In Vitro Efficacy of *Carica papaya*, *Chromolaena odorata* and *Piper betle* Leaves Extracts against *Sarcoptes scabiei* var. *canis*

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Abstract

Increasing concern on the efficacy of antimicrobials has spurred a demand for research on natural-based alternatives. This study assessed the efficacy of Carica papaya, Chromolaena odorata and Piper betle leaves extracts against mites (Sarcoptes scabiei var. canis). Efficacy was defined as the percent reduction in the number of live mites after 6 h of exposure to the treatments. The study utilized 100% fresh crude extract (FCE), 50% fresh crude extract in water (FCW), 50% ethanolic extract reconstituted in 60% ethanol (EEE) and 50% ethanolic extract reconstituted in distilled water (EEW) of the plants for the in vitro assay against S. scabiei var. canis. All plants exhibited varying acaricidal efficacy at different extraction procedures. Their FCW, EEE and EEW showed excellent acaricidal efficacy with P. betle showing maximum efficacy within 30 min of exposure. Thus, the three plants are potential sources of natural-based products for the treatment of canine sarcoptic mange. Further pharmacological and in vivo studies are recommended to validate their acaricidal efficacy.

***Keywords**:* acaricide, *Carica papaya*, *Chromolaena odorata*, *Piper betle*, in vitro

1. Introduction

Sarcoptic mange is a parasitic condition in the skin caused by microscopic mites known as *Sarcoptes scabiei*. It is a ubiquitous parasite affecting more than 100 species of mammals (El-Aleem *et al*., 2015). It is highly contagious causing intense pruritus and disruption of dogs' aesthetic appearance. It also leads to debilitation and even causes death if left untreated. There are also

reports that this condition is potentially zoonotic to humans (Bandi and Saikumar, 2013).

Commercial acaricidal products are available in the market today. However, they are expensive, chemical-based – which could build resistance to target species – and potentially toxic that may cause deleterious effects on the environment (Viste *et al.,* 2013; Luo *et al.,* 2015). The use of natural products has been widely accepted and plays an important role in the discovery of approved therapeutic drugs derived from natural sources (Preeti *et al.,* 2015). However, the use of herbal medicine against mange has not been explained at length.

Carica papaya and *Piper betle* are common plants with a variety of domestic uses, while *Chromolaena odorata* is considered as a noxious weed (Aravind *et al.,* 2013; Ikewuchi *et al.,* 2013; Rekha *et al.,* 2014; Sripradha, 2014). The use of *C. papaya* (Basalingappa *et al*., 2018; Nandini *et al*., 2020; Srivastava and Singh, 2016), *P. betle* (Peddapalli, *et al*., 2020; Sakinah *et al*., 2020), and *C. odorata* (Bhuyan *et al*., 2019; Mugwedi, 2020) as medicinal plants have been well documented. They are traditionally used on both skin and systemic lesions in various preparations. These plants are also utilized as insecticide and acaricide. However, there is a dearth of evidence on its efficacy against sarcoptic mites.

Thus, this study aimed to develop a new and alternative effective treatment against canine mange by using the leaves extracts of *C. papaya*, *C. odorata*, and *P. betle* tested in vitro. This work is directed towards stimulating the development of natural-based acaricidal products with a major focus on their organic, economical, effective, safe and environment-friendly benefits. Moreover, the findings of this study will provide information to the community on the use of these selected herbal plants as an alternative natural medication against canine mange.

2. Methodology

2.1 Selection of Dogs

Dogs that were naturally infested with *S. scabiei* regardless of age, sex, breed and nutrition were selected in the study as sources of mites. The selected dogs

were not previously treated with any acaricidal products (e.g., amitraz). Sarcoptic mange positive dogs were confirmed through skin scrapings. The mange-positive dogs were housed during the duration of the study. Throughout the trial, these dogs were fed with commercial dog food and table food twice a day and provided with adequate water ad libitum. The pens were cleaned regularly to remove feces and urine.

