

Effectiveness of Madre De Cacao Leaves Extract *Gliricidia Sepium*, Guava Leaves Extract *Psidium Guajava* & Natural Vinegar Dilute Acetic Acid in Eradicating Mosquito wrigglers *Culicidae* Diptera

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Research Article

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ABSTRACT

This study aims to create an organic mosquito repellent which helps to lessen the mosquitoes in the environment. Using this product, we assure you that our experiment is effective because of their organic properties and uses which keeps our environment safe and free from diseases. Madre de Cacao leaves has the following properties: antimicrobial, anti-scabies, nematicidal, insecticidal, antiviral, acaricidal. Guava leaves include antimicrobial properties as well as tannins, which can help with everything from gastrointestinal problems to chronic diseases like cancer. Natural Vinegar is traditionally made through a long fermentation process, rich in bioactive components like acetic acid, gallic acid, catechin, epicatechin, caffeic acid, and more, giving it potent antioxidant, antimicrobial, and many other beneficial properties. Madre de Cacao and Guava leaves are among the most effective plants used by healers. We made some concentrations to know the level of Madre de Cacao and Guava leaves extract on how effective they are as mosquito repellent. Mosquitoes are among the common insects that endanger animals and other mammal's health by spreading diseases through bites. To lessen this insect, we provide an organic mosquito repellent which we cannot spend more money and it is very effective. The researchers are aware of the risk of high toxic chemical compounds and become interested in using an organic mosquito repellent derive from plants as means of destroying pest hence the conduct of this study.

INTRODUCTION

Rationale of the Study

Mosquitoes are one of the most frequent insects that affect human health by transmitting diseases through bites. Mosquitoes are responsible for millions of deaths each year around the world. Female mosquitoes need blood to nurture their eggs, breed, and transmit diseases by bloodsucking. Dengue and hemorrhagic fever is a dreadful disease cause by a virus and transmitted by mosquitoes. To prevent the spreading of mosquito bite diseases and to improve the quality of environment and public health, mosquito control essential. There are a variety of mosquito control strategies that may be used to minimize the quantity of mosquitos^[1]. Local councils may use commercial larvicides (pesticides that can kill the larvae) which prevent mosquitoes from maturing to adults. The researchers studied the effects of Madre de Cacao (*Gliricidia sepium*), guava leaves extract (*Psidium guajava*), and natural vinegar (Dilute acetic acid) on mosquito larvae in an effort to assist the government in combating the disease and eliminating mosquito carriers. According to studies, the plant Madre de Cacao acts as an insect repellent and has the potential to eliminate a variety of insects that can carry a variety of diseases. Commercial and manmade chemicals are still widely used to eliminate pests that are potential carriers and are pricey. Because of the dangers of high-toxic chemical compounds, the researchers became interested in employing natural pesticides derived from plants as a means of pest control, which led to the creation of this study. The main objective of the study was to determine the effectiveness of Madre de Cacao (*Gliricidia sepium*), Guava leaves extract (*Psidium guajava*), and natural vinegar (Dilute acetic acid) in eradicating mosquito wrigglers (*Culicidae* diptera)^[2].

The Problem

Statement of the problem

The study aims to determine if the solution of Madre de Cacao leaves extract, guava leaves extract, and natural vinegar is effective in eradicating mosquito larvicide. Its goal is to provide answers to the following questions:

1. What is the level of effectiveness of Madre de cacao leaves extract, guava leaves extract, and natural vinegar as a mosquito larvicide?
2. Is there a significant difference in the level of effectiveness between the Madre de cacao leaves extract, guava leaves extract, and natural vinegar as a mosquito larvicide solution at different concentrations?

Null Hypothesis

H₀₁: The 100% concentration of Madre de cacao leaves extract, guava leaves extract, and natural vinegar has a higher level of effectiveness as a mosquito larvicide.

H₀₂: There is no significant difference in the level of effectiveness of Madre de cacao leaves extract, guava leaves extract, and natural vinegar as a mosquito larvicide at different concentrations.

