Retama raetam's In-Vitro Acaricidal Action on The *Hyalomma aegyptium* Tick, Infecting *Testudo Graeca Tortoises* in Laghouat, Southern Algeria

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Abstract
This research explores the efficacy of plant extracts from Retama raetam, which are essential for treating the tick Hyalomma aegyptium, parasitizing Testudo graeca This tortoise species lives in the Laghouat region of Southern Algeria. Various solvents, such as methanol, ethanol, and acetone, are used to extract the active compounds from the Retama raetam plant. The Hyalomma aegyptium tick is an ectoparasite that poses a threat to other animals' health, particularly tortoises and related species. *Solvents, including methanol, ethanol, and acetone, extract the Retama raetam plant.* These extracts were tested for their efficacy against Hyalomma aegyptium ticks. Ethanol-based Retama raetam extracts demonstrated the highest efficacy, with 100% mortality of ticks after 15 days of exposure. The acetone-based extract resulted in a mortality rate of 33%, while the methanol-based extract achieved a rate of 42%. Medium lethal concentration (LC50) tests determined the concentration required to cause 50% mortality in Hyalomma aegyptium ticks. The LC50 concentrations measured were 70 mg/mL for ethanol-based extracts, 80 mg/mL for methanol-based extracts, and 95 mg/mL for acetone-based extracts. The ticks can be effectively eliminated using ethanol-based plant extracts, which contributes to animal well-being and conservation efforts.
Keywords: Retama raetam; Tick; *solvents; LC50; mortality; Laghouat*

Introduction

The alarming decline of spur-thighed tortoise (*Testudo graeca*) populations in the Laghouat region of southern Algeria is primarily attributed to habitat loss, bacterial and parasitic infections, as well as the impact of ticks such as *Hyalomma aegyptium*. Meanwhile, the local plant *Retama raetam*, known for its various medicinal properties, is generating growing interest for its potential as a natural acaricide.
The species *Testudo graeca*, commonly referred to as the Moorish tortoise, is native to Laghouat in Southern Algeria and belongs to the family Testudinidae (Fritz et al., 1996). Originally described by Linnaeus in 1857, this species inhabits diverse ecosystems, including humid areas, arid regions, and coastal zones at high altitudes. However, its population has experienced significant declines, with some local extinctions reported. The International Union for Conservation of Nature (IUCN) has classified this decline as alarming (Tiar et al., 2019). *Moorish tortoises face numerous health challenges*, including bacterial infections and parasitic infestations. These health issues are exacerbated by pathogens such as *Hyalomma aegyptium* ticks, which are prevalent in Southern Algeria. These ticks act as vectors for diseases like *Rickettsia aeschlimannii* and Crimean-Congo hemorrhagic fever, posing severe threats to the survival of *Testudo graeca* populations (Bitam et al., 2009; Kautman et al., 2016). Additionally, infections caused by pathogens such as *Borrelia*, responsible for cat scratch disease, further compromise their health and raise concerns about the potential role of tortoises in disease transmission (Bouamer and Morrand, 2000; Bouaamer et al., 2003; Díaz et al., 2015; Ernst and Lovich, 2009; McBride et al., 2009). In recent years, plant-based extracts have garnered attention for their medicinal properties and potential to combat microbial infections and parasitic diseases. These extracts, inspired by traditional medicine practices, contain bioactive compounds with therapeutic effects. Such compounds include phenolics, terpenes, alkaloids, and steroids, which exhibit antibacterial,

