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https://dx.doi.org/10.4314/nvj.v43i4.3 ORIGINAL ARTICLE

IN VITRO EVALUATION OF THE ACARICIDAL EFFICACY OF AQUEOUS EXTRACT AND ESSENTIAL OIL OF *MOMORDICA CHARANTIA* L. AGAINST *RHIPICEPHALUS (BOOPHILUS) ANNULATUS* TICKS.

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ABSTRACT

Ticks are a significant threat to livestock production, and the synthetic acarcides used to control them have had negative effects on the environment, non-target organisms and the animals being treated. As a solution to this problem, it is necessary to explore alternatives that are safer for humans, animals, and the environment. The use of medicinal plants offers a promising solution. In this study, the acaricidal efficacy of *Momordica charantia*, a medicinal plant, was evaluated using the adult immersion test (AIT). The aqueous extract and essential oil (EO) of *M. charantia* leaves were evaluated. A positive control (2% cypermethrin) and negative control (distilled water) were used. Different concentrations (2.5, 5, 10, and 20%) of both aqueous and EO of plant were tested with 10 ticks per group. Tick mortality, reproductive index (RI), and inhibition of oviposition (IO %) were measured at 24 hours, 7 days, and 14 days post-treatment (PT). Statistical analysis was conducted using the probit method, with a significance level of $p < 0.05$. The results showed that both the aqueous extract and EO of *M. charantia* exhibited low acaricidal activity. Even at the highest EO concentration (20%), only 45% tick mortality was observed. The effect on oviposition was negligible, as the aqueous extract did not inhibit oviposition in live engorged female ticks. Consequently, *M. charantia* may not be a suitable alternative to synthetic acaricidal agents available on the market.

Keywords: *Momordica charantia,* acaricides, medicinal plants, Adult Immersion Test, Ticks

INTRODUCTION

Ticks are external parasites that thrive by feeding on the blood of birds, reptiles, amphibians and mammals (Jamil *et al.*, 2022). They serve as vectors for economically important viruses (such as flavi viruses), bacteria (such as *Borrelia burgdoferi*), and protozoan (*Babesia*, *Theileria*, and *Anaplasma* species) (Jaswal *et al.*, 2014; Salih *et al.*, 2015; Kasaija *et al.*, 2021). Animals do not only maintain tick cycles, but can either be clinically affected by the same zoonotic pathogens as humans and/or play a role as reservoir hosts (Springer *et al.,* 2021). In addition, certain tick species (*Dermacentor andersoni*, *Ixodes rubicundus*, and *Ixodes holocyclus*) can cause paralysis in animals and humans through their saliva (Hurtado and Giraldo-Ríos, 2018).

In Africa, ticks and tick-borne diseases (TBDs) are considered the most important animal disease challenge (Kasaija *et al.*, 2021).The favorable climatic conditions suitable for livestock production also support large tick populations, which enhance transmission of TBDs (Singh *et al.*, 2000). Direct and indirect economic loss due to TBDs in cattle globally costs an estimated \$13.9 to \$18.7 billion annually (Manjunathachar *et al.*, 2014). In Australia, losses due to the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* were approximately \$62 million, while in Brazil, losses were around \$2 billion per year (Valente *et al.*, 2014). Various methods have been employed to manage ticks, with synthetic acaricides (e.g., organophosphates, pyrethroids, and carbamates) being the primary control method (Sudhakar *et al.*, 2013; Adenubi *et al.*, 2020). However, the continued use of synthetic acaricides is discouraged due to acaricidal resistance and the presence of residues in animal by-products and the environment (De Meneghi *et al.*, 2016). Therefore, the search for safe and environmentally friendly alternatives is necessary. Medicinal plants have long been used for treatment and management of disease conditions around the world (Kasilo *et al.*, 2017). The use of medicinal plants is a basic part of African culture, one of the oldest and most diverse cultures (Mahomoodally, 2013). Rural and semi-urban farmers have limited access to veterinary care, information about animal diseases, therapeutic veterinary medicines and vaccines and therefore rely heavily on the use of medicinal plants (herbal medicine). *Momordica charantia* L. (Family: Cucurbitaceae), commonly known as bitter melon, is a tropical and subtropical vine that originated in Africa and has spread to Asia (Lad *et al.*, 2021). It is used in herbal medicine for various purposes, such as antidiabetic, abortifacient, anthelmintic, contraceptive, emmenagogue, antimalarial, galactogogue, laxative, and pain reliever (Poolperm and Jiraungkoorskul, 2017). The insecticidal activity of this plant has been reported in Haiti and Panama (Gandhi *et al.*, 2017). *Momordica charantia* is rich in saponins, including momordicin, momordin, momordicoside, karavilagenin, karaviloside, and kuguacin, which contribute to its biological properties (Poolperm and Jiraungkoorskul, 2017). In Odeda LGA, Adenubi *et al.* (2019) reported that the leaves of the plant are crushed with black soap and used to bathe tick-infested animals. This study therefore seeks to validate this ethnobotanical information by evaluating the acaricidal activities of *M. charantia* leaves *in vitro* as a preliminary study in the search for novel, effective and safe plant-based acaricide.