This research was carried out in accordance with the Animal Welfare Law of the Philippines; it secured a permit from the Institutional Animal Care and Use Committee of Central Mindanao University under study protocol 2016-27B.

2.2 Collection of Mites

Mites (*S. scabiei* var. *canis*) were collected through skin scrapings. The skin scrapings were done once a day for a maximum of 10 sites per dog with an area of 2 cm^2 per skin scrape. The collected scab and crusts were placed in a clean and dry petri dish. Live adult mites collected from these skin scrapings were separated using a binocular stereomicroscope (Wild M3B, Heerbrugg, Switzerland) and teasing needle; they were then isolated in an untreated petri dish until ready for selection.

2.3 Selection of Mites

A total of 540 live adult mites were collected. The criteria for the selection of mites were based on the uniformity of motility and inspection of adult mites under a binocular stereo microscope. The movability of mites was ensured before they were transferred to the treated petri dish. The characteristics of the adult *Sarcoptes scabiei* var. *canis* were based on the descriptions of Taylor *et al.* (2015) and Taylor (2015).

2.4 Identification, Collection and Preparation of Plant Samples

The plants utilized were *C. papaya*, *C. odorata* and *P. betle*. Fresh, matured and insect-bite-free leaves were collected early in the morning before sunrise from Dologon, Maramag, Bukidnon, Philippines. The collected leaves, approximately 3 kg, were packed instantly in polyethylene bags to avoid decomposition of the bioactive compounds. The leaves were then rinsed with water to remove dirt and foreign material. The leaves intended for 100% fresh crude and 50% fresh crude extraction in water were air-dried for at least 1 h until the water was drained off from the leaves at room temperature. On the

other hand, the leaves for ethanolic extraction were air-dried at room temperature for seven days.

2.5 Preparation of 100% Fresh Crude Extracts

About 50 g of fresh leaves of each plant were cut into small pieces using a knife or scissor, processed using mortar and pestle, and then strained using a cheesecloth. The filter paper was then used to remove the remaining solid particles. The filtrates were mixed using a vortex mixer (Fisher Vortex-Genie 2, Scientific Industries, United States). The resulting filtrates were capped and stored in a refrigerator at 4 °C (Moyo and Masika, 2013).

2.6 Preparation of 50% Fresh Crude Extraction in Water of Leaves

The fresh leaves (50 g) of each plant were cut into small pieces using a knife or scissors. Distilled water (50 mL) was added to the leaves then processed using mortar and pestle. The extracts were strained using a cheesecloth. The filter paper was used to remove the remaining solid particles. Containers for the filtrates were capped and stored for about an hour until use.

2.7 Preparation of Ethanolic Extraction of Leaves

A total of 400 g dried leaves were powdered and suspended in 2,000 mL of 95% ethanol (1:5 ratio) for two days at room temperature. The resulting mixture was filtered using a cheesecloth; the filtrates were concentrated via rotary evaporation (Stuart RE300, Keison Products, United Kingdom). The final extracts were completely dried and placed in a tightly closed container until they were ready to use. One preparation used 60% ethanol solution for reconstitution to a 50% concentration, while the other preparations utilized distilled water.

2.8 Treatments

C. papaya, *C. odorata*, and *P. betle* extracts were prepared according to the following treatments: 100% fresh crude extract (FCE), 50% fresh crude extract in water (FCW), 50% ethanolic extract reconstituted with 60% ethanol solution (EEE) and 50% ethanolic extract reconstituted with distilled water (EEW). Amitraz in 0.025% concentration was used as a reference compound with the acaricidal property. The 60% ethanol solution was used as a negative control. Each treatment had three replicates.

2.9 Application of Treatments and Control

A total of 540 live mites were subjected in vitro. Each treatment utilized 30 mites; 10 mites were used per replicate.