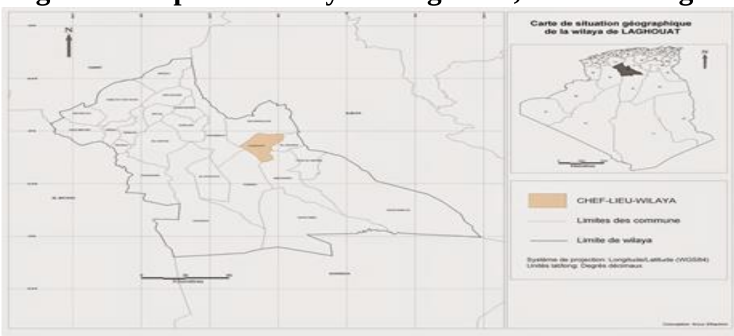
antiviral, antioxidant, anti-inflammatory, and antiparasitic properties (Hoste et al., 2006; Athanasiadou et al., 2007). Among these plants is *Retama raetam*, a perennial shrub native to Southeast Asia but also found in Algeria. Traditionally used for treating conditions such as diabetes mellitus, rheumatism, hypertension, inflammation, eczema, and snake bites (Hammouche-Mokrane et al., 2017; González-Mauraza et al., 2014; El-Toumy et al., 2011), *Retama raetam* has demonstrated pharmacological activities through its aqueous extracts. Studies suggest that these extracts possess diuretic properties and the ability to reduce blood glucose levels in diabetic rats (Belayachi et al., 2013; Edziri et al., 2008; Omara et al., 2009b; Koriem et al., 2010). Despite its documented pharmacological potential, limited research has explored the acaricidal properties of *Retama raetam* or its application in controlling tick infestations in *Testudo graeca*. This study aims to evaluate the efficacy of *Retama raetam* extracts in managing tick infestations affecting tortoises in the Laghouat region of Southern Algeria. By investigating the plant's bioactive compounds and their potential acaricidal effects, this research seeks to contribute to both conservation efforts for *Testudo graeca* and the broader understanding of plant-based treatments for parasitic diseases.

1. Experimental

1.1Investigation of the Geographical Region

The Laghouat region in southern Algeria lies 400 kilometers south of Algiers. Covering an area of approximately 25,052 square kilometers, it is home to a population of 520,188 residents. The northern part of the region experiences a continental climate with annual precipitation ranging from 300 to 400 mm. In contrast, the central and southern parts transition to a Saharan and arid climate with significantly lower rainfall levels—around 150 mm in the central area and only about 50 mm in the south. According to a report by Andi in 2013, winters are marked by white frosts, while summers are characterized by high temperatures and frequent sandstorms.

Figure 1: Map of the wilaya of Laghouat, Southern Algeria



1.2 Sampling of the *Retama raetam* Plant

The sampling points for the *Retama raetam* plant were meticulously selected to capture the diversity of its habitat and growing conditions, ensuring the collection of high-quality data for this study. Researchers also identified additional potential growth sites for *Retama raetam*, which facilitated the collection of leaf samples from a wide range of locations. Key environmental parameters such as soil type, sun exposure, altitude, and rainfall frequency were systematically analyzed to better understand the plant's ecological requirements. Comprehensive field surveys were conducted to assess the distribution and abundance of *Retama raetam* in its natural habitat. For this study, only healthy plants were selected, while dead or isolated specimens were excluded to ensure consistency in sample quality. Plants were harvested carefully to obtain samples with comparable characteristics across all sites. Each sample was meticulously labeled with essential information, including location details and environmental conditions, to support accurate and reliable analysis during subsequent research phases.

1.3 Extraction of Plant Materials

Dr. Salim Zarrok from the Department of Biology at the University of Laghouat, Southern Algeria, conducted this study on the *Retama raetam* plant to investigate the taxonomical implications of its leaves. To prepare the test medium, specific extraction solvents were used to isolate chemical compounds from the plant material. The leaves were first dried in sheltered areas for 7–10 days at a controlled temperature range of 25–37°C to preserve their chemical integrity. Once dried, they were ground into a fine powder using a mortar and pestle to ensure uniformity in sample preparation. For chemical extraction, solvents such as ethanol and acetone were employed under experimental conditions outlined by Kumar et al. (2011). The powdered plant material was soaked in these solvents for at least 16 hours to effectively extract bioactive compounds. Following this step, the extracts were separated from residual plant material through filtration after a boiling process lasting 16 hours. To ensure purity, the filtrates were passed through anhydrous sodium sulfate to remove any traces of residual alcohol, as described by Tabassum et al. (2008). This methodology allowed for the efficient isolation and preparation of chemical compounds for further analysis.