MATERIALS AND METHODS

Collection of plant material

Healthy *M. charantia* leaves were collected from their natural habitat in Alabata and Igbogila areas of Ogun State. The plant species was identified and authenticated at the Nigeria Natural Medicine Development Agency, Lagos State where a voucher specimen number (NH/2021/2494) was given.

Aqueous extraction

Healthy *M. charantia* leaves was rinsed with clean water and air-dried at the Veterinary Pharmacology and Toxicology laboratory for about three weeks, then blended to fine particle size. The blended leaves were thereafter soaked in a 1:10 ratio with distilled water (Khaliq *et al.,* 2012). After 48 hours, the mixture was first sieved using a muslin cloth, then through Whatman No. 1 filter paper. The resulting filtrate was then evaporated to dryness in a water bath at 40^{o}C. The crude extract was kept at 4^{o}C until use (Muchirah *et al.,* 2018).

Essential oil (EO) extraction

The EO from the plant sample was extracted using all Glass Clavenger Apparatus by the hydrodistillation method (Ogunwande *et al.*, 2019) at the Chemistry laboratory, College of Physical Sciences, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State. The fresh plant material was pulverized, weighed and transferred into the extraction flask containing water. The flask was then placed on the heating mantle and the system heated at 100^{o}C. Essential oil was released with the vapour, which was then carried to the condenser where it cooled off, separated from the vapour and was collected over n-hexane. The EO extracted was dried over sodium sulphate crystals and stored at 4^{o}C in a vial bottle to avoid possible loss by evaporation.

Collection and housing of ticks

Engorged female *R. (B.) annulatus* ticks were carefully handpicked periodically from herds of cattle at Lafenwa abattoir, Abeokuta, Ogun State.The ticks were put in perforated labelled sample bottles, to allow air and moisture exchange, these were transported to the Veterinary Pharmacology laboratory, College of Veterinary Medicine, FUNAAB, Ogun State. The bottles were sealed with rubber rings to prevent the ticks from escaping. The ticks were identified to the species level using both taxonomic descriptions and morphological keys (Walker *et al.*, 2003). They were kept in a tick rearing chamber at a temperature of 25^{o}C (±1^{o}C), 70-80% relative humidity with 14:10 hour light/dark cycle (Thorsell *et al.*, 2006).

Experimental procedure

Adult Immersion Test (AIT) was employed following the methodology outlined by Adenubi *et al.* (2021). Engorged adult female ticks were cleaned, dried, weighed, and individually marked. Each tick was then placed in a plastic bowl lined with tissue paper. Test samples of varying concentrations (20, 10, 5, and 2.5% w/v) were prepared and the ticks were immersed in these solutions for two minutes while gently agitating them. As controls, distilled water and cypermethrin were used as negative and positive controls, respectively. All tests were performed in duplicate. To achieve the desired concentrations, the aqueous extract was reconstituted. Each group of ticks, after recording their individual weights, was treated with the respective sample concentration. The ticks were divided into six groups (n=10) and immersed in the test samples. After treatment, each tick was placed individually in transparent bowls lined with tissue paper, referred to as the Post-Treatment (PT) condition. These bowls containing the ticks were then placed in a tick-rearing chamber maintained at a humidity of 70-80% and a temperature of 25°C ±1. During the PT period, the bowls were regularly monitored on an hourly basis to observe the tarsal reflex and response to light, following the procedure described by Akande *et al.* (2020). Subsequently, the tarsal reflexes and sensitivity to light were assessed every 24 hours until the fifth day, when oviposition was observed consistently. Engorged females that laid eggs were considered alive, while those that failed to oviposit were considered dead. The bowls were checked on hourly basis PT to monitor the tarsal reflex and response to light, as described by Akande *et al.* (2020). Thereafter, they were checked every 24 hours for tarsal reflexes and photosensitivity until the fifth day when oviposition was observed across board. Engorged females that oviposit are considered alive and those that did not oviposit are considered dead. Mortality was determined at 24 hours, 7 days and 14 days PT. On day 14, the eggs were collected and weighed, and the reproductive index (RI) and percentage inhibition of oviposition (IO%) were determined for the treatment groups and controls using the following formula:

$$RI = \frac{\text{weight of eggs}}{\text{Initial weight of engorged females}}$$

$$IO(\%) = \frac{\text{IO negative control} - \text{IO extract}}{\text{IO negative control}} \times 100$$

The same procedure was repeated using the *M. charantia* EO dissolved in 4% dimethylsulfoxide (DMSO) at four different concentrations of 20, 10, 5 and 2.5%.

