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Development and Evaluation of Acaricidal Soap Formulated from *Azadirachta indica* (Neem) and *Carica Papaya* (Papaya) Leaf Extracts Using Bio-Assay Guided Approach

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Abstract: The environment, human health, and animals are all at risk as a result of the pervasive use of chemical-based products to control ticks and lice in animals, mostly dogs. This investigation was designed to assess the efficacy and physicochemical properties of an acaricidal soap for canines that was formulated using *Azadirachta indica* and *Carica papaya* leaf extracts. The soap was intended to be effective against *Rhipicephalus sanguineus* (dog ticks). Following an experimental design, the investigation was consistent with Sustainable Development Goal #9 on Industry, Innovation, and Infrastructure. Assessing the acaricidal activity of the extracts, developing the detergent, evaluating its physicochemical characteristics, and determining its median Lethal Dose (LD_{50}) were all components of the process. While soap development took place at the University of Northern Philippines, other experiments were carried out at a reference laboratory. The final concentration of the soap was 20 mg/g, achieved by combining 13 mL of extracts (100 mg/mL each) with 65 g of soap constituents. The soap had a greenish-brown color, a smooth texture, a light, pleasant smell, and a pH of 7.9. There was a 14.2 cm height of foam, a 5.2 cm of foam retention, 20% moisture content, 29% total fatty matter, and 0.20% of alkali content. The soap exhibited an earlier onset of acaricidal activity (7–9 hours) compared to a commercial herbal soap (10-12 hours), and both soaps achieve 100% tick mortality within 15 hours. The combined extract's LD_{50} was 2,933 mg/g body weight. The soap that has been developed is safe, efficacious, and has the potential for further refinement and commercial development.

Keywords: Lethal Dose 50 (LD_{50}), Total Alkali Content, Foaming Index, Moisture Content, Sustainable Development Goal # 9

Introduction

Commercially available chemical-based acaricides have been the primary method of controlling and managing ectoparasites, including the brown dog tick (*R. sanguineus*). These chemical preparations, which include fipronil, ivermectin, amitraz, carbaryl, and synthetic pyrethroids such as deltamethrin, permethrin, and cypermethrin, are extensively employed in veterinary practice (Varloud & Fourie, 2015; Suraj *et al*., 2019; Siriporn *et al*., 2023). They are applied in a variety of forms, including spot-on treatments, impregnated collars, shampoos, sprays, dips, and powders (Dryden, 2024). While these conventional products are effective in reducing tick infestations, they are costly, exhibit long-term residual activity, and contribute significantly to environmental contamination and toxicity risks. Moreover, the development of resistance in tick populations has resulted from their indiscriminate and excessive use, which presents significant obstacles to the long-term control of ticks (Bai *et al*., 2024; Rosario-Cruz *et al*., 2024). Target site mutations, which are resistance mechanisms, have been documented in numerous species, including *R.* (Boophilus) *microplus*. However, *R.*

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sanguineus remains inadequately understood (Dzemo *et al*., 2022). Consequently, there is a rapidly increasing global apprehension regarding the sustainability of chemical acaricides and a compelling necessity to create alternative tick management strategies that are economically viable, environmentally friendly, and effective.

The presence of naturally occurring bioactive compounds, known as phytochemicals, has led researchers to focus on plant-based alternatives as a response to these concerns. These alternatives offer encouraging acaricidal potential (Abdel-Ghany *et al*., 2024). These plant-derived substances are recognized for their ability to disrupt the life cycle and physiology of arthropods without causing damage to non-target organisms, such as humans and pets (Matos *et al*., 2023). Malak *et al*. (2024) underscored the significance of recognizing botanical acaricides as ecologically benign alternatives that mitigate the disadvantages of synthetic chemicals. *A. indica* and *C. papaya* are distinguished among the plants investigated for their acaricidal effects by their documented insecticidal properties and diverse phytochemical composition. Neem, a member of the Meliaceae family, contains azadirachtin, a potent compound that functioned as a feeding deterrent, growth regulator, and oviposition inhibitor for over 200 arthropod pests (Gareh *et al*., 2022). On the other hand, papaya is also acknowledged for its pesticidal and larvicidal properties, which have been demonstrated in numerous in vitro studies that target tick (Dhabale *et al*., 2025).

