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Rauvolfia Serpentina and *Peperomia Pellucida* as Antiparasitic Spray Against *Rhipicephalus Sanguineus Latreille*

[1]Mahmooda Aziza Bhatti, [2]Mutahir Saeed, [3]Marites Hugo & [4]Jerico Guevarra

Abstract

Rauwolfia serpentina (serpentina) and peperomia pellucida (pansit-pansitan) were used as the raw materials for creating different mixtures to determine which concentration is faster in exterminating Rhipicephalus sanguineus latrille (common ticks). The process used was decoction, the harvested extract was used for the creation of the spray mixtures. The process used three different preparations of mixture concentration with variants concentration ratio of serpentina/pansit-pansitan as: mixture 1 with 50%/50%; mixture 2 with 75%/25%, and mixture 3 with 25%/75%. The study recorded the testing times in exterminating the ticks as: mixture 1 had the results in 1.5 hours (sprayed once), 1.33 hours (sprayed twice), and 1.25 hours (sprayed thrice); mixture 2 had the results in 3.5 hours (sprayed once), 3.42 hours (sprayed twice), and 3.33 hours (sprayed thrice); and mixture 3 had the results in 1.5 hours (sprayed once), 1.42 hours (sprayed twice), and 1.33 hours (sprayed thrice). The experiment showed that mixture 1 and mixture 3 almost had the same results, if not for a few second differences, mixture 1 was always faster than mixture 3, while mixture 2 was left behind for a few hours. Therefore, mixture 1 is the best concentration to exterminate the ticks in a controlled environment.

Keywords: *Serpentina, Pansit-Pansitan, Ticks, Antiparasitic*

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About the authors:
[1]Corresponding author. An alumna of City of San Jose del Monte National Science High School and currently taking up Bachelor of secondary Education Major in English Minor in Mandarin at Bulacan State University. Corresponding email: azizahbhatti@gmail.com
[2]Graduate of Biology, Naturopathy Health Science, and Alternative Medicine. Worked as an Associate Researcher under the Department of Health on Herbal and Natural Medicine.
[3]Teacher 3 at City of San Jose del Monte National Science High School and Junior High School department coordinator. Currently pursuing her Masters degree in Educational Managaement at Polytechinic University of the Philippines.
[4]Special Science Teacher I at City of San Jose del Monte National Science School and currently the Senior High School research coordinator. Pursuing Masters Degree in Education Major in Chemistry at Bulacan State University.

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1. Introduction

Rauwolfia serpentina has been used since the pre-vedic period to treat many infections and diseases. It is a large glabrous herb or shrub belonging to the family Apocynaceae and found in Assam, Pegu, the Himalayas, Java, Tenasserim, Deccan, Peninsula, Bihar, and the Malay Peninsula. It is a source of many phytoconstituents including alkaloids, carbohydrates, flavonoids, glycosides, phlorotannins, phenols, resins, saponins sterols, tannins, and terpenes (Chauhan et al., 2017). In a study by members of the Department of Chemistry at HNB Garhwal University, they used the roots of Rauwolfia Serpentina against Salmonella Typhimurium, Escherichia Coli (E-Coli), Citrobacter freundii, Proteus Vulgaris, Enterococcus faecalis, and Staphylococcus Aureus. The researchers have commented that “the research supports folklore” (Negi et al., 2014). On the other hand, pansit-pansitan, scientifically known as peperomia pellucida, is a medicinal plant with anti-gout properties. Its anti-gout properties help lower the uric acid amount in the blood. It was one of the ten medicinal plants which underwent clinical testing as per orders of the Department of Health (DOH).

