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Insecticidal effects of methanolic extracts of tephrosia vogelii, tithonia diversifolia, azadirachta indica, and ocimum americanum on fleas

Lusekelo Msomba Mwangengwa[1*], Lusajo Mwaikambo[1], Julius John Medardus[2]

Abstract

Background: Fleas are important ectoparasites of veterinary and public health significance. Currently, there is growing interest in plant-based alternatives to synthetic insecticides for their control. This study aimed to experimentally assess the insecticidal effects of extracts from plants traditionally used in Tanzania to manage ectoparasites.

Methods: An experimental study was conducted over four months in the Morogoro Region. Plant collection and processing were carried out in June and July 2025, while extract preparation and testing were conducted between January and April 2026. Different concentrations of leaf extracts from Tephrosia vogelii, Tithonia diversifolia, Azadirachta indica, and Ocimum americanum were evaluated for their insecticidal activity against fleas. The extracts (100mg/ml) were tested at concentrations of 25%, 50%, 75%, and 100% following a single exposure to determine their effectiveness. Flea mortality was monitored over time, and survival rates were analyzed using the Kaplan–Meier test.

Results: All four plant species demonstrated insecticidal activity, causing complete flea mortality at varying concentrations and post-exposure times. T. vogelii exhibited rapid activity, with 100% mortality achieved within 2 hours at 100% concentration and within 3 hours at 50% and 75% concentrations. T. diversifolia showed high efficacy, with 25%, 50%, and 75% concentrations causing 100% mortality within 2 hours, while the 100% concentration achieved complete mortality after 4 hours. A. indica produced 100% mortality within 4 hours at 25%, 50%, and 100% concentrations, whereas the 75% concentration required 6 hours. O. americanum caused complete mortality after 3 hours at 75% concentration, after 4 hours at 25%, and after 12 hours at both 50% and 100% concentrations.

Conclusion: Overall, all plant extracts were highly effective against fleas and performed comparably to the positive control, indicating their potential as environmentally friendly botanical insecticides for flea management.

Keywords: Plant extract, Exposure, Fleas, Insecticidal effects, Mortality, Tanzania

Correspondence: Lusekelo Msomba Mwangengwa (usamwange@sua.ac.tz)
[1]Department Department of Veterinary Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O. Box 3017, Morogoro, Tanzania.

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Background

Ectoparasites remain a major challenge affecting the well-being and productivity of domestic animals [1, 2]. Ectoparasites live and feed on the animal's skin, causing irritation, pruritus, hypersensitivity, dermatitis, skin necrosis, and discomfort [3, 4]. Also, causes anemia, poor growth, secondary bacterial infection, and reduced productivity [3, 4]. Common ectoparasites affecting livestock include ticks, fleas, lice, and mites [4]. Some ectoparasites, such as lice and mites, are species-specific. Others, including fleas, infest a wide range of animals, including cattle, sheep, goats, pigs, dogs, cats, and poultry [4, 5]. Although conventional acaricides are widely used against the ectoparasites of livestock, their high costs and limited availability reduce reliance on them. Also, some resistant strains of ectoparasites are emerging, reducing the effectiveness of drugs [6, 7]. This threatening situation highlights the need for more effective and less costly treatment drugs. Medicinal plants with acaricidal properties are increasingly regarded as potential alternative drugs requiring exploration. The medicinal plants already in use against insect pests traditionally lack scientific evidence of their effectiveness [8, 9]. An ethnobotanical survey conducted in Kongwa and Karatu, Tanzania, revealed several plant species used traditionally as insecticides [8, 9]. Tephrosia vogelii, Tithonia diversifolia, Azadirachta indica, and Ocimum americanum were frequently mentioned as plants for insect management, warranting scientific investigation [8, 9]. T. vogelii (Fabaceae) is a shrub of 1–3 m in height, described by hairy stems, branches and leaves with 6–15 pairs of elliptic to oblanceolate leaflets that are sparsely hairy above and densely

hairy beneath. It bears large, white flowers in dense, terminal heads and produces long, hairy pods that can reach up to 15 cm. The species is distributed in tropical areas of Africa, the Americas, Southeast Asia, and Malaysia [10]. T. diversifolia (family Asteraceae), commonly known as Mexican sunflower, is a shrub-like annual or perennial plant growing 1–3 m tall and has alternate, lobed, pubescent leaves with palmate venation. It possesses solitary yellow flower heads on long peduncles. The plant has anomocytic stomata, dorsiventral mesophyll, angular collenchyma in stems, and glandular and non-glandular trichomes. The plant is found in North and Central America, Africa, Asia, and Australia [11, 12]. O. americanum (Family Lamiaceae) is an erect, much-branched, aromatic herb or soft shrublet growing up to 80 cm. It has pubescent, gland-dotted leaves with a strong citrus scent and small white to purplish flowers arranged in whorled spikes. It is found in the tropical areas of East, West, South, and Central Africa, Asian and American countries [13]. Traditional use relies on its flea repellent properties. A. indica (Family Meliaceae) is a tropical-growing plant distributed worldwide. It is 15–30 meters tall, with a wide, spreading crown and deep roots; evergreen but may drop leaves in drought. The plant bark is dark grey, reddish inside, deeply fissured in older trees, and exudes a colorless, sticky sap with a pungent odor. The leaves are alternate, pinnately compound (20-40 cm long) with 8-19 serrated, lance-shaped leaflets that are glossy and have pointed tips; young leaves are reddish. Flowers are small, white, fragrant, and arranged in drooping clusters (panicles) in the leaf axils; bisexual and male flowers occur on the same tree. The plant's fruits are small, smooth, olive-like drupes that are yellow when ripe, with a bitter, fibrous pulp surrounding a single seed [14]. This study investigated the insecticidal potential of T. vogelii, T. diversifolia, A. indica, and O. americanum against fleas under Tanzanian conditions.