The brown dog tick (*R. sanguineus*) is a particularly significant target due to its broad host range and distinctive behavior. It is frequently observed infesting domesticated canines on a global scale, but it is also capable of feeding on humans and wild animals (López-López *et al*., 2023). *R. sanguineus* can serve as a vector of a variety of pathogens, such as *Rickettsia conorii*, *Ehrlichia canis*, *Brucella canis*, and Leishmania spp., which can cause a variety of zoonotic and animal diseases, despite the fact that its bite is typically asymptomatic and frequently going undetected (Zahri *et al*., 2025). Additional epidemiological significance is conferred by the tick's capacity to transmit pathogens through both transovarial and transstadial routes (Van Wyk *et al*., 2023). In addition to treating tick infestations on the host, the current challenge is to address environmental reservoirs where ticks can flourish and continue their life cycle (Zając *et al*., 2023). This emphasizes the necessity of implementing integrated control strategies that are effective in both the animal and the adjacent environment (Lagunes-Quintanilla *et al*., 2024).

The acaricidal potential of plant-based products has been emphasized in a variety of studies over the years. For example, Corpuz *et al*. (2025) demonstrated that both *A. indica* and *C. papaya* extracts exhibit acaricidal properties against *R. sanguineus* in vitro. The efficacy of the combination of the two extracts was further enhanced. Their results established the foundation for subsequent research that aimed to enhance the delivery method of these extracts. Anholeto *et al*. (2024) investigated the acaricidal properties of essential oils from lemongrass (*Cymbopogon citratus*), geranium (*Pelargonium asperum*), savory thyme (*Thymus saturejoides*), and white thyme (*Thymus zygis*) against *Ixodes scapularis*. They further explored the nature of interactions—synergistic, antagonistic, or additive—among binary mixtures of these oils. These discoveries not only advocate for the inclusion of botanicals in acaricidal formulations but also indicate that the combination of extracts could enhance the acaricidal effect, providing an alternative to synthetic products.

The integration of botanical constituents into consumer products, such as soaps, has experienced a surge in popularity in recent years. In particular, herbal soaps are becoming more popular due to their safety, user-friendliness, and compatibility with the epidermis of both humans and animals (Kouam *et al*., 2015; Nyahangare *et al*., 2019). The hot process is the most effective method for producing transparent or translucent soaps with excellent cleansing power, good moisturizing properties, long-lasting fragrance, and reduced irritant effects. They are produced by integrating plant oils or extracts into soap bases by employing a variety of methods (Hidayat *et al*., 2022). The functional and therapeutic properties of soap products are improved by herbal constituents, while adverse effects are minimized, as emphasized by Bronaugh and Maibach (2020). Thus, the advancement of herbal-based acaricidal soap offers a novel approach that not only regulates tick infestations but also enhances compliance by integrating treatment with standard sanitation practices.

This study sought to develop and assess an innovative acaricidal soap that incorporates combined leaf extracts of *A. indica* and *C. papaya*, in accordance with the principles of research and development, which entail systematic exploration to generate new products, processes, or services (Sarpong *et al*., 2022). The importance of this product is its capacity to provide a tick control solution that is sustainable, eco-friendly, effective, and low-cost. This solution can be readily implemented during routine pet grooming. The investigation aimed to resolve the obstacles of acaricide resistance, environmental toxicity, and economic constraints that are associated with conventional tick control methods through the use of naturally derived compounds.