A study by Akinnibosun et al. (2021) on the antibacterial activity of pansit-pansitan against three-gram negative bacterial isolates showed that the plant indeed has antibacterial properties. The plant undergoes a decoction process, and its extract was added to solvents, namely water, and ethanol. The results varied with the two solvents, wherein peperomia pellucida with ethanol is much more effective than peperomia pellucida with water. While the study focused only on bacteria and viruses using pansit-pansitan and serpentina separately, this study investigated the possibility of mixing the two (2) herbal medicines and finding out if they have powerful and effective effects in exterminating ticks specifically the brown ticks. Brown ticks, scientifically referred to as rhipicephalus sanguineus latrille, is a common tick found almost in any dog. This type of tick is usually reddish-brown in their adulthood, with no specific marks, unlike other species. Brown dog ticks often travel into houses on canines, their preferred hosts, or cats. Because they are found deep within the hair of animals, homeowners may not immediately see them. Adult ticks typically embed themselves to a dog’s ears and between its toes, while larvae and nymphs typically attach to the back of the dog. If brown dog ticks do not have a host to feed upon, they will readily seek out humans for them to survive. The pests attach themselves to an animal's skin to feed on its blood and lay eggs in its fur. After entering homes, they breed and can spread onto residents and other pets (Orkin, 2021).

This study aims to concoct a substance made from serpentina and pansit pansitan that exterminates the brown ticks among the animals. This uses variations of solutions and durations of tick exposure to the mixture. Although these plants have antibacterial properties and ticks are not bacteria, the study used these plants to remove ticks found on domestic cats and dogs, to provide alternative herbal antiparasitic spray without harmful chemicals thus lessening the environmental hazard and health hazard for both animals and human.

2. Literature review

2.1. Serpentina

The existence of enormous therapeutic properties makes Rauvolfia serpentina an essential medicinal plant in the pharmaceutical world (Khurshid et al., 2022). Because of the inclusion of alkaloids, carbohydrates, flavonoids, glycosides, phlobatannins, phenols, resins, saponins, sterols, tannins, and terpenes, the plant is used to treat a variety of ailments (Malviya & Sason, 2016). High blood pressure (Lobay, 2015), emotional agitation, epilepsy, traumas, anxiety, excitement, hysteria, sedative insomnia, and insanity (Ali et al., 2022) have all been treated with plant bits, seeds, and rhizomes for centuries in Ayurvedic medicine (Khurshid et al., 2022).

Rauwolfia Serpentina is noted to have reserpine, a substance that is used to cure hypertension (Lobay, 2015). In a study by the Department of Chemistry at HNB Garhwal University, they used the roots of Rauwolfia Serpentina against Salmonella Typhimurium, Escherichia Coli (E-Coli), Citrobacter freundii, Proteus vulgaris, Enterococcus faecalis, and Staphylococcus Aureus to quantify the reserpine content of the plant and test the antimicrobial effectiveness of its menthol extract. The menthol extract of Serpentina inhibited the growth of several bacterial species and concluded that the study supports the folklore claims of the plant species (Negi et al., 2014). Rauwolfia serpentina has been used since the pre-vedic period for the treatment of a lot of infect more than 50 distinct alkaloids in the plant. Ajmaline, ajmalicine, ajmalimine, deserpidine, indobine, indobinine, reserpine, reserpiline, rescinnamine, serpentine, and yohimbine are the most important alkaloids.

Antimicrobial, antifungal, anti-inflammatory, antiproliferative, antidiuretic, and anticholinergic properties are also known for serpentina. Because of its societal acceptability, greater compliance with the human body, and fewer side effects, herbal medicine is now the source of primary health care for 75–80 percent of the world's population (Ekor, 2014; Welz et al., 2018). As a result, naturally occurring remedies are now alternatives to save millions of patients around the world. The current study attempts to examine the different pharmacological, phytochemical, and medicinal effects of serpentina as a result of both of these properties (Kumari et al., 2013).