Methods

Study area, design and duration

An experimental study was conducted in the Morogoro Region, Tanzania. Plant collection and processing were carried out in June and July 2025, while extract preparation and testing were conducted between January and April 2026. Tephrosia vogelii, Tithonia diversifolia, Azadirachta indica, and Ocimum americanum. were collected from areas around Morogoro Municipality. T. vogelii and T. diversifolia were collected from the highlands of Uluguru Mountain. A. indica and O. americanum were collected from low-lying lands. A. indica leaves were collected from trees scattered in the Municipality, together with O. americanum, which were uprooted from farms where they grow and dominate as weeds. Testing of extracts on fleas was carried out in the Pharmacology and Toxicology Laboratory in the College of Veterinary Medicine and Biomedical Sciences (CVMBS) at SUA.

Plant collection and processing

A total of 10 kg of each of T. vogelii, T. diversifolia, A. indica, and O. americanum were collected and transported in bags to the laboratory, where they were dried under shade until they became brittle. The dried plant materials were ground into powder using an electric grinder and stored in airtight bags until extraction. The plant extraction process was carried out according to [15], with some modifications, using 70% methanol as the extraction solvent. Briefly, 500 g of powdered plant material from each plant was used for extraction. To maximize extraction efficiency, the 500 g was divided into five equal portions of 100 g, with each portion placed in a separate 250-mL conical flask. Each 100-g portion was extracted using 1.5 L of 70% methanol. The mixture was shaken once daily and, after 72 h of extraction, filtered through gauze and cotton wool. The resulting filtrates were concentrated in a water bath at 39 °C to evaporate the solvent and obtain a solid extract. The solid extracts were then stored in a refrigerator at 4 °C until use.

Collection of fleas for in vitro studies

The fleas used for in vitro testing were collected from dogs that were brought to the dipping facilities at the Animal Hospital, SUA. The collection involved scrubbing the animal's skin on the abdomen area with a hard brush. The brushed-off materials were collected into a Petri dish containing 1% ethanol. Ethanol was used to sedate the fleas for easy handling and identification. Samples of fleas were identified using a stereomicroscope on a Petri dish. The dominant flea species were Ctenocephalides canis and Ctenocephalides felis, which normally infest a wide range of hosts, including pigs, small ruminants, dogs, and cats.

In vitro assessment of the plant extracts on fleas

From the stored extracts of T. vogelii, T. diversifolia, A. indica, and O. americanum, a stock solution of 100 mg/mL was prepared and diluted to 100%, 75%, 50%, and 25% using distilled water, following the protocol by [15] with some modifications. The four concentrations of each plant extract were used for in vitro studies. Distilled water served as a negative control, and 0.1% DuoDIP (Chlorpyrifos 500 g/L + Cypermethrin 50 g/L at a 1:1000 dilution) served as a positive control. In vitro studies involved six groups of fleas for each plant extract: the negative control fleas (n = 5) immersed in distilled water, the positive control (n = 5) immersed in DuoDIP, and four other groups (n = 5 per group) immersed in 100%, 75%, 50% and 25% concentrations of plant extracts, respectively. Before exposure, the treatment solutions, including the plant extracts and positive and negative controls, were prepared in six test tubes. Fleas were randomly selected from the holding tube using a pipette and allocated to the treatment groups without considering species differences. Five fleas were assigned to each treatment group and immersed once in the respective treatment solution for 5 minutes. Also, appropriately trimmed filter papers were immersed in the corresponding treatment solutions, allowed to dry for 30 seconds, and then placed in six separate test tubes to mimic residue effects. After the 5-minute immersion period, the fleas were transferred to the test tubes containing the corresponding treated filter papers, and mortality was assessed at predetermined time intervals. The test tubes were covered with fine wire gauze to allow adequate aeration while preventing flea escape and were maintained in an incubator at 25 °C. During assessment, fleas were considered dead if they showed no movement or jumping, whereas those that were actively moving or jumping were considered alive. The assessments were done at various time intervals of 0.5, 1, 2, 3, 4, 6, and 12 hours from the point of exposure using a stereo microscope. Mortality rate (MR) at a particular time was calculated as follows:

Data analysis

Microsoft Excel 2016 was used for data handling, cleaning, mortality calculations, and preparation of figures. Mortality was calculated as the number of dead fleas relative to the total number of fleas assessed. For survival analysis, flea death outcome at any observation time was recorded as an event (coded 1), whereas no death outcome was considered a censored observation (coded 0). Kaplan–Meier survival analysis was performed using IBM SPSS Statistics. Pairwise comparisons between treatment groups were conducted using an appropriate post-hoc adjustment for multiple comparisons. Statistical significance was set at $P \leq 0.05$.