The objectives of this study were as follows:

a) To create an acaricidal soap formulation that incorporates the combined leaf extracts of *A. indica* and *C. papaya*
b) To evaluate the physicochemical properties of the formulated soap, including color, odor, appearance, pH, moisture content, foam height, foam retention,

total alkali content, alcohol-insoluble matter, and total fatty matter;

c) to compare the acaricidal efficacy of the developed soap against *R. sanguineus* by comparing it to a control herbal soap and measuring tick mortality at three-hour intervals over a 15-hour period
d) To determine the median lethal dose (LD_{50}) of the plant extracts used in the formulation

Materials and Methods

Prior to extraction, the fresh leaves of *C. papaya* and *A. indica* were collected, washed thoroughly with distilled water, and air-dried. The foliage extracts were prepared using 95% ethanol as solvent. *R. sanguineus* specimens were obtained from canines that were naturally infested and subsequently maintained in a controlled laboratory environment. To ensure uniform exposure throughout the spray application procedure, Petri dishes, forceps, filter paper, and spray bottles were used. The negative control was distilled water, while the positive control was a permethrin solution (Obaid *et al*., 2022; Davidson *et al*., 2024). These resources and techniques made it easier to systematically assess the acaricidal properties of *A. indica* and *C. papaya* leaf extracts.

Design of the Study

This study involved the production and assessment of an acaricidal soap generated from *A. indica* and *C. papaya* leaf extracts, utilizing an experimental research design and a bioassay-guided methodology (Pednekar *et al*., 2023).

The research was conducted in two primary phases:

(1) Formulation and development of the acaricidal soap
(2) Evaluation of its acaricidal efficacy

The central framework of the investigation was the bioassay-guided approach, which enabled the systematic evaluation and screening of plant extracts according to their biological activity against *R. sanguineus (*Gross *et al*., 2024). This method guaranteed that the final soap formulation contained only the most effective concentrations and combinations of extracts. Ethanol was employed as a solvent to collect, process, and extract fresh leaves of *A. indica* (Tavares *et al*., 2021) and *C. papaya* during the development phase (Nugraha *et al*., 2023; Taychaworaditsakul *et al*., 2024). Standard soap-making procedures were employed to create the best soap formulation based on the result of the acaricidal activity of the combined extracts (Nabilla, 2023), which is the first phase of the study (Corpuz *et al*., 2025).

At the assessment phase, the acaricidal activity of the soap that was formulated was evaluated using a Completely Randomized Design (CRD). The investigators conducted bioassays by exposing larval ticks to this soap formulation in controlled laboratory environments.

Mortality of the ticks was monitored at predetermined intervals and contrasted between treatment groups. The negative and positive controls were distilled water and permethrin solution, respectively.

The reliability and reproducibility of the findings were guaranteed by the implementation of statistical analysis to ascertain the significance of differences among regimens. The empirical evidence of the plant-based soap's acaricidal potential was provided by this design.

Plant Extraction

Ninety-five percent (95%) ethyl alcohol was employed as the solvent for plant extraction, utilizing the Soxhlet method. Prior to extraction, the fresh leaves of *C. papaya* and *A. indica* were air-dried and pulverized into a fine powder. A thimble containing approximately 100 grams of pulverized leaves was inserted into the Soxhlet apparatus. The phytochemical constituents were extracted thoroughly by maintaining a consistent temperature of 78–80°C (the boiling point of ethanol) for a total of 6 hours. The resulting extracts were filtered, concentrated under decreased pressure with a rotary evaporator, and kept in amber vials at 4°C until needed.

In order to guarantee controlled and standardized conditions, all procedures were implemented at a reference laboratory. The leaf extracts were returned to the College of Health Sciences Laboratory at the University of Northern Philippines for soap formulation post-extraction.

Acaricidal Soap Preparation

The acaricidal activities of the plant extracts were evaluated during Phase 1 of the investigation (Corpuz *et al*., 2025). The soap preparation and development process involved the combination of 39 g of oil constituents and 2 g of glycerin in a beaker. 6.5 mL of each of the 100 mg/mL leaf extracts from *A. indica* and *C. papaya* were mixed with 24 g. of NaOH separately. A uniform consistency was obtained by heating the oil to 44°C, pouring it into the beaker containing the extract-sodium hydroxide mixture, and blending it. The mixture was subsequently permitted to harden by being poured into molders. After molding, the soaps were subjected to a 14-day aging or curing period in a well-ventilated, sheltered area at ambient room temperature (approximately 25–28°C) to guarantee complete saponification and the appropriate consistency prior to physicochemical and acaricidal inspection (Nabilla, 2023).