Each treatment utilized 0.5 mL of its respective preparation including positive and negative control. The amount was placed on a petri dish (diameter: 10 cm; height: 2 cm) using a syringe. A fine and non-absorbable brush was used to uniformly coat the extracts of surfaces on the bottom and side of the petri dish. A total of 10 mites were placed immediately in the treated petri dish and were observed for the next 6 h at room temperature.

2.10 Assessment of Acaricidal Activity of Each Leaf Extract and Control

The time intervals for the mite assessment were adapted from the method described by Luo *et al.* (2015). After all the mites were transferred to the petri dish, they were then assessed at 30 min, 1, 2, 4 and 6 h of post-exposure.

The acaricidal activity was assessed based on the criteria of Khan *et al.* (2012). The test mites were inspected from their respective Petri dishes. The assessment was done by stimulating the mite with a teasing needle 10 times to determine the presence or absence of visible movements. Each replicate was observed for 2 min. This was done under dark field microscopy at 400x magnification. The criteria to determine a dead mite were based on the following: (1) lack of response upon stimulation with teasing needle, (2) absence of motility, (3) persistent immobility (i.e., no limb and body movements). Mites that were found alive after 6 h of the trial were considered non-susceptible to the treatment.

Equation 1 was used to determine the percent efficacy of plant extracts and controls.

$$\% \, Efficacy = \frac{A - B}{A} \times 100 \quad (1)$$

where A is the total number of mites per replicate and B is the number of remaining live mites per replicate.

The treatments with acaricidal efficacy of more than 80% are highly effective based on the standard criteria for acaricidal efficacy presented by Tabije *et al.* (2013).

2.11 Statistical Analysis

The study was laid out in a completely randomized design (CRD). Significant differences ($p < 0.05$) between treatments, plants and control groups were computed using an F-test or one-way analysis of variance (ANOVA). Tukey's honestly significant difference (HSD) test was used for post-hoc data analysis.

3. Results and Discussion

Table 1 shows the mean percentage efficacies of *C. papaya* in varying treatments at different times of exposure. For FCE, the acaricidal activity of the plant exhibited a mean percentage efficacy of 3.33% after 6 h of exposure. The FCW exhibited 6.67% efficacy after 2 h of exposure. However, EEE and EEW displayed 100% efficacy by 6 h – exhibited as early as 30 min post-exposure. The acaricidal efficacy of *C. papaya*, using its seed, was also evident in the study of Shyma *et al.* (2014) against *Rhipicephalus microplus* which caused adult mortality at 93.33%. A 100% acaricidal efficacy was also established by Sudha Rani *et al.* (2018) against sheep ticks.

Table 1. Mean percentage (%) efficacy of *C. papaya* leaf extracts against *S. scabiei* var. *canis* after a given time of exposure

Treatment	*n*	Mean percentage (%) Efficacy ± SD/Time of exposure				
		0.5 h*	1 h*	2 h*	4 h*	6 h*
100% fresh crude extract (FCE)	3	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{b}	3.33±0.58^{b}
50% fresh crude extract in water (FCW)	3	0.00±0.00^{c}	0.00±0.00^{d}	3.33±0.58^{c}	3.33±0.58^{b}	6.67±0.58^{b}
50% ethanolic extract (in 60% ethanol) (EEE)	3	3.33±0.58^{c}	40.00±3.46^{c}	63.33±3.46^{b}	93.33±1.15^{a}	100.00±0.00^{a}
50% ethanolic extract (in Distilled water) (EEW)	3	43.33±2.52^{b}	70.00±1.00^{b}	86.67±1.00^{ba}	100.00±0.00^{a}	100.00±0.00^{a}
60% ethanol solution$^{(-)}$	3	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
Amitraz$^{(+)}$	3	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

* – significant at $p < 0.05$; $^{-}$ – negative control; $^{+}$ – positive control; h – hour; SD – standard deviation; means in a column with the same letter are not significantly different, $p \geq 0.05$.