Significance of Study

This study aims to determine if Madre de Cacao leaves extract, guava leaves extract and natural vinegar solution can be utilized to eradicate mosquito larvae and can be a potential substitute for commercial larvicides. The researcher believes that the following will benefit from this research.

Environmental health workers

The study's findings will provide fundamental knowledge and information about the components of Madre de Cacao leaf extract, guava leaf extract, and natural vinegar solution as a potential mosquito larvicide. It will guide environmental health workers to find alternative ways to lessen the number of mosquitoes in the community and prevent certain mosquito-borne diseases^[3].

Government

The result of this study will help lessen government expenses intended in eradicating mosquitoes

Community

This study will provide people with natural and inexpensive mosquito larvicide derived from Madre de Cacao leaves extract, guava leaves extract, and natural vinegar solution^[4]. This solution will help minimize victims of Zika virus, dengue, and malaria in the community, especially in mosquito-prone areas.

Researchers

The result of this study will serve as baseline information for those who are interested in conducting a further investigation related to the problem.

Review of Related Literature and Studies

Madre de Cacao possesses antifungal and antimicrobial effects. To repel insects such as mosquitoes, some farmers used Madre de Cacao leaf extract, guava leaf extract, and natural vinegar. *Gliricidia sepium*, or Madre de Cacao, is a fast-growing, leguminous, medium-sized, thornless tree that can replace *Leucaena leucocephala* as a source of fodder, fuelwood, and green manure, in hedges and living fences, and as a shade tree on tea, coffee, and cocoa estates^[5]. It grows well in low-lying tropics with mean annual temperatures of 22-30 degrees Celsius and rainfall of 800-1700 mm, and it is more easier to establish than *leucaena*. The leaves contain 20-30% crude protein, and current research discussed in this publication shows the effectiveness of *gliricidia* as a meal supplement for mosquito wrigglers. Madre de Cacao (*Gliricidia sepium*) is a thornless, small to medium-sized leguminous tree that grows to a height of 10-12 m. With basal diameters of 50-70 cm, it commonly branches from the base. The bark is smooth, and the hue ranges from light grey to deep red-brown^[6]. Spreading crowns may be seen on the trees. The leaves are oddly pinnate, alternating, sub-opposite, or opposite, and 30 cm long. The leaves are 5-20 cm long, 2-7 cm broad, and ovate or elliptic in shape. Red stripes can be found on the midrib and rachis of leaflets. Inflorescences are 5-15 cm long and appear as clustered racemes on fresh and old wood's distal parts. Flowers are produced individually in racemes of 20-40, and the calyx is glabrous, green, and commonly tinged red, with tints ranging from vivid pink to lilac, colored with white, and usually with a diffuse pale yellow patch at the base of the standardpetal. The standardpetal is 20 mm long, round, and erect; the keel petals are 15-20 mm long and 4-7 mm wide. Fruit is green when unripe, reddish-purple when ripe, light yellow-brown when mature, thin, 10-18 cm long, 2 cm wide, valves twisting in dehiscence; seeds are 4-10, yellow-brown to brown, practically spherical, thin, 10-18 cm long, 2 cm wide, valves twisting in dehiscence^[7].

Growers in certain States use *gliricidia* to keep insects away from their crops. The leaves are blended with water after being mashed up. Following that, the animal is soaked in the paste. According to some of the farmers, repeating this process every 7-14 days reduces the frequency of torsalo (tropical warble fly) illnesses. This assertion was not backed up by any published research. However, following interviews with farmers and inspections of goats in Honduras, it was shown that goats cleaned with *Gliricidia* had just 2-3 torsalos, whilst others had 10 or more. In addition, one study discovered that *Gliricidia*'s heartwood contains chemicals that both attract and poison bugs (e.g., southern army worm, cabbage looper, yellow woolly bear, and *Glyptotermes dilatatus*, a termite^[8]).

Gliricidia was discovered to suppress the development of numerous strains of *Neisseria gonorrhoea* in vitro testing in another investigation. These studies were conducted using tinctures produced from the leaves. perhaps more^[9].