Results

The mortality rates and survivability of fleas exposed to different concentrations of leaf extracts (100 mg/mL stock solution) from T. vogelii, T. diversifolia, A. indica, and O. americanum are described in Figures 1,2,3,4,6,7 and 8, respectively. All four plant species produced crude leaf extracts with insecticidal activity against fleas. However, their efficacy varied with extract concentration and the duration of observation following a single exposure, with mortality generally increasing as concentration and exposure time increased. For T. vogelii, the 100% extract concentration achieved 100% flea mortality within 2 hours of exposure. The 50% and 75% concentrations also resulted in 100% mortality, but after 3 hours, comparable to the positive control. In contrast, the 25% concentration reached 100% mortality only after 6 hours of exposure (Figure 1). Kaplan–Meier survival analysis (Figure 2) showed a significant overall difference in flea survival among the treatment groups (χ^2 = 12.339, P = 0.030). Pairwise comparisons indicated that flea survival differed significantly between the negative control and the 100% (χ^2 = 5.628, P = 0.018), 75% (χ^2 = 6.269, P = 0.012), 50% (χ^2 = 6.260, P = 0.012), and 25% (χ^2 = 4.000, P = 0.046) extract concentrations. However, no significant differences in survival were observed among fleas exposed to the different extract concentrations or between any extract-treated group and the positive control (Figure 2). For the Tithonia extract, the 25%, 50%, and 75% concentrations caused 100% flea mortality within 2 hours of a single exposure. In comparison, the positive control and the 100% extract concentration achieved 100% mortality after 3 and 4 hours, respectively (Figure 3). Kaplan–Meier survival analysis (Figure 4) showed no significant overall difference in flea survival among the extract-treated groups and the positive control (χ^2 = 9.749, P = 0.083). However, significant differences existed between the negative control and fleas exposed to the 100% extract (χ^2 = 4.358, P = 0.037), 75% extract (χ^2 = 5.628, P = 0.018), 50% extract (χ^2 = 5.628, P = 0.018), 25% extract (χ^2 = 6.298, P = 0.012), as well as against the positive control (χ^2 = 6.260, P = 0.012). For A. indica, the 25%, 50%, and 100% extract concentrations achieved 100% flea mortality within 4 hours of exposure, whereas the 75% concentration reached 100% mortality after 6 hours (Figure 5). Kaplan–Meier survival analysis (Figure 6) showed no significant overall difference in flea survival among the treatment groups (χ^2 = 9.184, P = 0.120). Similarly, no significant differences were observed among the extract-treated groups or between any extract-treated group and the positive control. However, pairwise comparisons revealed significant differences in flea survival between the negative control and the 25% (χ^2 = 4.506, P = 0.034), 50% (χ^2 = 4.506, P = 0.034), and 75% (χ^2 = 5.000, P = 0.025) extract-treated groups, as well as between the negative control and the positive control (χ^2 = 6.260, P = 0.012). Different concentrations of O. americanum extract achieved 100% flea mortality at different times following exposure (Figure 7). The 75% concentration achieved 100% mortality within 3 hours, which was comparable to the positive control. The 25% concentration achieved 100% mortality after 4 hours, while the 50% and 100% concentrations reached 100% mortality after 12 hours (Figure 7). Kaplan–Meier survival analysis (Figure 8) revealed a significant overall difference in flea survival among the treatment groups (χ^2 = 13.160, P = 0.022). Pairwise comparisons showed significant differences in survival between the negative control and the 25% (χ^2 = 5.890, P = 0.015), 50% (χ^2 = 4.000, P = 0.046), 75% (χ^2 = 8.110, P = 0.004), and 100% (χ^2 = 5.000, P = 0.025) extract concentrations. However, no significant differences in flea survival were observed among the extract-treated groups or between any extract-treated group and the positive control (Figure 8).

