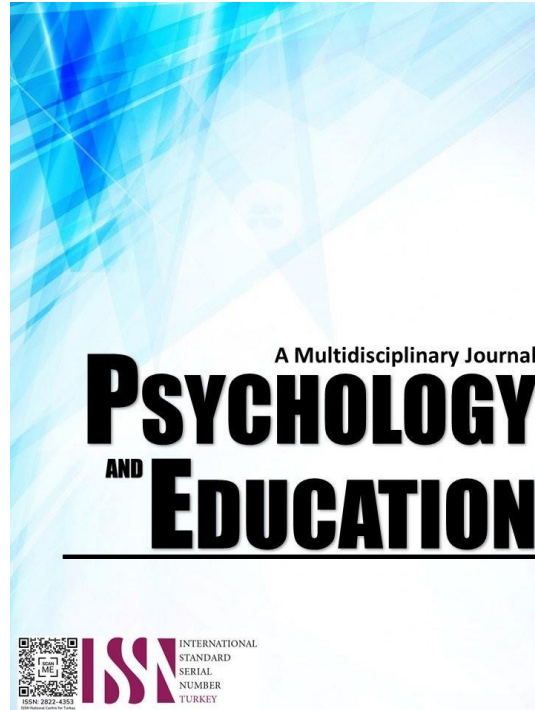


GUYABANO (ANNONA MURICATA, LINN.) SEED EXTRACT AS AN ALTERNATIVE PEDICULUCIDE FOR HEAD LICE



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Guyabano (*Annona muricata*, Linn.) Seed Extract as an Alternative Pediculucide for Head Lice

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Abstract

AI is everywhere. Popular list of various digital tools and resources that teachers and students can use in the academe, including e-learning platforms, digital pinboards, collaborative tools, and lesson planning apps. Artificial Intelligence is a booming technological domain capable of altering every aspect of social interactions. In education, AI has begun producing new teaching and learning solutions that are now undergoing testing in different contexts. AI requires advanced infrastructures and an ecosystem of thriving innovators. Additionally, it has a profound and increasingly important role in education. Its applications are transforming how education is delivered, making it more personalized, efficient, and effective. It is inevitable in the education aspect that teachers were informed and aware on how AI works. Teachers nowadays must be adaptive, flexible, and relevant in this tech driven world. AI in the education sector provides personalization, automation, accessibility, and data-driven insights. These features contribute to improved learning outcomes, increased teacher efficiency, and a more responsive and effective education system, ultimately benefiting students and educators alike. To address the research gaps, this study explores the status, challenges and future directions of AI in teaching practice. Since the field of AI-based instruction is still developing, this study can contribute to the development of comprehensive AI-based instruction that allow teachers to participate in the working process.

Keywords: *guyabano, seed extract, alternative, pediculucide*

Introduction

Head lice have been one of the most common problems that people have in their heads. Having many head lice can be so irritating. Head lice crawling and sucking the bloods in the scalp causing itchiness and irritation. It also lays hundreds of eggs that become additional to the problems. Head lice also affect the self esteem of a person. If other people see a person with hairs having many head lice they might avoid him/her.

In the Philippines, having head lice is very common in children, especially those who living in rural areas. Many local people use herbal plants as remedy to head lice infestation.

One of the best known herbal plants used in getting rid of head lice is the Guyabano, scientifically called as *Annona muricata*. *A. muricata* is a very powerful tree. Some of the folkloric beliefs are the following; decoction of its leaves is believed to kill bedbugs and insects and head lice. They extract the *A. muricata* leaves and then apply to their head thoroughly. The leaves can also be made as a tea to help detoxify the body and cleanse the liver. Meanwhile, its bark, if boiled, can be used to treat cough.

The foregoing stated belief about *Annona muricata* led the researchers to conduct a study aimed to determine the effectiveness of *Annona muricata* seed as a pediculucides.