Acaricidal Evaluation of the Prepared Soap (In Vitro Assay)

After the soap has aged, it was submitted back to the reference laboratory for the evaluation of its acaricidal activity and physicochemical characteristics (Sharma *et al*., 2022; Arhin *et al*., 2024). The commercially available herbal acaricidal soap, which contained Madre de cacao

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(*Gliricidia sepium*) leaf extract, was compared to the formulated soap for comparative analysis.

The ticks in their larval stage used for the acaricidal test were collected from native infested dogs. The harvesting was conducted manually, without regard to sex, and with meticulous care to prevent damage to their rostrum. Ticks were measured on a "Denver Instrument" scale with a capacity of 210 g and a sensitivity of 0.001 g. Their length was measured utilizing millimeter paper. The mean weight and length were 0.05 ± 0.01 g and 6.5 ± 0.04 mm, respectively.

Each bar soap had a weight of 65 g and contained 13 mL of the combined leaf extract (100 mg/mL), that is, a dose of 20 mg/g. This concentration was tested against the ticks using a base soap without extract as negative control and Madre de cacao herbal dog soap as positive control.

The tests involved assessing the acaricidal activity of the soap solution through direct contact with ticks. Using a pipette, 10 mL of the soap solution at a concentration of 100 mg/mL was evenly spread across petri plates with an area of 63.61 cm^2. Each treatment consisted of three repetitions, each containing 10 ticks placed in one of the aforementioned Petri dishes (Kouam *et al*., 2015).

The counting of dead ticks was conducted every three hours. The death rate for each dish was determined using the methodology provided by Miegoué *et al*. (2014):

Mortality rate = (Number of deceased ticks) × 100 / (Total number of ticks).

Physicochemical Assessment of the Soap (Gana et al., 2019; Devipriya Nisha, 2021; Sharma et al., 2022)

A. Determination of the Physical Characteristics: The physical characteristics of the acaricidal soap that were evaluated are the color, odor, and texture.
B. PH Determination: The pH value was assessed utilizing a pH meter.
C. Determination of Moisture Content: The moisture content was utilized to determine the percentage of water in the soap by drying it to a constant weight. The soap was measured and documented as the "wet weight of sample" and subsequently dried at temperatures ranging from 100 to 115°C using a drier. The sample was chilled and weighed to get the "dry weight of the sample." The moisture content was calculated with the formula:

$$\text{Moisture content in \%} = \frac{\text{Initial weight }(100)}{\text{Final weight}}$$

D. Determination of Foam Height: Approximately 0.5 grams of the soap sample was distributed in 25 mL of distilled water. The solution was subsequently transferred to a 100 mL graduated cylinder, and the volume was adjusted to 50 mL using pure water. Twenty-five (25) strokes were administered and permitted to remain until the aqueous volume reached 50 mL, at which point the foam height above the aqueous fluid was recorded.
E. Retention of Foam: A 1% soap solution was made, and 25 mL of this solution was transferred to a 100 mL measuring cylinder. The cylinder was sealed with a hand and agitated for 10 minutes. The foam volume was monitored at 1-minute intervals throughout a duration of 4 minutes.
F. Alcohol-Insoluble Matter: Five grams of the material were placed in a conical flask. Subsequently, 50 mL of warm ethanol was introduced and agitated briskly until the material was entirely dissolved. The solution was passed through a tared filter paper with 20 mL of warm ethanol and subsequently dried at 105°C for one hour. The mass of the desiccated paper was recorded.
G. Total Fatty Matter (TFM): The Total Fatty Matter (TFM) is determined by reacting soap with acid in hot water and quantifying the resultant fatty acids. Ten (10) grams of the manufactured soap were dissolved in 150 mL of distilled water and subsequently heated. Subsequently, 20 mL of 15% sulfuric acid was introduced while heating until a transparent solution was achieved. Fatty acids present on the surface of the resultant solution are saponified by the addition of 7 grams of beeswax, followed by reheating. Subsequently, it was permitted to solidify. The cake was extracted, wiped to remove moisture, and weighed to calculate the Total Fat Matter (TFM) using the formula (Das *et al*., 2024):

$$\%\text{TFM} = \frac{(\text{weight of the cake} - \text{weight of the wax in g})\text{ X }100}{\text{weight of the soap in g}}$$