In a study, Alshahrani et al. (2021) explored the antibacterial activity of R. serpentina. Ethanolic extract of root was evaluated using the well-diffusion method. Two Gram-positive (Bacillus subtilis and Staphylococcus) and three gram-negative bacteria (Klebsiella pneumoniae, Pseudomonas aeruginosa, and Salmonella typhimurium) were used for the activity of which only three bacteria Klebsiella pneumonia, Staphylococcus, and B. subtilis bacteria are found susceptible. Similarly, Negi et al. (2014) studied the antibacterial activity of methanolic extract of roots (MREt) of R. serpentina. Antibacterial activity was evaluated using the agar well diffusion method against gram-positive and gram-negative bacteria for the determination of minimum inhibitory concentration (MIC) and the diameter of the zone of inhibition (ZOI). The study revealed that Staphylococcus aureus shows the highest ZOI (13 mm) with the lowest MIC (625 µg) and Escherichia coli possess the highest MIC (10 mg), whereas Proteus vulgaris was observed resistant to tested extracts up to 10 mg. Hence, R. serpentina exhibited strong antibacterial activity. Nigussie et al. (2021) used methanolic and chloroform extracts of the leaf and root of R. serpentina for antibacterial activity. The activity was assessed against S. aureus, E. coli, P. aeruginosa, B. subtilis, and K. pneumonia by disk diffusion method. 50 µl/ml concentrations of leaf and root chloroform extracts showed no ZOI against S. aureus and B. subtilis. Maximum zone inhibition was observed at 15.0 mm and 15.5 mm against E. coli for leaf and root extract, respectively. 100 µl/ml concentration showed maximum zone inhibition against all test organisms for both leaf and root extract. All the bacteria were more susceptible to methanolic extract than chloroform ((Chauhan et al., 2017).

Azmi and Qureshi (2012) determined the phytochemistry and effect of Rauwolfia serpentina methanolic root extract (MREt) on diabetic alloxan-induced male mice. Mice were categorized into diabetic (distilled water at 1 mL/kg), negative (0.05 percent dimethyl sulfoxide at 1 mL/kg), positive (glibenclamide at 5 mg/kg) controls, and three test classes (MREt at 10, 30, and 60 mg/kg). For 14 days, all medications were given orally. MREt included alkaloids, carbohydrates, flavonoids, glycosides, cardiac glycosides, phlobatannins, resins, saponins, hormones, tannins, and triterpenoids qualitatively, while the extract contained complete phenols quantitatively. Root powder was also tested for flavonoids, saponins, and alkaloids. When opposed to diabetic treatment, MREt was shown to be successful in increasing body weights, glucose and insulin levels, insulin/glucose ratio, glycosylated and total hemoglobin in research groups. Total cholesterol, triglycerides, low-density lipoprotein (LDL-c), and relatively low-density lipoprotein (VLDL-c) cholesterol levels were all found to be significantly lower in the test groups. Both research groups' liver tissues showed significant lipolysis and increased glycogenesis. All of the groups had standard ALT levels. In alloxan-induced diabetic mice, MREt increases glycemic, antiatherogenic, coronary risk, and cardioprotective indices.

In another study, Santhosh et al. (2016) analyzed antibacterial activity and preliminary phytochemical screening of Endophytic Fugal Extract of Rauvolfia serpentine. Sarpgandha (Apocynaceae) is a common medicinal plant that is best known for its numerous phytochemicals. The key goal of this research was to see whether Rauvolfia serpentina L. had some antifungal action against Alternaria alternata, Aspergillus flavus, and Mucor rouxii, which are all phytopathogenic fungi. The antifungal function of aqueous extracts of the entire plant, stem, and roots of Rauvolfia serpentina L. was investigated using an agar well diffusion assay. Rauvolfia serpentina L. aqueous root extract had significantly greater antifungal efficacy than the other extracts tested against Alternaria alternata and Aspergillus flavus. Their research explicitly demonstrates Rauvolfia serpentina L.'s antifungal properties, implying that it may be used to combat pest control in a variety of plants and animals. Endophytic fungi isolated from Rauvolfia serpentina, a well-known Indian medicinal herb, are used in Ayurveda for the treatment of many diseases (Santhosh et al., 2016). The antibacterial behavior of isolated endophytes against pathogenic bacteria was tested. Twenty fungal isolates were recovered from various sections of the host plant and characterized for their morphological features using Scanning Electron Microscopy (SEM). They were classified into eight genera based on observations: Fusarium sp., Phomopsis sp., Colletotrichum sp., Cladosporium sp., Aspergillus sp., Xylaria sp., Alterneria sp., and Gleomastix sp. The examination of the extract against the target bacteria exposed the secret of the medicinal plant's fungal endophytes. Colletotrichum sp. (Rs-R5), Fusarium sp. (Rs-R1), (Rs-R7), and Cladosporium sp. (Rs-S4) extracts were shown to be selective against human pathogenic bacterial strains E. coli (ATCC 25922), Gram-negative bacteria, and S. aureus (ATCC 25323), Gram-positive bacteria. The most effective sample was an ethyl acetate extract of an active fungal isolate (Colletotrichum sp; Rs-R 5) against E. coli and S. aureus, with maximal inhibition zones of 16 mm and 14 mm and minimum MICs of 25 g/ml and 36.5 g/ml, respectively. They found eight endophytic fungal genera in R. serpentina, according to Santhosh et al., (2016): Fusarium sp., Alternaria sp., Phomopsis sp., Xylaria sp., Gleomastix sp., Aspergillus sp., Cladosporium sp., and Colletotrichum sp. Out of 20 fungal isolates tested, four showed antibacterial activity: Fusarium sp. (Rs-R1, Rs-S7), Cladosporium sp. (Rs-R5), and Colletotrichum sp. (Rs-R5). Using ethyl acetate extract, the inhibition zone and MIC were detected. Against E. coli, the maximal inhibition region (16 mm) and minimum MIC (25 g/ml) were observed. Secondary metabolites such as alkaloids, polyphenols, flavonoids, hormones, and saponins were present in abundance in the ethyl acetate sample.