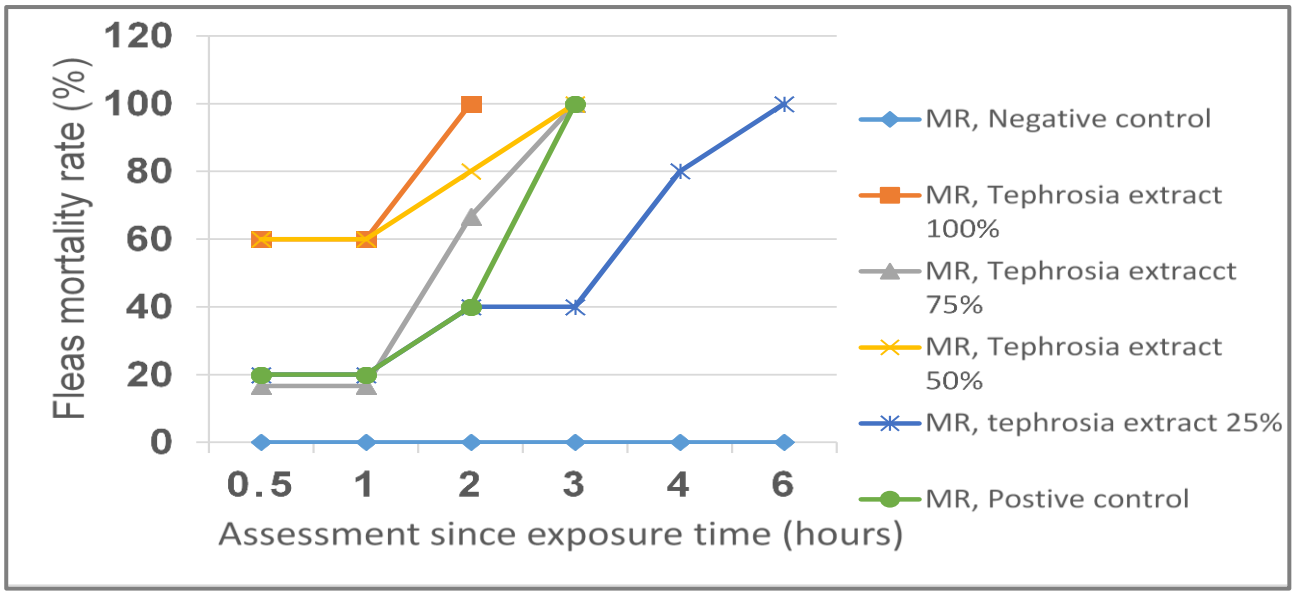

Figure 1. Fleas' mortality rate at varying time intervals of assessments following a single exposure to Tephrosia vogelii methanolic extracts

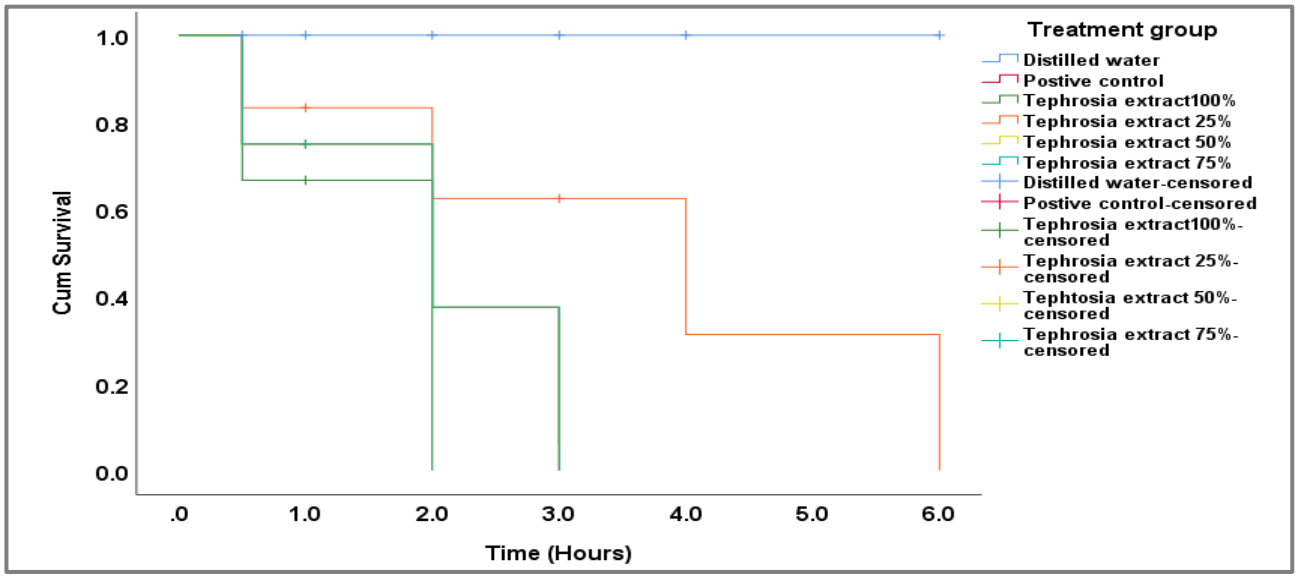

Figure 2. Fleas' survival function curves at varying time intervals of assessments following a single exposure to Tephrosia vogelii methanolic extracts