Research Questions

The general objective of the study was to determine the effectiveness of *Annona muricata* seed extracts as an alternative pediculucide. Specifically, it sought answers to the following questions:

1. What are the phytochemical components of *Annona muricata* seed?
2. What dosage level of *Annona muricata* seed extract is effective as an alternative pediculucide?
3. Is there a significant difference between and among the treatments (formulations) of *Annona muricata* seed extracts as an alternative pediculucide?

Literature Review

Guyabano

Annona muricata is a member of the Annonaceae family and is a fruit tree with a long history of traditional use. *Annona muricata*, also known as soursop, graviola and guanabana, is an evergreen plant that is mostly distributed in tropical and subtropical regions of the world (Moghadamtousi, S. Z. et al., 2015).

Known as a sedative, a nerve tonic, and used to maintain proper intestinal health, guyabano is just one medicinal tool stemming from the graviola tree. Throughout history, each part of the graviola tree, such as the bark, leaves, roots, fruit, and seeds have been used for medicinal purposes. According to Kedari et al., (2014), the extracts and metabolites from this plant exhibit pharmacological properties such as anti-inflammatory, antiulcer, anthelmintic, antibacterial, and free radical scavenging activity.

Description

Wagner et al., (2014) gave the following description of the *Annona muricata* tree, a small tree ranging from 7.5-9 meters tall, new growth have reddish brown hairs. The leaves are narrowly elliptic, 6.5-20 cm long. The fruit is a large, fleshy syncarp to 30 cm long, 15 cm long, ovoid to oblong, often somewhat curved, the surface bearing regularly well-spaced, soft, conical, curved spines 2-3 mm long, the flesh white, juicy and with cotton-like fibers, surrounding the numerous seeds. Seeds light to dark brown, 13-17 mm long, 9-10 mm wide, ellipsoid, compressed, with low marginal ridge; endosperm ruminate.

Origin and Distribution

Annona muricata is of tropical American origin, and it is probably native to Central America and north Southern America. Based on evidences, it indicates that *Annona muricata* was one of the first fruit trees came from America to the old world tropics. It was introduced to Asia by way of the Spanish galleon trade and now widely cultivated across Asia Pacific Region for its edible fruit (Datiles, M. J. et al., 2015).

Usefulness/Benefits

All portions of the *Annona muricata* tree are extensively used as traditional medicines against an array of human ailments and diseases, especially cancer and parasitic infections. The leaves are employed to treat cystitis, diabetes, headaches and insomnia. The crushed seeds are believed to have anthelmintic activities against external and internal worms and parasites (Moghadamtousi et al., 2015).

Kedari et al., (2014) reported that the fruit and juice is used against worms and parasites, to cool down fevers, to increase lactation after childbirth. While Hanelt (2001) reported some of its uses in traditional medicine, its leaves for fever, its bark as tonic, roots as antispasmodic and parasitidal, its flower as bechic (relieving coughs), unripe fruit as antiscrobutic, and seeds as insecticidal, astringent, and as fish – poison.

In addition, the leaves of this plant were used as an extract at varying concentrations as an antibacterial agent against oral pathogens (Pai, B. M. et al., 2016).

Pediculicidal Activity

In the study conducted by Intaranongpai J, et al., (2006), the result showed that the seed oil from *Annona muricata* seeds can kill head lice. A petroleum ether extract of seeds was prepared as 20% w/ oil in water cream and tested for anti- head lice activity in school girls. After a 3 hour- hair exposure to 20 g of freshly prepared custard apple cream, 95.34+/-1.96% of head lice were killed.

In support, a published article by BlaBlaWriting (2018), reported that the chromatographic and spectroscopic techniques revealed that two major compounds of the hexane seed extract were oleic acid and triglyceride with one oleate ester. The yields of these compounds were 13.25% and 7.74% dry weight, respectively. The compounds were tested in vitro against head lice, comparing to the crude hexane extract diluted with coconut oil 1:1. These compounds were found to kill all tested head lice in 49, 11, and 30 minutes, respectively.