At 30 min and 1 h of exposure, the EEW of *C. papaya* exhibited 43.33 and 70% acaricidal efficacy, respectively, and showed significant differences with the other treatments and control groups. On the other hand, the EEE at the same exposure time as the previous is also significantly different from the other treatments and control groups. It exhibited 3.33 and 40% efficacy, respectively. As exposure time increased, specifically at 2 h of exposure, the efficacy of EEW increased by 86.67% and showed no significant difference with the positive control. At the same exposure time, the EEE, having 63.33% efficacy, showed no significant difference with EEW but showed a significant difference with control groups. The onset of activity for FCW was observed at 2 h of exposure with 3.33% efficacy and showed a significant difference with the other treatments and the positive control. At 4 h of exposure, the EEW exhibited a 100% acaricidal efficacy and showed no significant difference with the positive control. Moreover, the EEE also showed no significant difference with the positive control. At 6 h of exposure, the EEE and EEW of *C. papaya* were not significantly different from the positive control. Thus, the acaricidal activities of the ethanol extract of *C. papaya* were comparable with the 0.025% amitraz solution against *S. scabiei* var. *canis*.

The bioactive compounds found in the leaves of *C. papaya* include alkaloids, flavonoids, glycosides, tannins and saponins (Ikeyi *et al.*, 2013). The presence of alkaloids, terpenes and flavonoids could be associated with the acaricidal activities of the plants. Alkaloids are known to intercalate with the DNA by inserting between the adenine-tylosin pairs or guanine-cytosine pairs, inhibiting further functions of the cell (Wink, 1998). The presence of flavonoids could cause cell membrane destruction by binding with the cholesterol permanently which leads to the destruction of membrane integrity and allows permission of foreign materials into cells. It was also stated that these flavonoids are responsible for reducing the complement activation, thereby reducing the release of anti-inflammatory substances such as prostaglandins and autocoids. Saponins are polyphenols that complex with sterols in the cell membrane, affect its integrity, and allow the entrance of foreign materials into the cell (Radulović *et al.*, 2013). Wink (2015) added that the increase in fluidity and permeability of the membranes leads to either uncontrolled influx of ions and metabolites or cell leakage and consequently cell death.

In Table 2, the FCE and FCW of *C. odorata* showed a mean percentage efficacy of 20 and 3.33%, respectively, during 6 h of exposure. The EEE and EEW of *C. odorata* showed acaricidal efficacy of 93.33 and 100%,

respectively, both exhibiting effect as early as 30 min of exposure. The results of the present study are in consonance with the findings of Dougoud *et al.* (2019) on the action of *C. odorata* against coleopteran, lepidopteran and hemipteran pests but are contrary to the results of Ravindran *et al.* (2015) wherein the acaricidal activity of *C. odorata* was not found against adult ticks.

Table 2. Mean percentage (%) efficacy of *C. odorata* leaf extracts against *S. scabiei* var. *canis* after a given time of exposure

Treatment	*n*	Mean percentage (%) Efficacy ± SD/Time of exposure				
		0.5 h*	1 h*	2 h*	4 h*	6 h*
100% fresh crude extract (FCE)	3	0.00±0.00^{c}	3.33±0.58^{c}	3.33±0.58^{cd}	6.67±1.15^{c}	20.00±1.73^{b}
50% fresh crude extract in water (FCW)	3	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{c}	3.33±0.58^{c}
50% ethanolic extract (in 60% ethanol) (EEE)	3	3.33±0.58^{c}	13.33±0.58^{c}	26.67±0.58^{c}	50.00±1.00^{b}	93.33±0.58^{a}
50% ethanolic extract (in distilled water) (EEW)	3	26.67±2.08^{b}	43.33±3.06^{b}	73.33±2.08^{b}	90.00±0.00^{a}	100.00±0.00^{a}
60% ethanol solution$^{(-)}$	3	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{c}
Amitraz$^{(+)}$	3	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

* – significant at $p < 0.05$; $^{-}$ – negative control; $^{+}$ – positive control; h – hour; SD – standard deviation; means in a column with the same letter are not significantly different, $p \geq 0.05$.