The obtained extract was concentrated under reduced pressure at a controlled temperature of 45°C and a vacuum of 22–26 mmHg to remove excess solvents. This step ensured that only the concentrated residue remained, which was subsequently used for medicinal compound analysis. To prepare a methanolic aqueous extract, powdered plant leaves were mixed with a methanol-based aqueous solvent in a ratio of 70:30 (70 mL solvent to 30 g of leaf powder). The extraction process was carried out over 72 hours with intermittent agitation to maximize compound solubility. After filtration, the crude extract was obtained by evaporating the solvent using a rotary evaporator under controlled conditions. The resulting concentrated residue was diluted to prepare solutions at various concentrations for further pharmacological or biochemical testing. This methodology ensures efficient extraction and preservation of bioactive compounds for medicinal applications.

1.4 Ticks

Bloodstained adult female ticks were collected and identified from naturally infested turtles. The ticks were transferred to the Parasitology Laboratory on Petri dishes with perforated covers, allowing air circulation. The ticks were put through the Adult Immersion Test immediately after arrival (Cen-Aguilar et al., 1998).

1.5 Adult Immersion Test (AIT)

The Total Immersion Analysis (TIA) was used to analyze the effectiveness of killing blood-engorged female ticks (Drummond et al., 1967). The plant extract was tested on 210 ticks. These ticks were divided into seven groups, with ten individuals each. The extracts were diluted at 25, 50, 75, 100, 125, and 150 mg/ml, while a control group was treated with distilled water. Each group underwent three duplicates. The ticks in the experimental groups were submerged for 30, 60, and 120 minutes, respectively, whereas the ticks in the control group underwent the same treatment using distilled water. The ticks were affixed and placed in Petri dishes, where they were subjected to incubation at a temperature of 27 ± 1.5°C and relative humidity of 70-80%, for 14 days, followed by the protocols outlined by (Cen-Aguilar et al., 1998). The ticks were subsequently inspected using a stereoscope at three-day intervals. Ticks exhibiting darkened cuticles, absence of movement in the Malpighian tube, and skin lesions with bleeding were classified as deceased. The calculation of mortality was performed using the corrected mortality formula developed by (Abbott, 1925) following the guidelines of the Food and Agriculture Organization (FAO) in 2004.

1.6 Statistical Analysis

Excel was used to input and process data. Correlation analysis was used as a statistical method to examine the relationship between two or more category variables. MCA analyzes and interprets the qualitative data, while PCA analyzes and interprets quantitative variables. Maps show qualitative variables and observation distances. MCA extends PCA in situations with more than two variables.

2. Results

Figure 2 illustrates the LC50 and LC90 values, which represent the concentrations of plant extract required to achieve 50% and 90% mortality of ticks, respectively. The plant extracts were prepared using different solvents to test their efficacy against Hyalomma aegyptium ticks, which infest Testudo graeca. All extracts exhibited varying levels of toxicity against the Hyalomma aegyptium tick.. However, the ethanolic extract demonstrated the highest efficacy, with an LC50 recorded at 70 mg/mL and an LC90 at 140 mg/mL. This indicates that ethanol is more effective than acetone, as it requires lower concentrations to achieve 50% and 90% mortality of ticks. For acetone, the LC50 was recorded at 95 mg/mL and the LC90 at 146 mg/mL This indicates that the concentration of Retama raetam extract in acetone required to kill 50% of parasites was 95 mg/mL.

