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Azadirachta indica A. Juss., *Tinospora rumphii* Boerl. and *Vitex negundo* against *Sarcoptes scabiei in vitro*

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ABSTRACT

The study aimed to evaluate the acaricidal efficacy of the ethanol leaf extracts of *Azadirachta indica* A. Juss., *Tinospora rumphii* Boerl., and *Vitex negundo* against *Sarcoptes scabiei var. canis*. It also aimed to determine the concentration level of each leaf extract which has the highest acaricidal activity. Ethanolic leaf extracts from the plants were prepared in 10% and 50% concentrations. Mite mortality was not ed as percentage efficacy and was measured 0.5, 1, 2, and 6 hours post-exposure. The study utilized Completed Randomized Design and One-way Analysis of Variance (ANOVA) for the data. All concentration levels of the plant extracts showed acaricidal potential. Activity for 10% and 50% ethanolic extracts of *T. rumphii*, *V. negundo*, and *A. indica* were 73.33% and 93.33%, 63.33% and 93.33%, and 36.67% and 76.67%, respectively. As indicated, *T. rumphii* showed the best acaricidal activity followed by *V. negundo* and *A. indica*. It also showed that only the 50% concentration level of *T. rumphii* and *V. negundo* have comparable acaricidal effects with a commercial acaricidal solution. It is recommended that further studies will be conducted to determine the lowest effective concentration level for each leaf extract, its toxicity, its bioactive compounds, and *in vivo* trials.

Keywords: acaricide, ethanolic extract, sarcoptic mange

INTRODUCTION

Mange mites are one of the common causes of dermatological problems in a wide range of mammals, causing intense pruritus and unsightly dermal lesions of animals. One of these common skin mites is *S. scabiei*, which causes severe pruritic dermatitis in domestic animals (Ahmed et al., 2012), and is an especially common problem in dogs. Pruritus disturbs the regular activity of the dog and thus, affecting its general health. Due to this problem, anti-mange products are commercially available, but these products are considered expensive.

Natural remedies have been steadily receiving recognition in popular consciousness and pharmaceutical science (Doughari, 2012). Many plants were already ascribed to have considerable positive effects on the body. This effect can be homeostatic, rehabilitative, preventive, or even curative. While a significant number of natural treatments have been described as specific to treating dermal lesions in domestic animals, few of these studies had shown exclusive use for the treatment of mange in domestic animals. Three plants that have been used on scabies are *V. negundo*, *A. indica*, and *T. rumphii*. These three are widely available locally and had been mentioned in anecdotal evidence to have effects on canine mange (Agrawal, 2011; Hamid, 2013; Khan et al. in 2012).

The purpose of this study is to assess the acaricidal activity of the plants mentioned above by using different concentration levels of their ethanolic leaf extracts against *S. scabiei var. canis*. It also aims to compare the efficacy between the plants, as well as with a commercially available acaricide. The study seeks to serve as baseline data for further research and development of specialized products that exhibit acaricidal effect against mange mites in animals and humans.

METHODOLOGY

Study Animals as Source of Mites

The study required ten mange positive local dogs, regardless of age, sex, and breed. Natural infection was confirmed through skin scrapings. The mange positive dogs were housed exclusively in an Experimental Animal Station during the duration of the study. These dogs were fed once a day with a mixture of commercial dog food and table food and provided with adequate water. Accommodation for the dogs was cleaned regularly for feces and urine.

After the study, the dogs were treated with ivermectin (Ivomec, Merial, France), 10 mg/ml, given at 200 mcg/kg BW, two weeks apart for two to three

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treatments before they were returned to their owners or released for adoption. This study was approved by the Institutional Animal Care and Use Committee of Central Mindanao University, Philippines (2016-27B).

Mite collection

Skin scraping (Elsheikha and Wright, 2015) was done once a day. The scraping was done at least an hour, but not more than 12 hours before the application of treatments. A maximum of ten skin scrapes, each within an area of 2 cm2, was done. The collected scabs were placed in an untreated Petri dish and inspected for mites. Using the dark field of a stereo microscope, the Petri dishes were inspected thoroughly to collect the adult mites. A total of 360 adult mites were collected for this study.

Preparation and Extraction of Leaves

The plants for this research include; *A. indica*, *T. rumphii*, and *V. negundo*. The leaves were collected early in the morning, and only the fresh, mature, and insect bite free leaves are selected, approximately one kilogram per plant. The leaves were washed with tap water and were air-dried for seven days. The dried leaves were then finely ground using a grinder before submerging into 1.0 L absolute ethanol (99.8%). This solution was stirred twice a day for 48 hours to obtain the standard extract. The resulting solution was strained using fine-holed cheesecloth, and the strained fluid was the stock solution. The stock solution was subjected to the rotary evaporator to obtain the 100% ethanolic leaf extract. The 100% leaf extracts were reconstituted using sterile water to achieve the desired 10% and 50% concentration.