H. Total Alkali Content: The total alkali concentration was determined by titrating the surplus acid in the aqueous phase with a standard NaOH solution. Five milliliters of ethanol were added to one gram of the prepared soap. The mixture was heated until liquefaction occurred, after which 0.5 mL of 1M H2SO4 was incorporated and stirred thoroughly. The solution was titrated with 1.0M NaOH with phenolphthalein as the indicator. Total alkali was determined utilizing the formula (Oyaro and Makokha, 2014):

$$\% \text{ alkali } = \frac{(\text{VA } - \text{ VB})\text{X } 3.1}{\text{Weight}}$$

Where:

VA = is the amount before titration
VB = is the amount after titration

LD_{50} Determination

Twenty-one (21) Wistar rats were allocated into seven groups, each comprising three rats. They were provided with normal feed and given access to water ad libitum. The groups received graded doses of mixed *A. indica* and *C. papaya* leaf extracts (200, 400, 800, 1600, 2600, and 3600 mg/kg) subcutaneously, while the seventh group (control group) was administered distilled water.

Wistar rats were monitored for indicators of acute toxicity, including behavioral alterations and mortality, over a 72-hour period to determine the LD_{50} (median fatal dose). Autopsies were conducted to identify any significant pathological alterations. The maximum tolerable dose of the extract was ascertained. The LD_{50} was determined utilizing the formula (Timothy, 2022; Ramalho *et al.*, 2023):

$$LD_{50} = \text{HD} - (\text{Dd}) \ / \ \text{n}$$

Where:

n = Total number of animals in group
HD = The highest dose where death of laboratory animals was recorded
Dd = Dose difference x mean dead

Statistical Treatment of Data

The efficacy of the formulated plant-based detergent against *R. sanguineus* was determined by analyzing the data collected from the acaricidal bioassays using appropriate statistical tools. To identify statistically significant differences between means, mortality of the ticks were compared across treatment groups using one-way Analysis of Variance (ANOVA) and Tukey HSD post-hoc test. Mortality was expressed as mean ± Standard Deviation (SD). A significance level of $P < 0.05$ was utilized for the entire study.

The sample size was determined using standard protocols employed in comparable acaricidal studies, with ten ticks per replicate, resulting in a total of 30 ticks per treatment group. Each treatment was replicated three times. In order to evaluate the precision of the mortality estimates and gain a more comprehensive understanding of the potential efficacy of the detergent formulations, confidence intervals at the 95% level were computed.

The LD_{50} (median lethal dose) was determined during the acute toxicity phase using the arithmetic method of Timothy (2022) and Ramalho (2023).

Confidence intervals were calculated based on the dose difference and mortality distribution. To guarantee the reliability, reproducibility, and rigorous comparison of experimental outcomes, all statistical computations were conducted using Microsoft Excel 2016. In order to mitigate bias, randomization was implemented during the bioassay trials, and all mortality assessments were conducted in a blinded manner to guarantee objectivity.

Results and Discussion

Formulation and Preparation of the Acaricidal Soap

The acaricidal soap was created by modifying the cold saponification method, which was adapted from Nabilla (2023). In order to enhance user acceptability, the raw material quantities were adjusted, and a colorant and fragrance were incorporated. The final formulation's composition is illustrated in Table 1.

The oils (olive, coconut, and palm) were combined and gently heated. Subsequently, a sodium hydroxide solution was infused with a homogenized extract of *A. indica* and *C. papaya* leaves. The mixture was agitated until it was uniform, molded, and allowed to cure for two weeks. This curing procedure, which is a standard in cold-processed soaps, enhances the bar's hardness, longevity, and safety for topical use, as well as stabilizes the pH.

Physicochemical Evaluation of the Soap Developed

Table 2 provides a summary of the soap's physicochemical parameters, which are presented in its ultimate form in Fig. 1. The natural colorants and essential oils in the leaf extracts were responsible for the soap's solid and smooth texture, mildly pleasant odor, pH, greenish-brown color. Consumer standards were met by the bar's weight of 65 ± 2 grams.