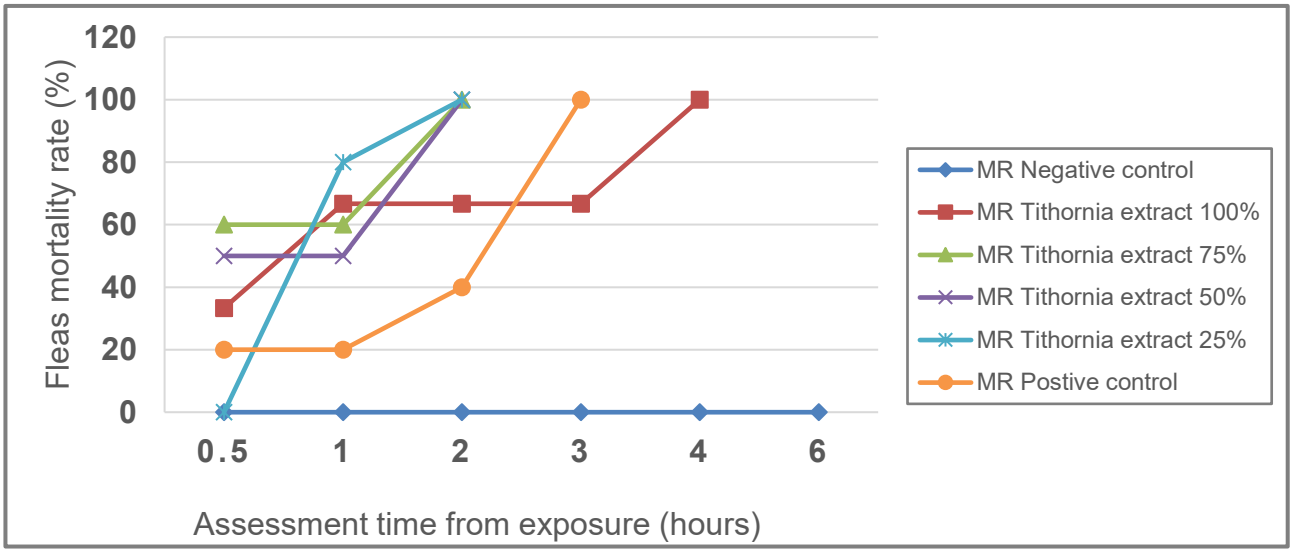

Figure 3. Fleas' mortality rate at varying time intervals of assessments following a single exposure to Tithonia diversifolia methanolic extracts

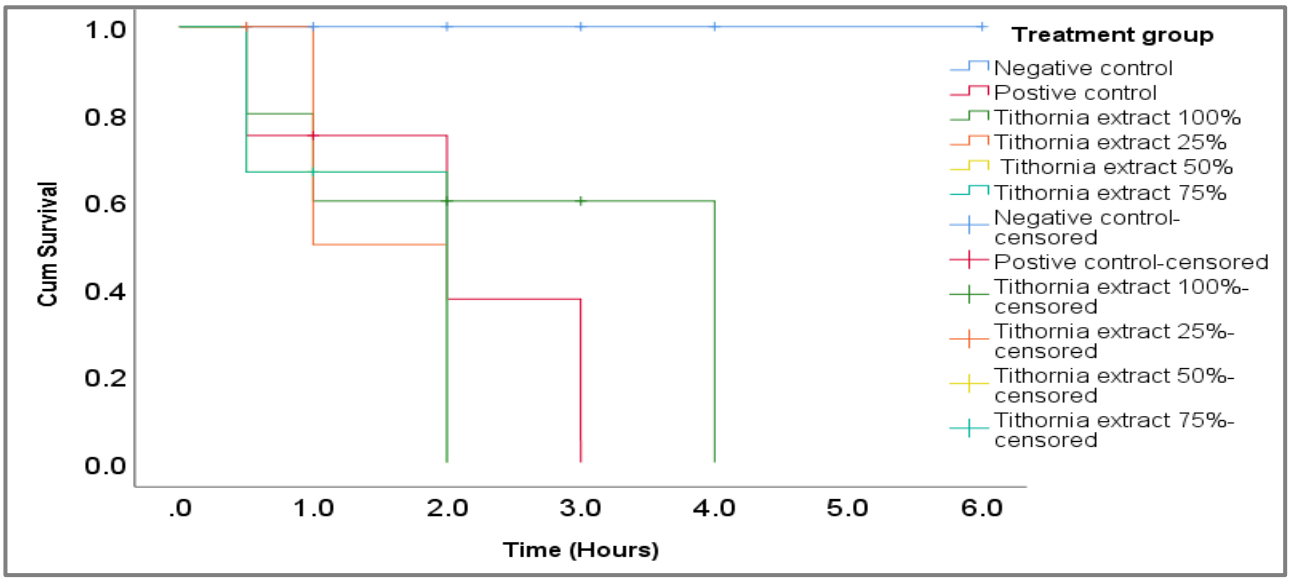

Figure 4. Fleas' survival function curves at varying time intervals of assessments following a single exposure to Tithonia diversifolia methanolic extracts