Data analysis

Data were recorded in Microsoft excel, and mean mortality and standard error of mean (Mean ± SEM) determined usingGraphPad Prism version 4.0, San Diego, CA, USA. Dose response data were analyzed by probit method (Lieberman, 1983) using GraphPad Prism and p value of ≤0.05 was considered statistically significant.

RESULTS

Yield of aqueous extract and essential oil of *Momordica charantia*

The aqueous extract was dark colored with a sharp odor. About 16% of the original grounded leaves was the percentage weight yield of the extract. The EO appeared golden yellow in color which appear creamy when the oil thickens with no particular smell. (Table 1).

Table 1. Yield of *Momordica charantia* leaves

Momordica charantia	**Yield (%)**	**Colour and consistency**
Aqueous extract	15.8	Dark brown color with a sticky consistency
Essential oil	1.8	Bright yellow color, low yield of EO which changes from oily to fatty when there is a change in temperature where it is kept

Effects of aqueous extract and essential oil of *Momordica charantia* on tarsal reflex of treated ticks

All the ticks treated with different concentrations of the aqueous extract were active within 24 hours PT, and responded to light and sound (Table 2). Similarly, ticks treated with graded doses of the EO (2.5 - 10%) were active within 24 hours PT, responding to light and sound. However, ticks treated with 20% concentration of the EO showed reduced reflex (weak or paralysed) 24 hours PT, comparable with the positive control (Table 3).

Table 2. Effects of *Momordica charantia* leaves aqueous extract on tarsal reflex of treated ticks.

RESPONSE	**SCORE**	**Concentrations of *Momordica charantia* aqueous extract**				**PC**	**NC**
		2.5%	**5%**	**10%**	**20%**		
No response	0	0	0	0	0	0	0
Moving 1-2 limbs slowly	1	0	0	0	0	1	0
Moving 1-2 limbs at a fast rate	2	0	0	0	1	3	0
Moving more than 2 limbs but slowly	3	1	1	2	2	5	0
Moving >2 but <8 limbs but slowly	4	1	2	3	3	1	1
Moving all limbs	5	8	7	5	4	0	9

PC- Positive control; NC- Negative control

Table 3. Tarsal reflex score of engorged female *R.* (*B.*) *annulatus ticks* treated with different concentrations of *Momordica charantia* essential oils.

RESPONSE	SCORE	Concentrations of *Momordica charantia* essential oil				PC	NC
		2.5%	5%	10%	20%		
No response	0	0	0	0	0	0	0
Moving 1-2 limbs slowly	1	0	0	0	3	2	0
Moving 1-2 limbs at a fast rate	2	0	4	1	2	5	0
Moving more than 2 limbs but slowly	3	0	1	2	2	3	0
Moving >2 but <8 limbs but slowly	4	6	1	2	3	0	0
Moving all limbs	5	4	4	5	0	0	10

PC- Positive control; NC- Negative control

Acaricidal activity of the aqueous extract and essential oil of *Momordica charantia* leaves.

The ticks showed a low response to the aqueous extract treatment, though there was a dose-response relationship as mortality increased with increasing concentration. At 10% and 20% concentrations, mortality was observed starting from day 4 and 5 PT, with about 15% and 30% tick mortalities respectively (Table 4).

Table 4. Percentage mortality, reproductive index, and inhibition of oviposition of engorged female *R. (B.) annulatus ticks* treated with different concentrations of aqueous extract of *Momordica charantia* leaves.

Test sample	Conc. (%)	MAM ±SEM (%)	Mass of eggs (mg)	RI± SEM	IO (%)
Momordica charantia aqueous extract	20	30.0±0.24	30	1.0 ± 0.4	69
	10	15.0±0.40	43	1.4 ± 0.3	57
	5	0.0	60	2.1± 0.6	36.4
	2.5	0.0	100	3.3 ± 0.2	0
Cypermethrin	2	100	0	0	100
Negative control		0.0	100	3.3± 0.7	0

MAM: Mean adult mortality; SEM: Standard error of mean; RI: Reproductive index; IO(%): Inhibition of oviposition; Data were not statistically significant ($p > 0.05$).

The aqueous extract of *Momordica charantia* leaves had a no effect on ovipositioning in the engorged female ticks (Figure 1).

Figure 1. Engorged female *Rhipicephalus (Boophilus) annulatus* ticks treated with 2.5% aqueous extract laying eggs.

For the EO, 2.5% concentration had a 10% mortality rate, while 5% concentration had a 20% mortality. 10 and 20% concentrations of the EO led to a 30 and 45% tick mortality rate at around day 6 PT (Figure 2).