Table 1: Formulation of the Acaricidal Soap

Ingredients	Quantity
Olive oil	15 g
Coconut oil	15 g
Palm oil	9 g
Glycerine	2 g
Sodium hydroxide	24 g
Combined *A. indica* – *C. papaya* leaf extracts (100 mg/mL)	13 mL
Colorant	5 gtts
Fragrance	15 gtts

The pH of the developed soap was 7.9, just below the standard range of 8–10 established by the National Agency for Food and Drug Administration and Control

(NAFDAC). However, it is still within acceptable limits for cutaneous application. Hashim *et al*. (2024) reported comparable results in their neem-based soaps, where a lower pH indicated a reduced propensity for skin irritation. This is consistent with the product's safety profile, particularly in the context of sensitive epidermis in humans and animals.

Table 2: Physicochemical Characteristics of the Soap

Parameters	Test Results	Control Results*
Color	Greenish-brown	Green
Weight	65 grams (+/-2)	85 grams as packed
Odor	Slightly pleasant	Pleasant smell (fragrant)
Texture	Solid and smooth	Solid and smooth
Appearance	Clear and transparent	Clear
Ph	7.9	7.87
Foam Height/Foaming Index	14.2 cm	20 cm
Foam Retention	5.2 cm	7.5 cm
Alcohol Insoluble Matter	9.0	21.0
Moisture Content	20%	15%
Total Fatty Matter	29%	36%
Total Alkali Content	0.20%	0.84%

**Control – Madre de cacao acaricidal dog soap purchased from a local store*

Fig. 1. *A. indica – C. papaya* bar soap developed by the researchers

The foam retention (5.2 centimeters) and foaming index (14.2 cm) were both lower than those of the commercial control soap. Although these values may not directly influence acaricidal activity, consumer preference frequently favors products with high frothing levels (Bhujbal *et al*., 2023). The Alcohol Insoluble Matter (AIM) content was 9.0%, which was substantially lower than the control. This indicates that the ingredient was well-solubilized and that there was minimal residue formation following its use.

The detergent has a moisture content of 20%, which is higher than that of the control (15%). This discovery is in accordance with the observations of Das *et al*. (2024), who observed that detergents that are abundant in plant extracts have a higher moisture retention rate as a result of the hygroscopic properties of specific phytochemicals. Nevertheless, this may result in a decrease in the shelf life of the product, as the hydrolysis of unsaponified lipids into free fatty acids and glycerol is facilitated by excess water.

Although the Total Fatty Matter (TFM) content was 29%, it was still comparable to that of other herbal soaps, despite being below the optimal benchmark of 30%. Owoicho (2021) suggest that residual unreacted sodium hydroxide may contribute to low TFM values. However, the saponification ratio could be refined to ameliorate this issue. The soap's alkali content was low at 0.20%, a positive indicator for product safety and mildness (Issa, *et al*., 2020). The soap is suitable for regular use on animals due to its low alkali content, which reduces the risk of dermal irritation.

Acaricidal Activity of the Developed Soap

The flood method was employed to evaluate the acaricidal efficacy of the detergent that was developed by Nyahangare (2019), with tick mortality being monitored at 3-hour intervals. Mortality commenced at 30% between the 7th and 9th hours, as illustrated in Table 3, and reached 100% by the 15th hour. This was significantly more rapid than the control soap (Madre de cacao-based) (Olorvida *et al*. 2013), which exhibited effects between the 10th and 12th hour and reached full mortality by the 15th hour. This implies that the detergent that has been developed has a more rapid onset of acaricidal action.