Plant products are gaining popularity as bactericides and fungicides (Sharanabasappa et al., 2015) due to their systemic efficacy and low phototoxicity. Sharanabasappa et al. (2015) attempted the antibacterial and pharmacological effects of various Rauvolfia serpentina extracts since a significant number of plants are recognized for their antibacterial and antifungal function. They tested the antibacterial efficacy of various Rauvolfia serpentina extracts against E. coli, Klebsiella, Pseudomonas, and S. Aureus. The components used are as follows: Nutrient agar medium, sterile Petri dishes, 0.1–0.2ml pipettes, cultures, nutrient broth, and sterile test tubes containing a proven concentration of the extract of the solutions. The cup plate method was used in the experiments, with a dosage of 1 mg/ml. Water and chloroform extracts were the most effective against bacterial strains E. coli and S. aureus. E. coli and P. aeruginosa Klebsiella and the rest of the Pet ether and ethanol extracts were also effective against P. aeruginosa and S. aureus. Various Rauvolfia serpentina extracts were examined for antifungal activity among various extracts of the Pet. Ether and ethanol extracts were found to be effective against A. flavus and A. niger. The operation of niger and the remaining extracts was low to moderate. The antifungal efficacy of plant extracts was compared to that of normal antifungal drugs fluconazole by cup plate process, with A. Flavus and A. niger as the fungi chosen for this. Rauvolfia serpentina extracts were tested for antibacterial and antifungal function. Rauvolfia serpentina extracts were tested for antibacterial and antifungal function. The water and chloroform extracts were the most effective against the bacterial strains E.coli and P. klebsiella, as well as the remaining Pet. ether and ethanol extracts were also effective against P. aeruginosa and S. aureus. The Pet. Ether and Ethanol extracts had excellent antifungal activity against A. flavus and A. niger, while the other extracts had low to moderate antifungal activity (Sharanabasappa et al., 2015).

2.2. Pansit-Pansitan

Pansit-pansitan, scientifically known as peperomia pellucida, is a medicinal plant valued for its anti-gout properties which help lower uric acid in the blood. It was one of the ten clinically tested medicinal plants endorsed by the DOH. It was reported that pansit-pansitan contained high amounts of toxic metals like lead (Pb) and cadmium (Cd) surpassing the limits allowed by the World Health Organization (WHO). To regulate the uptake of nutrient elements in pansit-pansitan, the use of hydroponic culture through non-aerated Hoagland's solution was studied (De Guzman, 2000).