Gliricidia leaves are cleaned and crushed in the Philippines to get the essence. It is then administered either once twice each day for one week to the region afflicted by parasitic infections. Gliricidia bark and leaves are used to cure human skin disorders in Guatemala.

Expectorant, insecticidal, rodenticidal, sedative, and suppurative properties have been described for Madre de Cacao. Madre de Cacao is used to cure alopecia, boils, bruises, burns, colds, cough, debility, eruptions, erysipelas, fever, fractures, gangrene, headache, itch, prickly heat, rheumatism, skin, sores, tumors, ulcers, urticaria, and wounds.

Guava leaves are equally as therapeutic as the nutritious superpower fruit with which they are associated. The guava tree's leaves are high in antioxidants, anti-inflammatory compounds, antibacterials, and even tannins, which can help with everything from stomach issues to chronic illnesses such as cancer ^[9].

The leaves of the popular tropical fruit, like the fruit itself, may be converted into drinks, jams, and other dishes. They may be steeped to produce tea, which releases useful compounds such as micronutrients and flavonoids such as quercetin ^[10].

Guava is a popular fruit in Asian countries, but it is also widely available in the United States. The fruit is round or pear-shaped, with a pale green, yellow, or maroon exterior and white or maroon flesh containing numerous small, hard edible seeds. When fully ripe, it has a creamy texture and a fragrant, musky scent. Guava is a great fruit because of its multiple health-promoting characteristics, in addition to its distinct flavor and scent. Guava trees' bark and leaves, in addition to the fruit, have several health benefits. Vitamins C and A are abundant in guava. In fact, a single guava fruit has four times the vitamin C of an orange and 10 times the vitamin A of a lemon ^[11].

The majority of us are aware of the guava fruit's health benefits. Guava leaves (Amrood ke Patte in Hindi) have a variety of medicinal properties and provide a variety of health benefits, which we are unaware of. Fresh guava leaves are a natural pain reliever because they are high in antioxidants, antimicrobial and anti-inflammatory compounds, and healthy tannins. Polyphenols, carotenoids, flavonoids, and tannins found in these leaves have been shown to be particularly useful in treating a variety of ailments. Because of its medicinal characteristics, guava leaves are used as a herbal remedy for a variety of ailments. Some of the health benefits of guava leaves are listed below ^[12].

Anti-Aging advantages

Guava leaves contain antioxidants that protect your skin from free radical damage, preventing aging and improving the tone and texture of your skin. To tighten the skin, a decoction of mature guava leaves can be administered.

Itching relief

If not treated promptly, skin itching can develop into a serious problem. Guava leaves, which contain allergy-blocking chemicals, provide a quick remedy for irritation ^[13].

Guava leaves are just as beneficial to one's health as the nutritional powerhouse fruit with which they grow. The guava tree's leaves are high in antioxidants, anti-inflammatory compounds, antibacterials, and even tannins, which can help with everything from stomach issues to chronic diseases like cancer.

Related Studies about the potentiality of Madre de Cacao (*Gliricidiasepium*)

Gliricidia extracts have been studied for their antifungal and antibacterial activities. The LC50 of medicarpin, one of the chemicals in the leaves and heartwood of gliricidia, was 454 ug.ml (CI 328-608) in a brine shrimp toxicity test, a common screening approach indicative of cytotoxicity and pesticidal action. In an antifungal investigation, gliricidia extracts barely inhibited Drechsleraoryzae germination by 6%. In another investigation, 50ug of stem chloroform extracts decreased Cladosporium cucumerinum growth and somewhat inhibited Candida albicans growth. Another study looked into the antibacterial properties of extracts from gliricidia bark. It worked well against bacteria that cause dermatitis. It was ineffective against enterobacteria and Candida albicans, though. The difference in Candida albicans efficiency could be due to the amount of plant extract utilized or the types of extracts employed. Leaf extracts were reported to be efficient against dermatophytes, microsporiumcanis, Trichophytonmenta grophytes, Varalgodonosa, and Trichophyton rubrum ^[14]. It was ineffective against Epidermophyton floccosum, M. gypseum or T.mentagrophytes vargranulare.