At 30 min of exposure, the EEW of *C. odorata* showed 26.67% acaricidal efficacy and the EEE solution showed activity with 3.33% efficacy. Both extracts showed a significant difference with the positive control. As time exposure increased, specifically at 1 h of exposure, the EEW and EEE also increased in activity showing 43.33 and 13.33% efficacy, respectively. Both extracts showed higher efficacy than FCE and FCW. At 2 h of exposure, EEW and EEE increased in activity and showed 73.33 and 26.67% efficacy, respectively. At 4 h of exposure, the EEW showed 90% efficacy and showed no significant difference with the positive control. On the other hand, the EEE showed an efficacy of 50%. At 6 h post-contact, the EEE and EEW were not significantly different from the 0.025% amitraz solution. Thus, the ethanolic extracts were comparable with the 0.025% amitraz solution.

The bioactive compounds present in the plants could attribute to their acaricidal efficacy. *C. adorata* contains tannins, saponins, glycosides, flavonoids, steroids, phenols, coumarins, terpenoids, tannins, alkaloids, steroids, phenols, saponins and anthraquinones (Vijayaraghavan *et al.,* 2013; Agaba and Fawole, 2016). The acaricidal efficacy of the essential oils from the leaves of this plant was tested against *Rhipicephalus lunulatus* (Tedonkeng *et al.,* 2004). On the other hand, insecticidal activity was reported against *Periplaneta americana*, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Sukhthankar *et al.,* 2014; Udebuani *et al.,* 2015), while Panda *et al.* (2010) disclosed the anti-helminthic activity of this plant.

As shown in Table 3, *P. betle* exhibited an outstanding result in all preparations with 100% acaricidal efficacy within 6 h. This was observed as early as 30 min of exposure with 100% mortality. At 30 min, the FCE, FCW, EEW and EEE of *P. betle* showed no significant difference with the 0.025% amitraz solution. Hence, the plant's extracts were comparable with the 0.025% amitraz solution.

Table 3. Mean percentage (%) efficacy of *P. betle* leaf extracts against *S. scabiei* var. *canis* after a given time of exposure

Treatment	*n*	Mean percentage (%) Efficacy ± SD/Time of exposure				
		0.5 h*	1 h*	2 h*	4 h*	6 h*
100% fresh crude extract (FCE)	3	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
50% fresh crude extract in water (FCW)	3	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
50% ethanolic extract (in 60% ethanol) (EEE)	3	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
50% ethanolic extract (in Distilled water) (EEW)	3	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
60% ethanol solution$^{(-)}$	3	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Amitraz$^{(+)}$	3	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

* – significant at $p < 0.05$; $^{-}$ – negative control; $^{+}$ – positive control; h – hour; SD – standard deviation; means in a column with the same letter are not significantly different, $p \geq 0.05$.

Similar results were obtained by Chaimanee *et al.* (2021) wherein among 11 plant species studied, the *P. betle* showed the highest acaricidal activity against *Tropilaelaps mercedesae* mites.

P. betle, on the other hand, has essential oil, tannin, alkaloids, carbohydrates, amino acids and steroidal components (Pradhan *et al*., 2013). The presence of saponins is also found in *P. betle* (Rekha *et al*., 2014). Phytochemical screening done by Kumari and Rao (2014) also revealed that its leaf contains anthraquinones, cardiac glycosides, glycosides and phlobatannins.

The positive control (0.025% amitraz solution) showed a mean percentage efficacy of 100% mortality as early as 30 min of exposure. On the other hand, the negative control (60% ethanol solution) showed a 0% efficacy after the given time of exposure.