Chemical Constituents

According to Stuart Jr. (2016), studies yielded tannins, steroids and cardiac glycosides as major phytochemical compounds. Seed contains poisonous alkaloid. Fruit flesh yielded cis-annoreticuin and sabadelin. Seeds yield lactones, annomonicina, annomontacina, annonacina, annomuricata, annonacinona, javoricina, among others. Study of freeze-dried seeds of *A. muricata* isolated murisolin, annoreticuin-9-one, cis- annoreticuin, sabadelin, β -sitosterol, stigmasterol, and triglyceride.

Furthermore, Kedari, et al., (2014) reported that the ethanolic leaf extract shows high phosphorous and iron content of 128 mg% and 1.075 mg% respectively. The ethanolic root extract shows highest calcium content of 22 mg% and the aqueous extract of seed contain high carbohydrate content of 11.025 mg%. And according to Moghadamtousi, S. Z. et al., (2015), the presence of different major minerals such as K, Ca, Na, Cu, Fe and Mg suggest that regular consumption of the *A. muricata* fruit can help provide essential nutrients and elements to the human body.

Based on the analysis of defatted seeds from ripe soursop led to the isolation of annonaceous acetogenins including annonacin, isoannonacin, isoanno- nain-10-one, goniothalamycin, and gigantetrocin which showed cytotoxicities for the A-549 lung carcinoma, MCF-7 breast carcinoma, and HT-29 colon adenocarcinoma human tumor cell lines (Schauss, A. G. 2010)

Plant Profile

Domain: Eukaryota, Kingdom: Plantae, Phylum: Spermatophyta, Subphylum: Angiospermae, Class: Dicotyledonae, Order: Annonales, Family: Annonaceae, Genus: *Annona*, Species: *Annona muricata*, Scientific Name: *Annona muricata* (Datiles, M. J. et al., 2010).

Head Lice

According to Ogg, B. (2014), head lice are small parasites, tan to brown colored insects. They are found close to the scalp and must feed frequently on blood. Their bites can itch and some people find them quite repulsive. Aside from they are fairly harmless and do not carry diseases. Head lice mostly lay their eggs on hairs, close to the scalp. The next generation usually hatches within one week.

The eggs laid by the head lice are called nits.

Head lice contamination is common worldwide and has been proposed as a major health problem not only in poor countries but also in developed and industrial countries. Every year, over 12 million Americans are contaminated by this parasite. Studies carried out in different parts of the world have reported different prevalence for head lice in children. For example, the rates of contamination have been estimated to be 16.59% in India, 13.3% in Yemen and 8.9% in Belgium (Salehi, S et al., 2014).

Life Span of Head Lice

Eggs laid by adult female lice are glued to strands of hair about 1/4-inch from the scalp. Eggs hatch in 7–10 days. After hatching, the eggshell (nit) remains firmly attached to the hairshaft. Head lice cannot fly or hop like fleas, but they can crawl quickly through the hair at a rate of 9 inches per minute. An adult female head louse lays an average of six to seven eggs per day and the average life span is about 32 days (Ogg, B., 2014).

Life Span on Objects or Without Food

In the study conducted by Sullivan, D. (2016) about the life cycle of head lice, the result showed that adult lice can't live longer than 24 hours or so on nonhuman surfaces like carpets, hardwood floors, clothing, furniture, sports helmets, headphones, or hair accessories. However, if you have identified lice in your home, isolate and wash those items and areas within at least 72 hours.

Symptoms

Itching on the scalp, neck and ears is the most common symptom. Lice may be visible but are difficult to spot because they're small, avoid light and move quickly. Nits stick to hair shafts. Incubating nits may be difficult to see because they're very tiny. They're easiest to spot around the ears and the hairline of the neck (Mayo, 2017).

In addition, often people who have head lice will have no symptoms for 4 to 6 weeks with first case. Other symptoms include crawling or tickling sensation on the scalp and scratch marks or small red bumps like a rash (What are the symptoms of head lice? 2016).

Causes

The infestation rate was found to be associated with low educational level of parents, long hair, family size, mother's job (housewife), father's job (worker/unemployed), using a common comb, lack of bathrooms in the house, and a low frequency of bathing (Moosazadeh, 2015).