A full-time professor from the University of Northern Philippines, Alfredo Rabena. Discovered coumarins from Madre de cacao leaves. The toxic substance is known to eliminate different kinds of insects and pets. Rabena stated that soaking one kilogram of Madre de cacao leaves overnight can produce seven gallons of "botanopesticides" (botanical pesticides), a pesticide formulated from botanical plants ^[15].

Fresh young stem scarpings and leaves of Madre de cacao were extracted and combined with the same amount of water in a study conducted in Zamboanga National High School Science Lab (2008) about the larvicidal potential of crude aqueous Madre de cacao extract against larvae. Ten mosquito larvae placed in 5.0 mL rainwater in different Petri dishes were separately treated, with 20 mL, 15 mL, and 15 mL of the two extracts. Five tests were conducted for all the setups. All the groups were left undisturbed for 24 hours. With both means of 9.4 (94 percent mortality, n=5), the highest mortality was observed in 20 and 15 mL leaf extract. Fresh leaves (CEE2), Crude ethanol extracts (CEE) of *Gliricidia sepium*'s dried leaves (CEE1), dried petioles (CEE3), and stem bark (CEE4) were examined for toxicity on late third instar larvae of *Anopheles stephansi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Sharma, N. et al., 1998). The findings show that all CEEs are detrimental to the larvae of all three species,

producing 100% mortality at doses of 16,000 ppm or less. Although the mortality rate is dose-dependent, the tolerance of various species' larvae at lower doses of CEE is genetically varied. The toxicity may be generated by more than one toxic principle, at least in certain instances. The findings also suggest that toxic elements vary quantitatively and/or qualitatively in different organs of the plant. Overall, In comparison to CEEs from fresh leaves and bark, CEE2 has higher toxicity while CEE3 has weaker toxicity^[16].

The aforementioned related literatures and studies allow the researcher to obtain with additional knowledge and insights in conducting the research study.

METHOD

Instrumentation

The materials and equipment used were:

- Graduated Cylinder- used to calculate the amount of extract and its % concentration.
- Madre de Cacao leaves- were cut, mashed, and removed with a sieve.
- Guava leaves- the leaves were chopped, pounded and extracted using a strainer.
- Mortar and Pestle- used in pounding the Madre de Cacao leaves and guava leaves to obtain the extract.
- Mosquito larvae- are wormlike eating forms that develop from domestic mosquito eggs.
- Petri Dish- utilized as a container for setting up and treatments.
- Strainer- used in separating the extract from the blended Madre de Cacao leaves and guava leaves.

Collection and Processing of Madre de Cacao Plant

The leaves of Madre de Cacao leaves were gathered from mountain areas like Matab-ang, Toledo City, Cebu, Philippines. The leaves were washed and pounded using mortar and pestle then the extracts were collected using a strainer. The extract was diluted to various quantities by adding distilled water.

Procedure

Culturing of mosquito larvae

For a period of five to seven days, mosquito larvae were cultivated. The container was filled with water, and handfuls of dung were added to feed the mosquitos. The mosquito eggs were tested on a regular basis. If there were little eggs on the surface of the water, they were transferred from the bucket to a container at room temperature with freshwater resources and provided with a fish net for easy capture until they reached their larval stage of development. The larvae appeared like lengthy insects that wiggled when disturbed at this stage. After seven days, the larvae were removed from the jar with a fish net and employed as research subjects.

Treatment

The concentration was varied to twenty five percent (25%), fifty percent (50%), seventy five percent (75%) and one hundred percent (100%) by volume respectively. There were five treatments used. Treatment (1) is the control with zero percent (0%) concentration of the extract. Treatment (2) use 25% concentration containing 10 ml of extract and 30 ml of distilled water. Treatment (3) use 50% concentration containing 20ml of extract and 20 ml of distilled water. Treatment (4) use 75% concentration containing 30 ml of extract and 10 ml of distilled water and lastly is treatment (5) 100% containing 40 ml of extract with no distilled water. Three (3) replicates of the prepared Madre de Cacao extract were placed in a petri dish for each treatment, for a total of fifteen (15) petri dishes. Thirty seven (6) larvae were added on each petri dish and were observed by the researchers for a duration of twenty four (24) hours. The researchers counted and recorded the number of dead larvae in each petri dish.