Peperomia pellucida (Linn.) is a piperaceae bush with a glossy or silvery appearance. This plant's ethnomedicinal applications include curing stomach pain, abscesses, inflammation, boils, colic, and exhaustion (Gomes et al., 2022). Abdulrazaq (2018) extracted from the air-dried leaves of P. pellucida the essential oils using a Clevenger apparatus and a hydro distillation process. The essential oil obtained was light yellow in color, had an unpleasant odor, and yielded 0.30 v/w. Elemol (9.32 percent), Neointermedeol (8.35 percent), 1H-3a,7-Methanoazulene- (5.60 percent), and Bicyclo [2.2.1] heptanes,2,2,3-trimethyl are the main constituents (5.08 percent). A large amount of the leaf oil was made up of sesquiterpenes and oxygenated sesquiterpenes (52.71 percent). The antimicrobial effects revealed that it has a Minimum Inhibition Concentration (MIC) on Pseudomonas aeruginosa and Bacillus subtilis at 0.01 percent oil concentration, with inhibition zones of 7.0 mm and 9.3 mm, respectively; however, MIC against Bacillus cereus was obtained at 0.1

percent oil concentration. Antifungal tests on Lasiodiplodia theobromae, Fusarium oxysporum, and Aspergillus tamari at percent levels that revealed the plant's essential oils had potent antifungal effects on all three fungi species. These findings suggested that the plant's essential oil could be used as an antimicrobial agent (Abdulrazaq, 2018).

The anticancer, antimicrobial, antioxidant, and chemical compositions of Peperomia pellucida leaf extract were also studied. In the study of Wei et al. (2011), the anticancer activity of P. pellucida leaf extract was determined using a colorimetric MTT (tetrazolium) assay against the human breast adenocarcinoma (MCF-7) cell line, and the plant extract's antimicrobial property was discovered using a two-fold broth microdilution system against 10 bacterial isolates. The plant extract's antioxidant activity was then determined using the DPPH radical scavenging process, and the chemical compositions were screened and classified using gas chromatography-mass spectrometry (GC-MS). The findings of this analysis revealed that P. pellucida leaf extract had anticancer properties, with an IC50 of 10.4±0.06 g/ml. The plant extract was found to inhibit the growth of Edwardsiella tarda, Escherichia coli, Flavobacterium sp., Pseudomonas aeruginosa, and Vibrio cholerae at 31.25 mg/l; Klebsiella sp., Aeromonas hydrophila, and Vibrio alginolyticus at 62.5 mg/l; and Salmonella sp. and Vibrio parahaemolyticus at 125 mg/l. The plant extract was observed to inhibit 30% of DPPH, a free radical, at a concentration of 0.625 ppt. The main compound in the plant extract was phytol (37.88%), followed by 2-Naphthalenol, decahydro- (26.20%), Hexadecanoic acid, methyl ester (18.31%), and 9,12 Octadecadienoic acid (Z, Z)-, methyl ester (9.12%). (17.61 percent). The results of this study showed that a methanol extract of P. pellucida leaf had a lot of potential as a medicinal drug, particularly in the treatment of breast cancer (Wei et al., 2011).

In the study of Bojo et al. (1995), fresh and air-dried Peperomia pellucida plants were subjected to a differential extraction method using three solvents: methanol-water (14:1), ethyl acetate, and hexane. The ethyl acetate extract yielded a strong antibacterial extract. A major fraction of the ethyl acetate extract was shown to have strong antibacterial efficacy against Staphylococcus aureus, Bacillus subtilis, and Pseudomonas aeruginosa that was more active than the penicillin norm, indicating its ability as a wide spectrum antibiotic. Meanwhile, the study of Apatas et al. (2020) aimed to see whether Pansit-pansitan (Peperomia pellucida Linn) aqueous leaf extract has anti-inflammatory properties in vitro.

Fresh leaves were gathered, air dried, and aqueous extracted before being prepared in various doses (200, 400, 600, 800, and 1000ug/mL). In-vitro anti-inflammatory activity was assessed using inhibitors of HRBC lysis and protein denaturation. The existence of secondary metabolites, which are believed to have anti-inflammatory properties, may be due to the aqueous extract's anti-inflammatory effect on the plant. However, these metabolites are not sufficient enough when used in low dosages.