Head lice are most common in children. They almost always spread through direct body contact, for instance when children play with their heads close together. Hair length has at most very little influence on your risk of getting head lice, but it is harder to find the lice in longer, thick and curly hairs (PubMed Health, 2015).

Preventions

Terry Dwelle, M.D. (2012) gave the following steps in managing and treating head lice: first careful inspection and screening of the hair and scalp to identify lice and/or nits correctly; second, use of a pediculicidal (head lice) product if live lice or viable nits are found; and third, cleaning of personal items and the environment.

Phytochemical Analysis

Based on the phytochemical analysis of *Annona muricata* seed extracts at NDDU Laboratory, General Santos City on August 11, 2018, the results were the following secondary metabolites: alkaloids, flavonoids, phenols and tannins.

Alkaloids

Aniszewski (2007), stress that alkaloids are any group of complex heterocyclic nitrogen compounds, which have strong physiological activity, are often toxic. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid - base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine, the stimulant caffeine, nicotine, the analgesic morphine, or the antimalarial drug quinine. Some alkaloids have a bitter taste.

Flavonoids

Flavonoids are biologically active low molecular weight secondary metabolites that are produced by plants, with over 10,000 structural variants now reported. Due to their physical and biochemical properties, they interact with many diverse targets in subcellular locations to elicit various activities in microbes, plants, and animals (Weston, L. A. et al, 2013).

The majority of flavonoids are present in the form of glycosides under natural conditions. Because of their diverse chemical structure and variety resulting from the attached substituents, they have a number of important functions in plants. They participate in plant

protection against biotic (herbivores, pathogens) and abiotic stresses (UV radiation, heat), and due to their antioxidative properties, they also maintain a redox state in cells. Flavonoids play an important role in the protection of plants against plant feeding insects and herbivores. Their presence can alter the palatability of the plants and reduce their nutritive value, decrease digestibility or even act as toxins (Mierziak, J. et al, 2014).

Phenols

According to Bhattacharya A, et al. (2010), Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress. The functions of phenolic compounds in plant physiology and interactions with biotic and abiotic environments are difficult to overestimate. Phenolics play important roles in plant development, particularly in lignin and pigment biosynthesis. They also provide structural integrity and scaffolding support to plants. Importantly, phenolic phytoalexins, secreted by wounded or otherwise perturbed plants, repel or kill many microorganisms, and some pathogens.

Phenol and Phenolic compounds cause irritation, odour and taste problem and are toxic in higher concentration. The essential factor that determines phenol toxicity is the reactivity of the compound with a cell's biomolecules and is related with easiness of donation of free electrons by phenol from oxidized substrate. The effect of their action is the formation of phenoxy radicals and intermediate metabolites-semiquinones and quinone methides that interact with biomolecules in the cell. Toxic influence of phenols is also related to the kind of substrate that comes into reaction, also its localization in cell and phase of cell proliferation (Parihar, S. 2016).

Tannins

According to Ashok, P. K. et al, (2012), tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from the tannins is that which causes the dry and puckery feeling in the mouth following the consumption of red wine, strong tea, or an unripened fruit. Interestingly, tannins are found almost in all plants and in all climates all over the world. Tannins are usually found in large quantities in the bark of trees where they act as a barrier for micro-organisms and protect the tree. Apart from tanning, tannins are also used in dyeing, photography, refining beer and wine as well as an astringent in medicines. Tannins have been also reported to have anti-viral antibacterial and antiparasitic effects.

Methodology

Research Design

The researchers used experimental method to determine the effectiveness of *A. muricata* seed extracts as an alternative pediculicides.

Respondents

The researchers collected the *A. muricata* seeds and conducted the production of *Annona muricata* seed extracts at Barangay Meta, Datu Unsay, Maguindanao. The researchers choose the seeds that come from ripe *Annona muricata* fruit.

