1 dead over 4 alive lice

TABLE 1.4 – Mortality rate of lice after 3 hours

After 3 hours	Trial 1	Trial 2	Trial 3
Treatment A (no treatment)	4/4	3/4	3/4
Treatment B (with licealiz)	4/4	4/4	4/4
Treatment C (with Atis extract 50g/25ml)	3/4	2/4	4/4
Treatment D (with Atis extract50g/50ml)	2/4	4/4	3/4
Treatment E (with distilled water)	2/4	4/4	3/4

After observing for three hours, With Controlled group, and the first container had 4 dead over 4 alive lice, the second container had 3 dead over 4 alive lice, while the third container also had 3 dead over 4 alive. In treatment B or with licealiz, container one, two and three had the same mortality of 4 dead over 4 alive lice. Treatment C or with 50 g/25 ml Atis extract after observing for two hours, In the 1st container, it showed 3 dead over 4 alive lice, in the 2nd container, it showed only 2 dead over 4 alive, while in the third container, it showed 4 dead over 4 alive lice. Treatment D with 50 g/50 ml Atis extract have an effect on the first container 2 dead over 4 alive , In the 2nd container 4 dead over 4 alive lice, while in the third container the effect is 3 dead lice over 4 alive. Last but not the least, treatment E with distilled water, they have observed in the first container that there is two dead lice over 4 alive while in the second container there is 4 dead over 4 alive lice while the third container showed 3 dead over 4 alive lice.

TABLE 1.5– Mortality rate of lice after 4 hours

After 4 hours	Trial 1	Trial 2	Trial 3
Treatment A (no treatment)	4/4	4/4	4/4
Treatment B (with licealiz)	4/4	4/4	4/4
Treatment C (with Atis extract 50g/25ml))	4/4	4/4	4/4
Treatment D (with Atis extract50g/50ml)	4/4	4/4	4/4
Treatment E (with distilled water)	4/4	4/4	4/4

After four hours of observation, they have observed that the lice regardless of the treatment, either no treatment, with 50 g/25 ml Atis extract, with 50 g/50 ml extract, with licealiz or with distilled water slowly become weak because of less movements as seen on their crawls and eventually died. It gave us a result of 100% mortality rate of lice which means 4 dead over 4 total lice with all of the containers from control group to treatment E.

Using analyses of variance, results show significant difference in the lice mortality among the control and the four treatments used (p=.000). There is also a significant difference in the lice mortality among the time of exposure to the treatments (p=.000). The interaction effect of the treatment and the time is also significant (p=.03). This shows that the treatments used has affected the mortality in conjunction with the length of exposure of the lice in the treatments applied. This is considered a main factor in the mortality rate of the lice in our experiment. The life of the lice is affected by the treatment and the time because both of this can affect in the death of the lice. This is a multifactor death of the lice, it may be considered because of the treatment and it may be considered as the life span that they harvest in the head of the respondents in the experiment. This is to conclude that the treatment and time is a cause of death of the lice and both are proven by the statistical data.

Table 2.1 Analyses of Variance: Tests of Between-Subjects Effects

Dependent Variable: Lice Mortality					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	167.79	24.00	6.99	11.92	0.00
Intercept	389.88	1.00	389.88	664.57	0.00
TREATMENT	36.99	4.00	9.25	15.76	0.00
TIME	111.92	4.00	27.98	47.69	0.00
TREATMENT * TIME	18.88	16.00	1.18	2.01	0.03
Error	29.33	50.00	0.59		
Total	587.00	75.00			
Corrected Total	197.12	74.00			
A	R Squared = .851 (Adjusted R Squared = .780)				

TREATMENT	Mean
Control(no treatment)	1.73 [a]
Treatment 3 (atis extract 50 g/50 mL)	1.93 [a]
Treatment 2 (atis extract 50 g/25 mL)	2.00 [a]
Treatment 4 (with distilled water)	2.07 [a]
Treatment 1 (with licealiz)	3.67 [b]

Legend: Means with different superscripts are significantly different

Duncan's post hoc test show Treatment B (with licealiz) had a significantly higher mean mortality compared to the controlled group and the other treatment groups. The other treatment groups: Treatment D, with atis extract (50 mg/50 mL); Treatment C, with atis extract (50 mg/25 mL) and Treatment E, with distilled water, however are not significantly different from the controlled group. On the other hand, results show that lice mortality is higher in Treatment E (with distilled water) than in Treatment D and Treatment C both with atis extract. This means that the treatment that has the fastest effect on killing the lice is the Licealiz and the 2nd is the distilled water followed by the atis extract 50 g/25 mL and followed by the atis extract 50 g/50 mL and lastly is the control group/ no treatment which means that the lice will die even though

there is no treatment but with a longer time than the time of the treatment D and C which is the experiment treatment.

Time of exposure	Mean
30 minutes	0.60^{a}
1 hour	1.47^{b}
2 hours	2.07^{c}
3 hours	3.27^{d}
4 hours	4.00^{e}

Legend: Means with different superscripts are significantly different

Duncan's post hoc test shows the effect of the length of exposure of the lice in the treatments. Data shows the highest mean mortality in 4 hours of exposure. Results show that the time of exposure is significantly different among each other. The longer the time of exposure, the higher is the mortality. The mortality increases as the length of exposure increases. This means that the life of the lice affects the period of the treatment and can affect the mortality under the effect of the treatment. The time of exposure is considered a factor that affects the mortality rate of the lice even with the treatment or no treatment applied on it.