2.3.Ticks

Rhipicephalus sanguineus is usually reddish brown in their adulthood, with no specific marks, unlike other species. Brown dog ticks often travel into houses on canines, their preferred hosts, or cats. Because they are found deep within the hair of animals, homeowners may not immediately see them. Adult ticks typically embed themselves in a dog’s ears and between its toes, while larvae and nymphs typically attach to the dog's back. If brown dog ticks do not have a preferred host to feed upon, they will readily seek out humans for their needed blood meals. The pests attach themselves to an animal's skin to feed on its blood and lay eggs in its fur. After entering homes, they breed and can spread to residents and other pets. Unlike other species, brown dog ticks can survive and complete their entire life cycle indoors. Warm temperatures help these pests develop and reproduce, causing infestations to spread quickly. Brown dog ticks may transmit canine-related diseases, such as canine ehrlichiosis and babesiosis. They are known transmitters of Rocky Mountain spotted fever to humans, but are not known to transmit Lyme disease. However, they are often mistaken for deer ticks, which are known carriers of Lyme disease (Orkin, 2021).

According to John et al. (2017), ticks are tiny crawling bugs in the spider family that feed by sucking blood from animals. They are second only to mosquitoes as vectors of human disease, both infectious and toxic. Infected ticks spread over a hundred diseases, some of which are fatal if undetected. They spread the spirochete (which multiplies in the insect's gut) with a subsequent bite to the next host. Among vector-borne diseases, the most common, Lyme disease, also known as the great mimicker, can present with rheumatoid arthritis, fibromyalgia, depression, attention deficit hyperactivity disorder, multiple sclerosis, chronic fatigue syndrome, cardiac manifestations, encephalitis, mental illness, name some of the many associations.

3. Methodology

This experimental study strictly followed scientific procedure. The series of steps were followed to generate accurate and substantial data collection.

1. Prepare and gather all materials that are required in the whole procedure of making the antibacterial spray.
2. Wash all the leaves with tap water.
3. Crush 20 leaves with a mechanical blender to get their extract.
4. Boil each 20 crushed leaves in separate pots with 400 ml water.
5. Stir the materials evenly for 30 seconds up to 1 minute.
6. Prepare 3 glass containers and label them from mixtures 1 to 3. Use the funnel and measuring cups to follow the percentages needed for different concentrations of the mixtures.

   Mixture 1: 200 ml of Serpentina and 200 ml of Pansit-pansitan Mixture

   Mixture 2: 300 ml of Serpentina and 100 ml of Pansit-pansitan Mixture.

   Mixture 3: 100 ml of Serpentina and 300 ml of Pansit-pansitan.

7. Stir the materials evenly for 30 seconds up to 1 minute.
8. Cool the mixture and transfer it to a spray bottle.
9. Prepare 9 plastic cups and fill them with 3 ticks each with the following label:

   Label 1 - Mixture 1 (spray once)

   Label 2 - Mixture 1 (spray twice)

   Label 3 - Mixture 1 (spray thrice)

   Label 4 - Mixture 2 (spray once)

   Label 5 - Mixture 2 (spray twice)

   Label 6 - Mixture 2 (spray thrice)

   Label 7 - Mixture 3 (spray once)

   Label 8 - Mixture 3 (spray twice)

Label 9 - Mixture 3 (spray thrice)

10. Every solution is sprayed in the labeled plastic cups of different concentration
11. After an hour from the first spray, record how many ticks were found dead.
12. This manner of checking is done until all the ticks are dead.

The duration of the experiment is as follows:

Week 1. The last days of week 1 will be fully devoted to the creation of the mixture. To be safe, the study used two (2) days for the preparation of the mixture.

Week 2. This is the period of experimentation and observation. Every hour, the researcher sprays the ticks in the petri dish with the mixture assigned to them. The experiment and observation are enclosed in a week.

The method of observation is descriptive, wherein the ticks were placed in a controlled environment and then exposed to the mixture of serpentina and pansit-pansitan in different amount of concentrations. It was observed for an hour and the observer took notes of all the changes that took place.

4. Findings and Discussion

This study experiments on Rauvolfia Serpentina (Serpentina) and Peperomia pellucida (Pansit-Pansitan) as an anti-parasitic spray against Rhipicephalus Sanguineus Latreille (Common Ticks). The first scientific query is the time required for the anti-parasitic spray treatment to suppress the ticks as shown in table 1.