Table 3: Acaricidal Activity of the Developed Soap

Treatment	Initial Count (Ticks)	Mean Dead Ticks Within the 15-Hour Observation Period						Dead Ticks at a Maximum Time	
		3	6	9	12	15	% Mortality within 15 hrs.	Maximum Duration	% Mortality
Negative Control (Soap prepared without the extract)	10	0	0	0	0	0	0%	74 hrs	100%
Azarirachta indica – Carica papaya Extract Soap Preparation (20mg/g)	10	0	0	3	4.3	2.7	100%	15 hrs	100%
*Commercial soap (Madre de Cacao Herbal Dog Soap)	10	0	0	0	4.3	5.7	100%	15 hrs	100%

These findings align with prior research demonstrating the efficacy of *A. indica* and *C. papaya* leaf extracts in treating ectoparasites. Mohammed *et al*. (2022) documented analogous results when utilizing neem-based formulations. They attributed mortality to the phytochemicals present in the extracts, including alkaloids, flavonoids, saponins, tannins, glycosides, and phenolic compounds. These bioactive agents are recognized for their ability to disrupt cellular membranes, induce vacuolation, and interfere with neurotransmission, resulting in neuromuscular failure and mortality in arthropods.

Cardoso *et al*. (2020) reported that plant-derived compounds inhibit acetylcholinesterase activity, which leads to the accumulation of acetylcholine in the synaptic cleft. The mechanism of action observed in this study is consistent with their findings. This results in convulsions, paralysis, and death as a result of excessive neural stimulation. This neurotoxic mode of action is further substantiated by the altered morphology of deceased ticks and the impaired movement of Malpighian tubules in this study.

The synergistic application of *A. indica* and *C. papaya* — a combination that has been minimally investigated in the existing literature - is what distinguishes this study. Although each plant has demonstrated moderate to strong acaricidal activity separately, their combined use may have an additive or even synergistic effect, thereby increasing efficacy and reducing resistance potential (Corpuz *et al*., 2025).

Median Lethal Dose (LD_{50}) of the Leaf Extract Used in Developing the Acaricidal Soap

The LD50, or Median Lethal Dose, is the quantity of a substance administered simultaneously that results in the mortality of 50% of the subjects.

The LD_{50} evaluation of the leaf extract utilized in the development of the product was conducted in a reference laboratory since the researchers were not capable of performing the test. Table 4 presents the LD_{50} result of the leaf extracts utilized in developing the acaricidal soap in this study.

The toxicity assessment revealed that laboratory animals receiving doses of the extract at 200, 400, and 800 mg/kg body weight displayed no significant signs of toxicity or mortality.

These results indicate that the dosage levels of the test compound were well tolerated by the Wistar rats. Conversely, the test animals' deaths were precipitated by the administration of higher doses—2,600 and 3,600 mg/kg - which induced severe toxic effects as observed in Table 5. Physiological distress and behavioral changes were among the observable symptoms of significant toxicity, which suggested systemic toxicity. These effects emphasize the point at which the compound's safety margin is exceeded.

The Maximum Tolerated Dose (MTD) is 1,600 mg/kg, which is the greatest dose at which no mortality or severe toxic effects were observed, according to the observed results in Table 5. This benchmark is essential in toxicological studies, particularly for the purpose of establishing safe dose ranges for future pharmacological or therapeutic evaluations Van Berlo *et al*. (2022).

In addition, the median lethal dose (LD_{50}) was determined to be 2,933 mg/kg body weight, which represents the dose at which 50% of the test population succumbed to the substances. Substances with an LD_{50} value exceeding 1,000 mg/kg are regarded as relatively safe by Adler-Flindt & Martin (2019) suggesting a significant difference between the therapeutic and toxic concentrations.

In addition, this evaluation is consistent with the classification of the World Health Organization, which regards natural product extracts with LD_{50} values exceeding 2,000 mg/kg as essentially safe for use (Muñoz *et al*., 2021). Consequently, the test extract can be classified as having a favorable safety profile and minimal acute toxicity, as indicated by an LD_{50} of 2,933 mg/kg.

Table 4: Median Lethal Dose (LD_{50}) of the Combined *A. indica* – *C. papaya* Leaf Extracts in Wistar Rats Using the Karber Method

Dose (mg/kg)	Dose Difference	No. of Dead Rats (n)	Mean Dead	Dose Difference x Mean Dead
200	0	0	0	0
400	200	0	0	0
800	400	0	0	0
1,600	800	0	0	0
2,600	1,000	3	1	1,000
3,600	1,000	3	1	1,000
Dist. Water (control)	0	0	0	0

LD 50 = 3,600 – (2,000 / 3) = 2,933 mg/kg body weight

The findings corroborate the assertion that the extract is relatively non-toxic and safe for further pharmacological or therapeutic exploration under the tested conditions, particularly when administered within or below the maximum tolerated dose of 1,600 mg/kg.