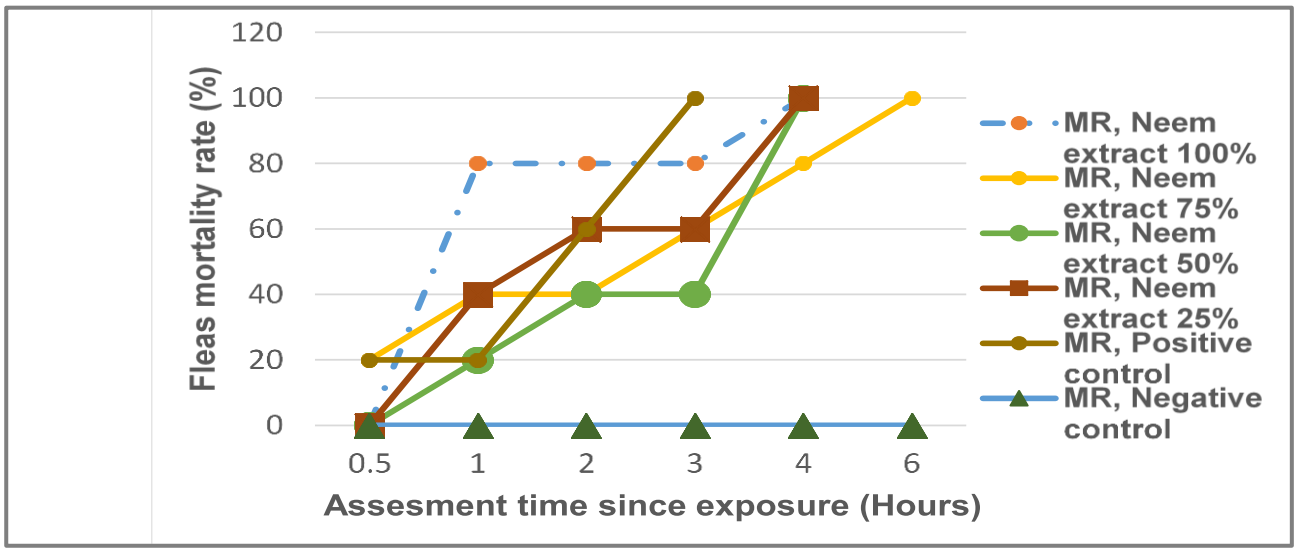

Figure 5. Fleas' mortality rate at varying time intervals of assessments following a single exposure to Azadirachta indica (Neem) methanolic extracts

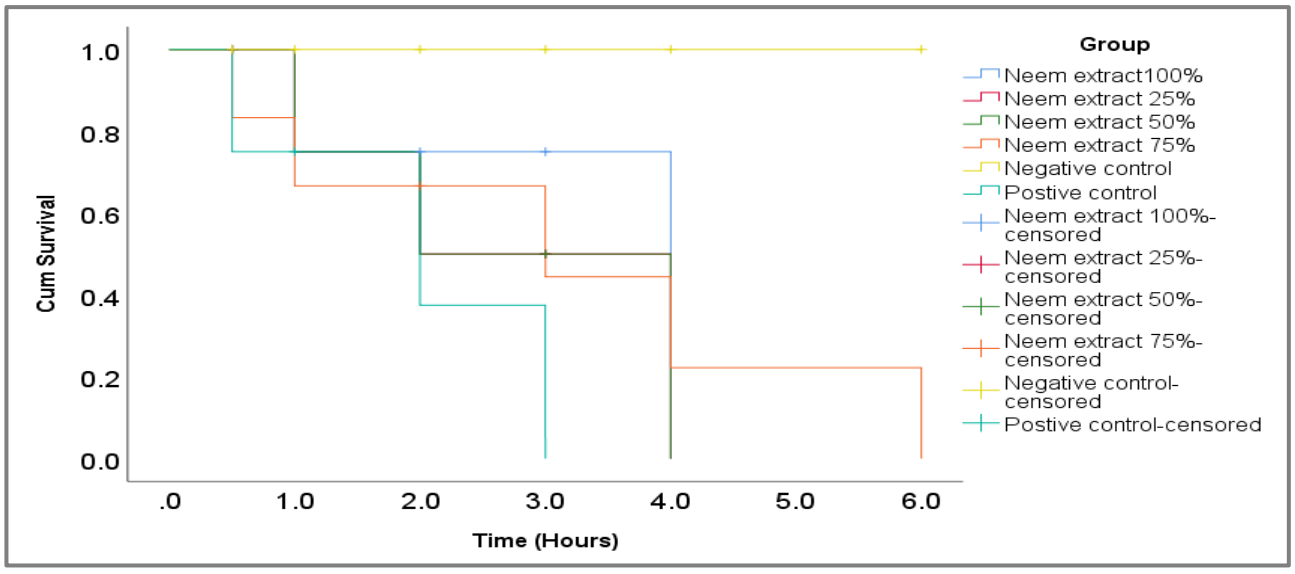

Figure 6. Fleas' survival function curves at varying time intervals of assessments following a single exposure to Azadirachta indica (Neem) methanolic extracts