The amitraz has been used commercially as insecticides and acaricides in animals. It is a triazapentadiene compound, a member of the amidine chemical family. It causes monoamine oxidase (MAO) enzyme activity and prostaglandin E_2 synthesis degrading neurotransmitters resulting in neurotoxicity and behavioral toxicity (Eizadi-Mood *et al.,* 2011; Nichols *et al.,* 2014). Amitraz also acts on the octopaminergic or alpha-2 adrenergic receptor of organisms causing hyper-excitement, paralysis, and death (Shitole *et al.,* 2010).

Some parameters may influence the efficacy of plant extracts which include the plant material, extraction procedure and solvent used for extraction (Pandey and Tripathi, 2014). Aside from the crude extract, the present study utilized water and alcohol extraction. Zhang *et al.* (2018) mentioned that solvent extraction is the most widely used technique for natural products extraction.

Water has the advantage of dissolving a wide range of substances aside from being non-toxic, non-flammable and cheap (Abubakar and Haque, 2020). Ali *et al.* (2015) demonstrated the good effect of aqueous extraction with the use of the neem plant against sarcoptic mite that is applied in vitro. The use of water has been used as a solvent for the extraction of compounds because it is safer to handle (Chew *et al.,* 2011). Water is referred to as the universal solvent and is considered highly polar (Tiwari *et al.,* 2011; Daley and Daley, 2013). Tiwari *et al*. (2011) mentioned bioactive compounds that can be extracted using water as a solvent such as tannins, saponins, anthocyanins and

terpenoids. Polar compounds are likely the frontliners resulting in the acaricidal efficacy of *P. betle* in fresh crude extraction in water.

However, in this study, the ethanol extract of the selected plants carried out their utmost potential as acaricides due to the possibility that the ethanol extraction was able to obtain the most possible potential bioactive compounds in each plant. Ethanol extraction is widely used to obtain extracts of bioactive compounds from plant materials for therapeutic reasons. The use of ethanol as a solvent for the extraction of bioactive compounds is relatively safe than other solvents like methanol and acetone (Wendakoon *et al.*, 2011). The principle "like dissolves like" means that the solvents would only extract compounds that have similar polarity with the solvents (Chew *et al.*, 2011). Ethanol possesses both polar and non-polar solubility (Daley *and Daley*, 2013). These are supported by the results of Egbunu *at al.* (2019) wherein it was shown that water has low extractive potential compared with ethanol. The ethanol extraction in their study showed high extractive value for flavonoids, tannins and steroids.

In this study, fresh crude extracts demonstrated variable efficacies between plants. Plant crude extracts contain phenols in low concentrations (Dai and Mumper, 2010). These are possible compounds attributed to the acaricidal efficacy of *P. betle* in fresh crude extraction.

Studies demonstrated that the selected plants exhibited bioactive compounds with potential acaricidal properties. According to Tripathi *et al.* (2014), the efficacy of plants may be due to the number of chemical compounds they may produce. It was probable that the differences of efficacies of the selected plants at extraction may involve the type and quantity level of bioactive compounds extracted that result in their acaricidal efficacy. As stated by Pandey and Tripathi (2014), the efficacy of extracted plants' phytochemicals depends on the nature of plant material, its origin, degree of processing, moisture content and particle size. Tiwari *et al.* (2011) added that variations in different extraction methods affect the quantity and secondary metabolite composition of an extract depending upon the type of extraction, time of extraction, temperature, nature of the solvent, solvent concentration and polarity of the metabolite.

4. Conclusion and Recommendation

The result showed varied efficacies among plants at different extraction procedures. Only *P. betle* exhibited outstanding results in all treatment preparations. However, all the studied plants in EEW and EEE exhibited excellent acaricidal efficacy against *S. scabiei* var. *canis*. Thus, these plants are potential sources of natural-based products for the treatment of canine sarcoptic mange. However, further pharmacological and in vivo studies are recommended to validate their acaricidal efficacy.

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