The results of this investigation are largely consistent with the existing literature, but they also present numerous improvements. Initially, the soap's superior efficacy in comparison to Madre de cacao-based products is indicated by its quicker tick mortality. Secondly, the integration of two phytochemical-rich plant extracts - *A. indica* and *C. papaya* – is a novel approach that has not been extensively tested. These plants were typically examined in isolation in previous investigations.

Table 5: Mortality and Behavioral Signs in Wistar Rats Injected with *Azadirachta indica* – *Carica papaya* Leaf Extract

Group	Dose (mg/kg)	Route	Sign/s of Toxicity	Mortality
1	200	*SC	No signs of toxicity were observed.	No death was recorded.
2	400	SC	No signs of toxicity were observed.	No death was recorded.
3	800	SC	No signs of toxicity were observed.	No death was recorded.
4	1,600	SC	Rats became over sleepy after 4 hours, and had no appetite for food and water but became active and regained appetite in 12 hours.	No death was recorded.
5	2,600	SC	Rats became over sleepy in 3 hours, with eyes slightly closed, rested for almost three hours, experienced a diminished appetite, and exhibited partial paralysis of the forelimbs.	All died after 72 hours.
6	3,600	SC	Rats became over sleepy within 3 hours with eyes closed, rested for almost three hours, experienced a diminished appetite, and exhibited partial paralysis of the forelimbs.	All died between 48 and 72 hours.
7	Dist. water	SC	No toxicity.	No death.

**SC = Subcutaneous*

The ecological implications of this endeavor are substantial. Synthetic acaricides have been associated with environmental toxicity, which includes detrimental effects on non-target organisms and soil and water contamination (Sabová, *et al*., 2022). Sustainable agriculture and veterinary care are promoted by the utilization of plant-based soaps as biopesticides. The soap's use is ecologically friendly and reduces the development of resistance, which is a growing concern with chemical acaricides, due to its biodegradability and lack of synthetic residues.

Additionally, the utilization of locally sourced plant materials encourages community-based production and diminishes its dependence on costly, imported acaricides. This is consistent with the worldwide initiative to promote eco-safe alternatives and green veterinary products, as specified in the sustainable pest control frameworks of the FAO and WHO (2020).

Conclusion

In summary, the current investigation demonstrated that the soaps produced from the combined extracts of *A. indica* and *C. papaya* leaves exhibited significant acaricidal efficacy against *R. sanguineus* in vitro. The findings suggest that the herbal detergent has the potential to function as a plant-based, effective alternative to traditional tick control products. The soap that was developed was generally comparable to the commercial control soap in terms of its physicochemical properties, including color, odor, texture, pH, foaming index, and total alkali content. At the same time, there is still room for improvement in specific aspects of the product to increase its stability and user appeal. In addition, acute toxicity testing demonstrated that the median lethal dose (LD_{50}) of the acaricidal soap that was developed is 2,933 mg/kg body weight. This finding suggests that the soap is essentially safe for topical use in canines and suggests a low level of toxicity. The feasibility of further developing and potentially commercializing this plant-based detergent as a safe and eco-friendly option for tick management in companion animals is supported by these findings. In order to optimize its practical implementation, future research should incorporate in vivo efficacy trials, long-term safety evaluations, and formulation refinement.

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Author's Contributions

Alfredo Vita Corpuz: conceived the study, supervised and conducted all the experimental procedures, performed data analysis, contributed to the manuscript, and revised the manuscript in compliance with the reviewers' suggestions.

Carmela Montoya Florentino: participated in the experimental procedures, contributed to the analysis of data and writing of the manuscript.

Ace Danielle C. Avero: participated in all phases of the experimental procedures and analysis of data.

Ethics

This article is a unique piece of work. The corresponding author confirms that the manuscript has been reviewed and approved in its final form by all co-authors and that the study is free of any ethical conflict or violations. It underwent ethical review by the University of Northern Philippines – Ethics Review Committee with an Approval Number 2023-067.

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