Table 1

Testing results of the mixtures

	Mixture 1	**Mixture 2**	**Mixture 3**
Once	1hr 30mins (1.5 hrs)	3hrs 30mins (3.5 hrs)	1hr 30mins (1.5 hrs)
Twice	1hr 20mins (1.33 hrs)	3hrs 25mins (3.42 hrs)	1hr 25mins (1.42 hrs)
Thrice	1hr 15mins (1.25 hrs)	3hrs 20mins (3.33 hrs)	1hr 20mins (1.33 hrs)

The table shows the number of times that the mixture has been sprayed on the ticks in a controlled environment. It also shows the length of time that the ticks exterminate after the mixture has been sprayed with Mixture 1 with the shortest period in the three instances of spraying. The longest period of extermination is Mixture 2 with minimum of 3 hours 20 minutes in three sprays.

Table 2

The number of ticks that exterminate

Mixture	Mixture 1	Mixture 2	Mixture 3
Number of times that the mixture has been sprayed	3	3	3
Several ticks died	3	2	2

Table 2 shows the number of times that the mixture has been sprayed on the ticks. In a span of 1 (one) hour and 30 (thirty) minutes, mixture 1 killed 3 ticks and mixture 2 killed 2 ticks.

The experiment used three solutions or three variants of concentration. The three mixtures used are: mixture 1 has 50%/50% of Serpentina and Pansit-Pansitan diluted with water; mixture 2 has 75%/25% of Serpentina and Pansit-Pansitan diluted with water; and mixture 3 has 25%/75% of Serpentina and Pansit-Pansitan diluted with water. The study experimented on the appropriate mixture for extermination with results shown in table 3.

Table 3

Summary of testing results

Mixture 1			Mixture 2			Mixture 3		
Times sprayed	Number of Hours	Number of ticks died	Times sprayed	Number of Hours	Number of ticks died	Times it sprayed	Number of Hours	Number of ticks died
1	1.5	3	1	3.5	2	1	1.5	2
2	1.33	3	2	3.42	2	2	1.42	2
3	1.25	3	3	3.33	2	3	1.33	2
Average	**1.36**	**3**	**2**	**3.42**	**2**	**2**	**1.42**	**2**

Table 3 shows the summary of the testing of the three (3) mixtures of serpentina and pansit-pansitan in different concentrations spraying against the brown ticks in a controlled environment. For mixture 1, the average time in hours that the brown ticks have been exterminated is 1.36 hours in relation to the number of times the mixture is sprayed. The brown ticks that have been killed are 3 from one (1) to three (3) sprays of the mixture. In mixture 2, the average time in hours that the brown ticks have been exterminated is 3.42 hours and it only kills 2 ticks with the different number of times that the mixture is sprayed. Lastly, mixture 3 has an average of 1.42 hours in relation to the number of times that the mixture was sprayed on the brown ticks. It exterminated 2 ticks in each number of times that the mixture was sprayed.

Table 4

Summary Result of the Three (3) Mixtures

Groups	Count	Sum	Average	Variance
Mix1	3	6.66	2.22	0.04120
Mix2	3	1.75	0.58	0.00023
Mix3	3	4.24	1.41	0.00723

Table 4 shows the number of times that the mixtures are sprayed on the brown ticks and the average time, in an hour, that the mixture exterminated the ticks. It also shows the computed variance of each mixture.

Table 5

Result of One-Way ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4.018289	2	2.009144	123.8514	**0.0000132**	5.143253
Within Groups	0.097333	6	0.016222			
Total	4.115622	8				

** Significant difference among groups (P-value < 0.05)*

Table 5 shows the result of the one-way analysis of variance (ANOVA). It shows the degree of freedom (df) which are 2 for between groups and 6 for within the groups, then the calculated F value which is 123.8514 along with the P-value of 0.0000132 while the critical F value is 5.143253. For the p-value, it is 0.0000132 in comparison to the level of significant value which is 0.05 implying significant difference in the mixtures.