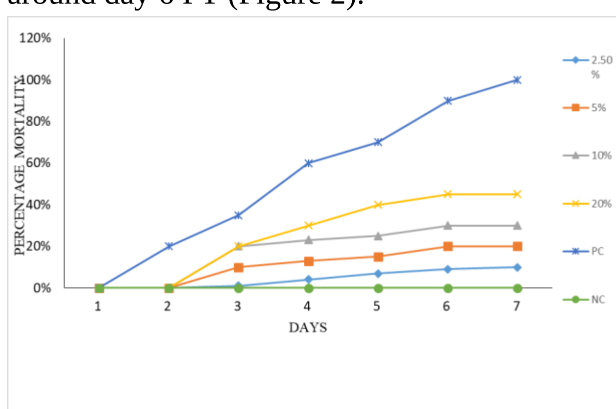

Figure 2. Acaricidal activity of the essential

oil of *Momordica charantia* leaves on engorged female *Rhipicephalus (Boophilus) annulatus* ticks.

DISCUSSION

The yield of both aqueous extract and EO of M. charantia in this study was found to be low. It is known that genetic factors, environmental conditions, and farming systems can significantly influence the yield and bioactive compounds in medicinal plants (Valyaei *et al.,* 2021). Other factors that can affect the adaptability and yield of *M. charantia* include the duration of the growth period, flowering time, fruit ripening, and other morphological and biochemical characteristics (Valyaei *et al.,* 2021). In this study, activity of both aqueous extract and EO of *M. charantia* showed less than 50%acaricidal efficacy and inhibition of oviposition of *R. (B.) annulatus* ticks. This is lower than the 66.1% acaricidal efficacy reported by Pedraza et al. (2020), who studied the acaricidal effect of ethanolic extract of *M. charantia* grown in Colombia against *R. (B.) microplus* ticks at a concentration of 160 mg/mL (16%). To the best of our knowledge, theirs is the only other study done on the anti-tick activity of *M. charantia,* to which these results are comparable. Different varieties of the same plant species can vary in their yield potential, content, and composition of bioactive chemicals due to changes in metabolic activity influenced by environmental factors. Several studies have demonstrated the ability of groups of secondary metabolites such as coumarins, condensed tannins, flavonoids, triterpenes, rotenoides, among others; to alter the biology of mites and insects (Gandhi *et al.,* 2017). Several of these secondary metabolite groups were found in the ethanolic extract of *M. charantia* (Pedraza *et al.,*2020). Mada *et al.*(2013) reported that the aqueous and ethanolic leaf extracts of *M. charantia* grown in northern Nigeria contains saponins, steroids, tannins, cardiac glycosides, alkaloids, and flavonoids. In a study in southwestern Nigeria by Oloruntola *et al.* (2021), high levels of tannins, flavonoids, phenols, saponins, alkaloids, and phytate were reported for the leaf powder of *M. charantia.* Plant tannins have been recorded to control bloat, endo and ectoparasites in ruminants raised on pasture (Huang *et al.*, 2018). The higher and faster acaricidal effect of *M. charantia* EO over the aqueous extract observed in this study may be attributed to its ability to diffuse more readily through the chitin of ticks (Giuntii *et al.,* 2023). Gonzalez *et al.* (2019) suggested that green-synthesized zinc nanoparticles of *M. charantia* have the potential to be used as an eco-friendly approach for controlling haematophagous parasites. Dantas-Neto *et al.* (2015) used an ointment-based formulation of *M. charantia* and observed 100% acaricidal activity against *Psoroptes ovis* and *Sarcoptes scabiei* mites on rabbits within 21 days. This may support the ethnobotanical use of the plant mixed with soap and applied topically on cattle as reported in Ọdédá LGA, Ogun State (Adenubi *et al.*,2021). The observed delay in the acaricidal activity of *M. charantia* may be due to a mechanism of action similar to that of amitraz, which induces various behavioral changes in ticks, including hyperactivity, leg waving, and detaching behavior. These behavioral effects are thought to be secondary to the actions on tick octopaminergic G protein-coupled receptors (de La Canal *et al.*, 2021). The results suggest that sub-lethal and behavioral effects may be more significant in the mechanism of action of *M. charantia* EO than lethality.

CONCLUSION

This study showed that the aqueous extract and EO of *M. charantia* leaves have low acaricidal effect against *R. (B.) annulatus* ticks, and may not be considered to be used in place of commercially available synthetic acaricidal agents. It is necessary that other parts of the plant (including the seeds and stem) should be studied, which could be hold promising phytochemicals absent in the leaves. Other extraction methods and testing of *M. charantia* leaves with an excipient is recommended as well as possible combination(s) with other medicinal plants.

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