The participants of the study were ten (10) girls who have reached the ages of 10 to 13 years old, having many head lice, and they were residents of Barangay Meta, Datu Unsay, Maguindanao.

Instrument

The materials used for the study were *Annona muricata* seeds, triple beam balance, petri dish, mortar and pestle, 200 ml beaker, water, pot and plastic bottles.

Phytochemical Procedures

Annona muricata Seed Extraction

The seed extraction was conducted by the researchers at the Natural Science Laboratory of Mindanao State University – Maguindanao on August 09, 2018.

First, the researchers weighted 200 grams of *Annona muricata* seeds and cracked them using the mortar and pestle. Then the cracked *Annona muricata* seeds was placed in a bottle and submerged into 250 ml of 95% ethanol for 48 hours.

Phytochemical Analysis

Alkaloids

The researchers took 10 ml of filtrate. The filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of yellow colored precipitates indicates the presence of alkaloids.

Flavonoids

The researchers took 10 ml filtrate. The extracts were treated with few drops of lead acetate solution. The formation of yellow color precipitate indicates the presence of flavonoids.

Phenols

The researchers took 10 ml filtrate. The filtrates were treated with 3-4 drops of ferric chloride solution. The formation of bluish black color indicates the presence of phenols.

Saponins

The researchers took 10 ml filtrate. The filtrate was diluted with 20ml distilled water and this was shaken in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam indicates the presence of saponins.

Tannins

The researchers took 10 ml filtrate. Then 1% gelatin solution containing sodium chloride was added to the filtrate. The formation of white precipitate indicates the presence of tannins.

Preparation of *Annona muricata* seed extracts

The specific mass of *Annona muricata* seeds were weighed and cracked using the mortar and pestle. After the seeds were fully cracked, it was put in a pot and 100 ml of water was added for the decoction. The decoction lasted only after the first boil.

Application of *A. muricata* seed extracts

The application of *Annona muricata* seed extracts is in vitro. First, the researchers prepared 25 head lice in a small container. Then, the researchers set the time and sprayed the *Annona muricata* seed extracts on the head lice. Afterward, the researchers recorded the number of head lice dead in duration of 10, 20 and 30 minutes, respectively.

Procedure

Before the study was conducted, the researchers prepared a letter for the approval of the adviser. When the request granted, the researchers briefed the participants. The researchers collected head lice from the participants and store it in a small container.

After ten (10) minutes of resting the head lice, the researchers put a total of 25 head lice in each container and apply the *Annona muricata* seed extracts. After applying the pediculucides, the researchers counted the number of head lice dead.

Data Analysis

The data were gathered and analyze using the percentage, mean and standard deviation as statistical tools for analysis.

Results and Discussion

This section presents the data gathered, analysis and interpretation of results. The data are presented in tables with the corresponding interpretations.

Phytochemical Analysis of *Annona muricata* Seed Extracts

The table below indicates the result of the phytochemical analysis of *Annona muricata* seed extracts conducted at the Research Laboratory of Notre Dame of Dadiangas University, General Santos City. The researchers examined the secondary metabolites of the samples on August 11, 2018.

Table 1. *Result of Phytochemical Analysis of Annona muricata Seed Extracts*

<i>Phytochemical</i>	<i>Results</i>
Alkaloids	Positive
Flavonoids	Positive
Phenols	Positive
Saponins	Negative
Tannins	Positive

Table 1 shows *Annona muricata* seed extracts contain alkaloids, flavonoids, phenols and tannins but negative for saponins.

Table 2. *Treatment levels of Annona muricata seeds and Decoction*

<i>Treatment</i>	<i>Seeds (g)</i>	<i>Decoction (ml)</i>
T1	10	100
T2	20	100
T3	30	100

As shown in Table 2, level of *Annona muricata* seed extracts ranges from 10, 20 and 30 grams. Decoction in all treatments was made in 100 ml of water.