Table 6

t-test Results between Mixtures

	Between Mixtures 1 & 2		Between Mixtures 1 & 3	
	Mix1	**Mix2**	**Mix1**	**Mix3**
Mean	2.22	0.583333	2.22	1.413333
Variance	0.0412	0.000233	0.0412	0.007233
Observations	3	3	3	3
Hypothesized Mean Difference	0		0	
Df	2		3	
t Stat	13.92663		6.348667	
P(T<=t) one-tail	0.002558		0.003953	
t Critical one-tail	2.919986		2.353363	
P(T<=t) two-tail	0.005116		0.007906	
t Critical two-tail	4.302653		3.182446	

Table 6 shows the t-test between mixture 1 and mixture 2. The mean of mixture 1 is 2.22 while mixture 2 is 0.58333 the calculated t-value is 13.92663 against the critical t-value is 2.919986 for one-tail and 4.302653 for two-tail and the degree of freedom (df) value is 2. Meanwhile, the result of t-test between mixture 1 and mixture 3 shows the mean for mixture 1 is 2.22 and for mixture 3 it is 1.413333, the degree of freedom value is 3. The calculated t-value is 6.348667 while the critical t-value for one-tail is 2.353363 and for two-tail is 3.182446.

Table 6

The Average Number of Ticks that Died per Hour

Mix1	Mix2	Mix3
2.00	0.57	1.33
2.26	0.58	1.41
2.40	0.60	1.50

Table 6 shows the average number of ticks died per hour after the mixture have been sprayed on the brown ticks. Mixture 1 had exterminated an average of two (2) ticks for an hour followed by mixture 2 with less than 1 tick exterminated and mixture 3 with an average 1 tick per hour.

After all the testing and calculations, the most effective variation in concentration out of the 3 mixtures used against Rhipicephalus Sanguineus Latreille is mixture 1, which consists of 50%/50% of Serpentina and Pansit-Pansitan diluted with water. It showed the fastest effect in suppressing Rhipicephalus Sanguineus Latreille with the shortest amount of time needed. As shown in the result in table 6, mixture 1 has exterminated an average of 2 brown ticks compared to the other two mixtures which exterminated less than 1 tick for mixture 1 and 1.5 ticks for mixture 3.

The results have also been confirmed using the one-way ANOVA. In comparison to the level of significance value of 0.05, the calculated p-value is 0.0000132 which is less than 0.05 and means that there is a significant difference among the groups of mixture that have been used. This only signifies that the most effective concentration of the mixture is 50% pansit-pansitan and 50% serpentina.

Further confirmation of the result, the study used the t-test between mixture 1 and 2 which shows that mixture 1 is still the most effective concentration having the t-value of 13.92663 which is way up higher than the critical t-value of 2.919986 for one-tail and 4.302653 for two-tail. Thus, the result shows that there is a significant difference between mixture 1 and mixture 2. In comparing mixture 1 to mixture 3, another t-test have been done to prove that mixture 1 is the most effective concentration. The calculated t-value is 6.348667 which is also higher than the critical t-value having 2.353363 for one-tail and 3.182446 for two-tail. The p-value also confirms the result, having the following value: 0.003953 for one-tail and 0.007906 in comparison to a 0.05 level of significance.

The results further showed that the most effective application out of the 3 durations provided against Rhipicephalus Sanguineus Latreille is thrice per hour. It showed the shortest amount of time in suppressing the tick in all three mixtures used. The result of the experimentation showed that Rauvolfia Serpentina (Serpentina) and Peperomia Pellucida (Pansit-Pansitan) as an antiparasitic spray against Rhipicephalus Sanguineus Latreille

(Common Ticks) is effective.

5. Conclusion

This study proved that the mixture of pansit-pansitan and serpentina can really exterminate the brown ticks as a result of physical observation and statistical analysis. Statistically, the mixture that has the most effective concentration in exterminating the brown ticks is mixture 1 for it shows that it can exterminate a minimum of 2 brown ticks in an hour compared to the other concentration can only exterminate less than 1 tick and 1.5 ticks for mixture 2 and 3 respectively in an hour. Even though all mixtures are effective in exterminating ticks, mixture 2, which has a 75/25 ratio of Serpentina and Pansit-pansitan, is the slowest followed by the mixture 3. Therefore, mixture 1 is the fastest option in terms of effectiveness and rapidity in the extermination of ticks.

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