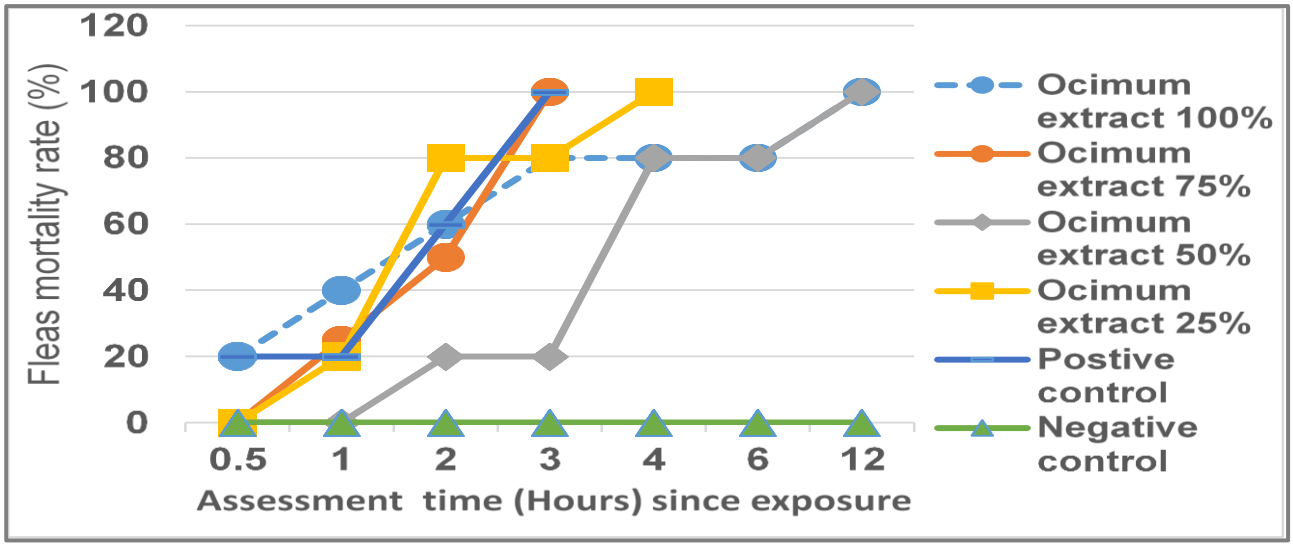

Figure 7. Fleas' mortality rate at varying time intervals of assessments following a single exposure to Ocimum americanum methanolic extracts

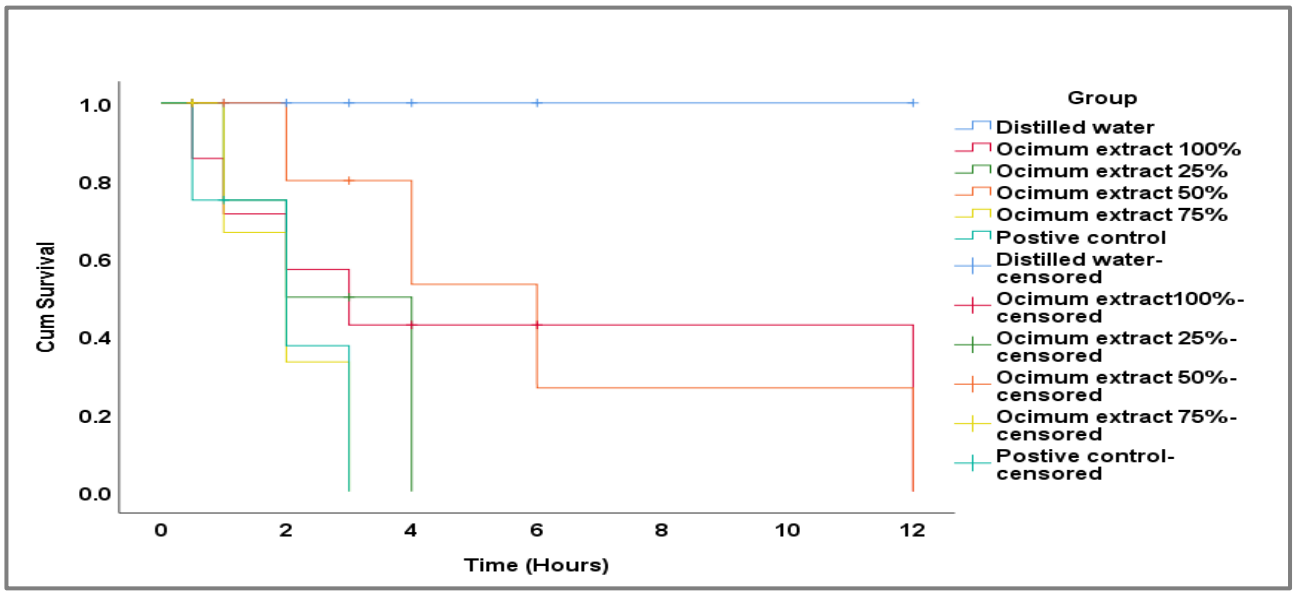

Figure 8. Fleas' survival function curves at varying time intervals of assessments following a single exposure to Ocimum americanum methanolic extracts