Discussion

As the researchers see in Table 1.1, the first observation was done after 30 minutes. The first was the controlled group or no treatment was done. According to Centers for Disease and Control, 2013 Adult lice can live up to 30 days or 1 month in a human's scalp but if they are not on their host then they can only live for 24 to 48 hours. They also need to be fed on blood every three to six hours (Louiseville Metro Department of Health, 2013). They can see in the table that the control group or the group of lice without treatment survived only four hours starting with the collecting time of lice of the children infested. The researchers can conclude that it is due to the following fact that first, the clean container was not their habitat but rather human's scalp and also according to the literature above, they need to be fed every three to six hours by 11:00 am, the lice are already starving and craving for blood. The second treatment was with Licealiz, this was the fastest among the rest of the treatments. After only

30 minutes, it killed 75% of the first container, 100% of the 2nd one while none on the 3rd one. According to (Mims, 2013) the content of Licealiz is pyrethrin, it is an extract found in Chrysanthemum flower. It is used as insecticide for already 160 years because it induces a toxic effect to insects that affect the nervous system. When applied on the scalp its effect would be after 10 minutes. As the researchers conducted our experiment, we have observed that the licealiz tends to effect on lice after a span of 30 minute period. Treatment C with 50 g/ 25 ml Atis leaves extract is less effective than licealiz because during the first observation, the extract still does not have effect on the lice, but after the second observation, it showed 25% or 1 dead lice over four lice mortality and it continued to increase as hours of observation passed by. According to a study conducted by Montoya, 2008 Atis has this insecticidal property called anthraquinones which is derived from anthracene which is effective in removing lice. The treatment with 50 g/25 ml Atis leaves extract's effectiveness has no difference with 50 g:50 ml Atis leaves extract as shown in Tables 1.1 – 1.5.

In contradiction with our experiment, there is this study of Olarte (2011), the pesticide was derived from the extraction of the 750 grams of Atis leaves. The following experimental set-ups had been made; 50% Atis leaves extract and 50% distilled water (25 ml atis leaves extract and 25 ml distilled water); 100% Atis leaves extract (50 ml pure atis leaves extract). Using these concentrations, the effectiveness was tested to some pests such as lice, ticks, termites, and hairy caterpillars in terms of mortality rate. T- test 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. The results of experimentation and data analyses showed that the experimental product is just as effective as the commercial pesticide in eradicating pests. Thus, the results showed organic pesticide from *Annona squamosa* leaves extract is just as effective with commercial synthetic pesticide (Organic Pesticide from Atis (Anona squamosa) Leaves Extract as an Alternative to Commercial Synthetic Pesticide; Olarte,2011). But in relation with the study commercially prepared pesticide is more potent or more effective than the natural or organic pesticide.

CONCLUSIONS and RECOMMENDATIONS

A great number of newly commercially available products for head lice containing chemicals are growing in the market. However, the evidence of its effectivity is markedly deficient. The safety also of these chemicals is all deficient. All commercially prepared products should undergo so much clinical trials and in vitro test to determine the effectiveness of it. Since the prevalence and degree of insecticide resistance of head lice to pyrethrin, permethrin and malathion is expected to increase, alternative topical therapies for pediculosis are needed. It is possible that, in the long run, plant extracts, or their constituent compounds, will replace chemical insecticides on the market (Canyon, 2007). Phytochemical analysis of insecticidal properties of the leaves of the Anona Squamosa, its wide distribution and the trends of Pediculosis in the Philippines serves as the basis of the researchers in utilizing it as the subject of the researchers.

Conclusion

The *Annona Squamosa* leaf extract is found to have a pediculicidal effect potential against lice found within the Philippines, specifically Brgy. San Roque, Sto. Tomas, Batangas. Results show that it is less effective compared with the commercially prepared Licealiz because as the researchers observed in just a span of four hours, the mortality of lice increased but the treatment with the fastest pediculicidal effect was with Licealiz. They also concluded that the habitat of lice is also a major factor. According to Louisville Metro Department of Health (2013) the life span of lice without on human's scalp is about 24 to 48 hours. However, as the researchers conducted the study, all of the lice became dead in just a span of four hours with or without treatment; this is because lice should be fed with blood every three to six hours. Food is an important factor in determining the length of lice. Insects that have well developed mouth will not live long if they do not receive food (Fundamentals of Entomology, 2010).

Recommendations

To the Public Health Nurses, the researchers recommend them to teach parents especially mothers on how to prepare the extract using only materials available at their homes. Researchers also want to suggest them regarding conducting of more awareness programs for the parents to manage and treat pediculosis effectively.

To the Community, the researchers recommend using Atis extract for removing head lice because it is much safer than the commercially prepared; it is much cheaper and accessible. They can plant Atis by means of seed dispersing and it can grow in months. If they don't have money to buy commercially prepared ones then Atis extract is best for an alternative, with continued use it can really help them in eliminating their child's lice.

To the LPU– SC Faculty, the researchers do recommend them to share knowledge and information about the efficacy of *Annona Squamosa leaf* extract as an effective treatment of Pediculosis to their future students.

To the LPU-SC College of Allied Medicine, researchers recommend them to promote and spread the new move towards managing the incidence of Pediculosis with *Annona Squamosa* leaves extract so that it will not be a problem anymore in the Philippines.

To the Students and for the future researchers, the researchers would like to recommend the enhancement of this study by researching for more information regarding *Annona Squamosa.* They recommend increasing the concentration of extract or making it more pure. Researchers also recommend having phytochemical analysis of other parts of Atis like the stem, roots, and fruits to know if it also has pediculicidal effect. They also want to suggest that they should have humans as their respondents to increase the validity of results.

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