Table 3. Number of Head Lice Dead from 10 minutes to 30 minutes of Observation

Treatments	Head lice	Number of minutes			Total	Percentage	Mean	SD
		(10)	(20)	(30)				
T1 (10 g A. muricata seeds in 100 ml distilled water)	25	15	18	22	22	88%	18.33	3.51
T2 (20 g A. muricata seeds 100 ml distilled water)	25	17	19	25	25	100%	20.33	4.16
T3 (30 g A. muricata seeds 100 ml distilled water)	25	17	25	25	25	100%	22.33	4.62

Table 3 shows the number of lice dead in each treatment with the corresponding number of minutes. In Treatment 1 (10 g of *Annona muricata* seeds in 100 ml distilled water), the number of head lice dead after 10 minutes is 15, after 20 minutes another 3 head lice died, and after 30 minutes 4 more head lice died, overall total of head lice dead was 22, which is equivalent to 88%.

Treatment 2 (20 g of *Annona muricata* seeds in 100 ml distilled water) showed more effectiveness compared to treatment 1. From the total of 25 head lice, 17 head lice died after 10 minutes, additional 2 after 20 minutes, and after 30 minutes all the 25 head lice died which is 100% by mortality.

Treatment 3 (30 g of *Annona muricata* seeds in 100 ml distilled water) showed the most effective dosage as an alternative pediculicides for head lice. Of the total 25 head lice, 17 after 10 minutes, and after 20 minutes, all the 25 head lice died resulting to 100% mortality.

Table 4. Comparison Between and Among Treatments

Treatments	Mean	SD	Computed t-value	Critical t-value	Description
T1 (10 g A. muricata seeds in 100 ml distilled water)	18.33	3.51	1.837	±2.011	Not Significant
T2 (20 g A. muricata seeds 100 ml distilled water)	20.33	4.16			
T1 (10 g A. muricata seeds in 100 ml distilled water)	18.33	3.51	3.447	±2.011	Significant
T3 (30 g A. muricata seeds 100 ml distilled water)	22.33	4.62			
T2 (20 g A. muricata seeds 100 ml distilled water)	20.33	4.16	1.608	±2.011	Not Significant
T3 (30 g A. muricata seeds 100 ml distilled water)	22.33	4.62			

Legend: Computed t-value to be significant at 0.5 level should be at least ±2.011

The results of the experimental test for the effectiveness of *Annona muricata* seed extracts showed that T1 and T2, and T3 and T4 have no significant differences. These are indicated by smaller computed t-values of 1.837 and 1.608 respectively, as compared to the critical value of ±2.011 at 0.05 level of significance. The results implied that the two treatments, T1 (10 g *Annona muricata* seeds in 100 ml distilled water) and T2 (20 g *Annona muricata* seeds 100 ml distilled water); and T3 (30 g *Annona muricata* seeds 100 ml distilled water) manifested almost the same level of effectiveness as an alternative pediculicides.

However, in the case of T1 (10 g *Annona muricata* seeds in 100 ml distilled water) and T3 (30 g *Annona muricata* seeds 100 ml distilled water), the result of computed t-value is significant. This is indicated by the computed t-value of 3.447 which is higher than the critical t-value of 2.011 at 0.05 level of significance. This means that the 30 g of *Annona muricata* seeds mixed with 100 ml of distilled water is more effective as pediculicides compared to 10 g of *Annona muricata* seeds mixed with 100 ml of distilled water.

Conclusions

Annona muricata seed extract was proven positive in toxic secondary metabolites, which are alkaloids, phenols and tannins. The *Annona muricata* seed extracts is effective as an alternative pediculicide for head lice at higher level of dosage. There is a significant difference between treatments 1 and 2, which indicated that treatment of higher amount of *Annona muricata* seed extract is more effective as pediculicide.

The following recommendations were offered.

First, *Annona muricata* seed extracts can be potentially used as an alternative pediculicide for head lice provided that the head has no wounds or other opening that may lead to the penetration of extracts.

Second, higher dosage of *Annona muricata* seed extracts is recommended for use.

Third, since the study is in vitro, further study may be conducted such as making *Annona muricata* seed extracts into shampoo and hair spray.

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