RESULTS AND DISCUSSION

Trial 1

Treatment 5 is the most successful, as seen in the table 1. The mosquito wrigglers are reduced by the 100% concentration of Madre de Cacao leaves extract, Guava leaves extract, and Natural Vinegar because of their potency as insect repellents. This is also effective because of their characteristics, which make mosquito wrigglers feeble. The higher the concentration of extract, the lower the survival rate of mosquito wrigglers.

Trial 2

Treatment 5 is the most successful, as seen in the Tables 1,2. The mosquito wrigglers are reduced by the 100% concentration of Madre de Cacao leaves extract, Guava leaves extract, and Natural Vinegar because of their potency as insect repellents. This is also effective because of their characteristics, which make mosquito wrigglers feeble. The higher the concentration of extract, the lower the survival rate of mosquito wrigglers.

Table 1. Treatment.

Treatment	Percentage	No. of wrigglers	Time	Survival
1	0%	6	30 min.	5
2	25%	6	30 min.	4
3	50%	6	30 min.	2
4	75%	6	30 min.	0
5	100%	6	30 min.	0

Table 2. Trial 1.

Treatment	Percentage	No. of wrigglers	Time	Survival
1	0%	6	30 min.	5
2	25%	6	30 min.	4
3	50%	6	30 min.	2
4	75%	6	30 min.	0
5	100%	6	30 min.	0

Table 3. Trial 3.

Treatment	Percentage	No. of Wrigglers	Time	Survival
1	0%	6	30 min.	6
2	25%	6	30 min.	6
3	50%	6	30 min.	4
4	75%	6	30 min.	2
5	100%	6	30 min.	0

Trial 3

According to trial 3, treatment (1) employed a 0 percent concentration of the extract with a survival rate of 6 out of 6 wrigglers. Treatment #2 used a 25% concentration containing ml of extract and 30 ml of distilled water, with 6 out of 6 wrigglers surviving. Treatment (3) employed a 50 percent concentration with 20 ml of extract and 20 ml of distilled water, with 4 out of 6 wrigglers surviving. Treatment (4) employed 75 percent of the concentration, with 30 ml of extract and 10 ml of distilled water, and two out of six wrigglers survived. Treatment (5) employed a 100% concentration of extract in 40 mL of distilled water, with a survival rate of 0 out of 6 no. of wrigglers. Treatment (5) used 100% concentration containing 40 ml of extract with no distilled water with a survival of 0 out of 6 no. of wrigglers. The more extract used, similar to treatments 1 and 2, the lower the survival rate of mosquito wrigglers.

Conclusion

The study's findings led to the following conclusions:

1. The maximum concentration of Madre de Cacao leaves extract, Guava leaves extract, and pure vinegar has the highest level of efficiency in killing mosquitos and protecting our surroundings from the risks and diseases usually caused by mosquitos.
2. Because of its parricidal qualities, which are excellent in eliminating mosquito wrigglers, the combination of Madre de Cacao leaves extract, Guava leaves extract, and Natural vinegar has a higher level of efficiency than the other concentrations.

Recommendation

We would like to propose this study to students and those who are interested in using Madre de Cacao, Guava leaves, and Natural vinegar as mosquito repellents, as well as finding other insects for whom the solution is relevant and conducting more research on the solution. Furthermore, this research can help young brains like ours develop an alternate and organic way to reducing the amount of mosquitoes around the country.

The following suggestions are made:

1. For a less pressing procedure, use the bark of Madre de Cacao instead of the leaves.
2. Instead of crushing, use a boiling procedure to ensure that the extract is as effective as possible.
3. Because of its mosquito-repelling capabilities, add more Guava extract to the mixture.

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