Treatments

The treatments were the following: T0- – sterile water, T0+ – 0.025% amitraz solution; T1 – 10% ethanolic leaf extracts for *A. indica*, T2- 50% ethanolic leaf extracts for *A. indica*, T3 – 10% ethanolic leaf extracts for *T. rumphii*, T4- 50% ethanolic leaf extracts for *T. rumphii*, and T5- 10% ethanolic leaf extracts for *V. negundo*, and T6- 50% ethanolic leaf extracts for *V. negundo*.

In vitro Application of the Leaf Extract and Controls in Petri Dishes

There were 36 Petri dishes used for this study. For each treatment, three petri dishes were alloted, and for each Petri dish, there were ten mites. At least one hour before the isolation of mites from the collected scabs, the inner base and sides of the Petri dishes with smaller diameter were coated with 0.5 ml reconstituted ethanolic leaf extracts (10% and 50% concentration levels) and the control groups, 0.025% amitraz solution for the positive control and sterile water for the negative control. The Petri dishes were covered and labeled accordingly.

Assessment of Acaricidal Activity of each Leaf extract and Controls

Assessment activity of acaricidal activity was measured by mite mortality, observed at 0.5, 1, 2, and 6 hours after exposure to the control extract. Post-treatment, the mites were inspected in every Petri dish for movement through stimulation with a teasing needle. The mites were touched ten times if they were found immobile. The mites were considered dead if no movements were noted after 5 minutes of visual inspection under a stereo binocular microscope.

The assessment of the efficacy of each leaf extract and control was modified from the formula used by Tabije et al. in 2013 through subtraction of the total remaining live mites after 6 hours per replicate from the total mites used per replicate divided by the total number of mites used per replicate multiplied by 100.

Statistical Analysis

The study utilized a complete randomized design. The significant differences in the acaricidal effects of different leaf extracts and their comparison with the commercially available anti mange bath product and sterile water were determined using the F-test or analysis of variance (ANOVA) and Tukey's honesty significant test. The comparison between the concentration levels was assessed using an independent sample t-test.

RESULTS AND DISCUSSION

Tables 1, 2, and 3 summarized the data on the acaricidal efficacy (%) of the ethanolic extracts of *A. indica*, *T. rumphii*, and *V. negundo*, respectively.

As shown in Table 1, the percentage efficacies of *A. indica* are 36.67% for 10% and 76.67% for 50%. The results supported the claims of Prashanth and Krishnaiah in 2014. The acaricidal efficiency of *A. indica* was also reported against *S. scabiei var. cuniculi* (Seddiek et al., 2013). Aside from its acaricidal potential, it had been well known to be a mosquito repellent (Agrawal, 2011). The effects might be slow-acting, but A. indica is relatively a potential source for organic insecticides against *S. scabiei var. canis*.

Tinospora rumphii showed a relatively high efficacy compared to the other plants against S. scabiei var. canis. In Table 2, it revealed the percentage efficacy of 10% and 50% ethanolic leaf extract of *T. rumphii* were 73.33% and 93.33%, respectively. This study utilized leaves, although the most studied part of *T. rumphii* is its stem, which is characterized by fleshy protuberances (Devprakash et al., 2011). Nevertheless, phytochemical analyses showed that the bioactive compounds found in stems could also be found in leaves (Hamid, 2013). There were fewer studies about the pharmacological importance of *T. rumphii* compared to other plants, but past researches and the results of this study showed that the plant could be a potent insecticide against adult *S. scabiei var. canis*.

The percentage efficacies of *V. negundo* for 10% and 50% ethanol extract were 63.33% and 93.33% (Appendix 2), respectively, as shown in Table 3. The results of this study conforms to the results of Khan et al. in 2012 that showed the potential of this plant against *S. scabiei* showing not less than 70% mite mortality in 40% methanol extraction.These acaricidal efficacy of the present study

Table 1

Mean percentage (%) efficacy and the total number of remaining live mites for 10% and 50% A. indica ethanolic leaf extracts against adult S. scabiei var. canis after a given time of exposure

Treatment	N	Total number of remaining live mites/time of exposure					Mean percentage efficacy
		30 min	1 hr	2 hr	4 hr	6 hr	
10% *A. indica*	30	30	28	28	23	19	36.67
50% *A. indica*	30	27	26	23	19	7	76.67
Amitraz**	30	0	0	0	0	0	100.00
Sterile water*	30	30	30	30	30	30	0.00

*- negative control, **- positive control, N- total number of mites per treatment, min-minutes, hr- hour.

Table 2

Mean percentage (%) efficacy and the number of remaining live mites for 10% and 50% T. rumphii ethanolic leaf extracts and control groups against adult mites (S. scabiei var. canis) after a given time of exposure.