Discussion

This study demonstrated that crude leaf extracts of T. vogelii, T. diversifolia, A. indica, and O. americanum possess insecticidal activity against fleas. No flea mortality was observed in the distilled water-treated group (negative control), indicating that the mortality observed in the treatment groups was associated with exposure to the plant extracts. Furthermore, the insecticidal efficacy of each plant extract varied across concentrations and observation time intervals following a single exposure. Regarding T. vogelii, the 100% extract concentration was the most effective, achieving 100% flea mortality within 2 hours of exposure. The 75% and 50% concentrations achieved complete mortality within 3 hours, whereas the 25% concentration required 6 hours to achieve the same effect. For T. diversifolia, the 25%, 50%, and 75% concentrations achieved 100% mortality within 2 hours, while the 100% concentration required 4 hours. Similarly, A. indica extracts at 25%, 50%, and 100% concentrations achieved 100% flea mortality within 4 hours, whereas the 75% concentration required 6 hours. In contrast, O. americanum showed the highest efficacy at the 75% concentration, which achieved complete flea mortality within 3 hours, comparable to the positive control. The 25% concentration achieved complete mortality after 4 hours, while the 50% and 100% concentrations required 12 hours. The findings of the present study are consistent with previous reports demonstrating the ectoparasiticidal properties of these plant species. For T. vogelii, Siame and colleagues [16] reported significant acaricidal activity against cattle ticks, with different extract concentrations exhibiting comparable efficacy. Likewise, a study by Isabirye and Macleod [17] found that 25%, 33.3%, and 50% (w/v) T. vogelii extracts provided prolonged protection against fleas in free-ranging chickens, achieving complete flea mortality within five days. Moreover, the efficacy of T. diversifolia observed in this study agrees with the findings of [18], who reported a 93% flea mortality within 48 hours following a single exposure to the crude plant extract. Also, the insecticidal activity of A. indica observed in the present study is supported by several previous investigations. A study by Pasipanodya and colleagues [19] reported that 5%, 10%, and 25% (w/v) aqueous neem fruit extracts exhibited significant acaricidal activity against Sarcoptes scabiei var. suis in grower pigs, suggesting that neem extracts provide a safer, cheaper, and environmentally friendly alternative to conventional acaricides. Similarly, effective insecticidal effects of aqueous neem seed extracts was revealed against goat fleas [20], and camel ticks, where the greatest efficacy was observed at the 20% concentration [21]. The findings for O. americanum are consistent with some previous studies involving Ocimum plant species that demonstrated its broad-spectrum insecticidal and acaricidal properties. Essential oils of Ocimum plants were reported to be effective against fleas [22], mosquitoes [23], and several species of ticks [24, 25]. The unexpected observation that some lower extract concentrations produced faster flea mortality than higher concentrations warrants further investigation. For example, the 25% concentrations of T. diversifolia and O. americanum extracts produced faster mortality than some higher concentrations. This finding may indicate a non-linear concentration–response relationship. It may also be associated with differences in the composition and interactions of bioactive phytochemicals at different concentrations. This is because plant extracts contain complex mixtures of multiple secondary metabolites with diverse biological activities [26–28]. However, these mechanisms were not investigated in the present study and therefore cannot be confirmed. The main limitation of this study was the small sample size (n = 5 fleas per treatment group). However, the absence of flea mortality in fleas exposed to distilled water, compared with mortality observed in the extract-treated groups, strengthens the evidence for the observed insecticidal effects. Nevertheless, future studies should use larger sample sizes and appropriate replication to confirm these findings. Also, flea species recovered from dogs were identified as Ctenocephalides canis and Ctenocephalides felis. However, sorting of fleas into the treatment groups did not consider species differences.

Conclusion

The present findings reinforce the potential of T. vogelii, T. diversifolia, A. indica, and O. americanum as promising botanical alternatives for flea control. Their high efficacy,

coupled with evidence from previous studies against a range of ectoparasites, suggests that these plant extracts could contribute to the development of environmentally friendly and sustainable flea management strategies. Future studies should focus on identifying the bioactive compounds responsible for their insecticidal activity, evaluating their safety in target animal species, and assessing their efficacy under field conditions.

Abbreviation

SUA: Sokoine University of Agriculture; 0C: Degrees Celsius; χ^2: Chi-square; %: Percentage; SUARIS: Sokoine University of Agriculture Research and Innovation Support.

Declaration

Acknowledgment

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request by emailing usamwange@sua.ac.tz.

Authors' contributions

All authors contributed equally in designing the study, collecting the data, performing the statistical analyses, interpreting of the data, critically revising the manuscript, and approving the final version. All authors read and approved the final manuscript.

Ethics approval and consent to participate

We conducted the research in accordance with the Declaration of Helsinki. This study was reviewed and approved by the Research Ethics Review Committee of Sokoine University of Agriculture (SUA) and was granted ethical clearance under reference number SUA/DPRTC/R/126/VET/3/2023/2027 on 11 February 2025.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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Author Details

[1]Department of Veterinary Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O. Box 3017, Morogoro, Tanzania.

[2]Department of Veterinary Anatomy and Pathology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3016, Morogoro, Tanzania.

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