Treatment	N	Total number of remaining live mites/time of exposure					Mean percentage efficacy
		30 min	1 hr	2 hr	4 hr	6 hr	
10% *T. rumphii*	30	30	27	27	19	8	73.33
50% *T. rumphii*	30	30	27	22	7	2	93.33
Amitraz**	30	0	0	0	0	0	100.00
Sterile water*	30	30	30	30	30	30	0.00

*- negative control, **- positive control, N- total number of mites per treatment, min-minutes, hr- hour.

conforms to the results of Nandini and Sriniwasa (2018).

In terms of percent efficacy, results show that at 10% concentration level, *T. rumphii* has the highest percentage efficacy followed by *V. negundo* and *A. indica* with the following percentage efficacies; 73.33%, 63.33%, and 36.67%, respectively. For the 50% concentration level, *T. rumphii* and *V. negundo* have the same percentage efficacies of 93.33% and *A. indica* has 76.67%.

In Table 4, it showed that 10% *A. indica* leaf ethanol extract (T1), *T. rumphii* (T3), and *V. negundo* (T5) are significantly different from amitraz solution, but *A. indica* is not significantly different with sterile water (T0-). Thus, none of these leaf extracts have comparable acaricidal effects with the amitraz solution.

Table 5 shows that the acaricidal activity of 50% A. indica (T3) is significantly different with amitraz solution and sterile water while the 50% of *T. rumphii* (T4) and *V. negundo* (T6) leaf ethanol extract is not significantly different with the acaricidal activity of amitraz (T0+) but are significantly different with sterile water (T0-). Therefore, 50% *T. rumphii* and *V. negundo* ethanolic leaf extracts are comparable with the acaricidal property of amitraz solution, but all the three-leaf extracts have more superior efficacy compared with sterile water.

CONCLUSION

All of the leaf extracts showed acaricidal property but varied in efficacy. Among the three mentioned plants, *T. rumphii* showed the promising potential in killing adult *S. scabiei var canis* with percentage efficacies of 73.33% in 10% concentration level and 93.33% in 50% concentration level, followed by *V. negundo* with percentage efficacies of 63.33% and 93.33% in 10% and 50% concentration levels, respectively. The least among the three extracts were *A. indica* with 36.67% for 10% concentration level and 73.33% for 50% concentration level. The control groups had the following percentage efficacies; the positive control (Amitraz) exhibited 100%, and the negative control (sterile water) had 0% in all treatments. In comparison between the concentration levels of each leaf extract, it was shown that a 50% concentration level has more significant acaricidal effects than a 10% concentration level. However, only the 50% concentration level of *V. negundo* and *T. rumphii* have comparable acaricidal effects to the amitraz solution.

Therefore, only the acaricidal activity of the 50% concentration levels of *T. rumphii* and *V. negundo* are comparable with a commercial anti-mange solution. However, the determination and quantification of the the bioactive components responsible for the acaricidal activity of the plant extracts is necessary to validate the potential use of the studied plants.

Table 3

Percentage (%) efficacy and the total number of remaining live mites for 10% and 50% V. negundo ethanolic leaf extracts against adult mites (S. scabiei var. canis) after a given time of exposure

Treatment	N	Total number of remaining live mites/time of exposure					Mean percentage efficacy
		30 min	1 hr	2 hr	4 hr	6 hr	
10% *V. negundo*	30	28	26	25	18	11	63.33
50% *V. negundo*	30	26	25	20	12	2	93.33
Amitraz**	30	0	0	0	0	0	100.00
Sterile water*	30	30	30	30	30	30	0.00

*- negative control, **- positive control, N- total number of mites per treatment, min-minutes, hr- hour.

Table 4

The Comparison between 10% concentration level of each Ethanolic leaf extracts with amitraz solution and sterile water.

Treatment	N	Total number of remaining live mites/time of exposure					Mean percentage efficacy
		30 min	1 hr	2 hr	4 hr	6 hr	
10% *A. indica*	30	30	28	28	23	19	36.67^{c}
10% *T. rumphii*	30	30	27	27	19	8	73.33^{b}
10% *V. negundo*	30	28	26	25	18	11	63.33^{b}
Amitraz**	30	0	0	0	0	0	100.00^{a}
Sterile water*	30	30	30	30	30	30	0.00^{c}

*- negative control, **- positive control, N- total number of replicates per treatment, min- minutes, hr- hour. Treatments with the same letters (superscript) are not significantly different p>0.05

Table 5

The Comparison between 50% concentration level of each Ethanolic leaf extracts with amitraz solution and sterile water

Treatment	N	Total number of remaining live mites/time of exposure					Mean percentage efficacy
		30 min	1 hr	2 hr	4 hr	6 hr	
10% *A. indica*	30	27	26	23	19	7	76.67^{b}
10% *T. rumphii*	30	30	27	22	7	2	93.33^{ab}
10% *V. negundo*	30	26	25	20	12	2	93.33^{ab}
Amitraz**	30	0	0	0	0	0	100.00^{a}
Sterile water*	30	30	30	30	30	30	0.00^{c}

*- negative control, **- positive control, R- number of replicates, min- minutes, hr- hour. Treatment with same letters (superscript) are not significantly different p>0.05

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