in comparison, an LC90 value of 146 mg/mL was required to kill 90% of parasites using acetone-based extracts.. For methanol-based extracts, the LC50 was recorded at 80 mg/mL, while the LC90 was recorded at 146 mg/mL. Although the LC50 value for methanol is slightly higher than that for ethanol, its LC90 value *is comparable to that of acetone*, indicating *similar efficacy between methanol- and acetone-based extracts.* The analysis of the results also indicates *ethanol demonstrated the highest efficacy in combating the Hyalomma aegyptium tick,. Followed by methanol with moderate efficacy, while acetone showed the least effectiveness against ticks and parasites.*

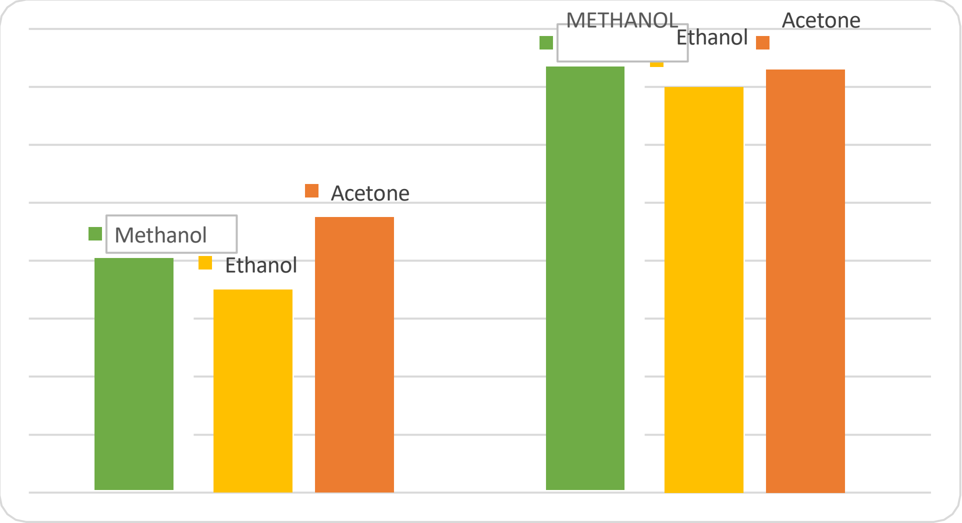


Figure 2: LC50 and LC90 of *Retama raetam* Extract Obtained for Different Solvents to fight against *Hyalomma aegyptium* Tick

2.1 Association for Computing Machinery (ACM) Analysis

*The purpose of the ACM analysis is to visualize both quantitative and qualitative factors on the map.*Following this research, the ethanolic extract has the highest efficacy rate, in comparison with the methanolic and acetone extracts. *In relation to the F2 axis, which explains 30.97% of the variance, and the F1 axis, which explains 48.39%, resulting in a cumulative variance of 79.35%.*

According to the research findings presented in Figure 3, *all extracts of Retama raetam demonstrated anti-tick properties against Hyalomma aegyptium ticks at all tested concentrations. It is important to note that all three types of extracts (acetone, methanol, and ethanol) show an increase in tick mortality as concentration increases.* It also indicates that all the extraction solvents efficiently extract chemicals *which harm the Hyalomma aegyptium tick. The acetone extract increases* the mortality of the tick as the concentration increases. It increases from 15% to 100% mortality with the increase in concentrations, ranging from 25 *mg/mL* to 150 *mg/mL*.

Similarly, a methanolic extract shows an increase in the mortality of ticks with increasing concentrations, ranging *from 15% to 100% mortality as concentrations increase from 25 mg/mL to 150 mg/mL.* in the same concentration range as the acetonic extract.The ethanolic extract follows the same trend, with a gradual increase in the tick mortality rate as the concentration increases, *reaching 100% mortality at 150 mg/mL. The negative control showed no mortality (0%) at any concentration. Confirming that observed mortality in other groups was due to the extract's effect and not external factors.*

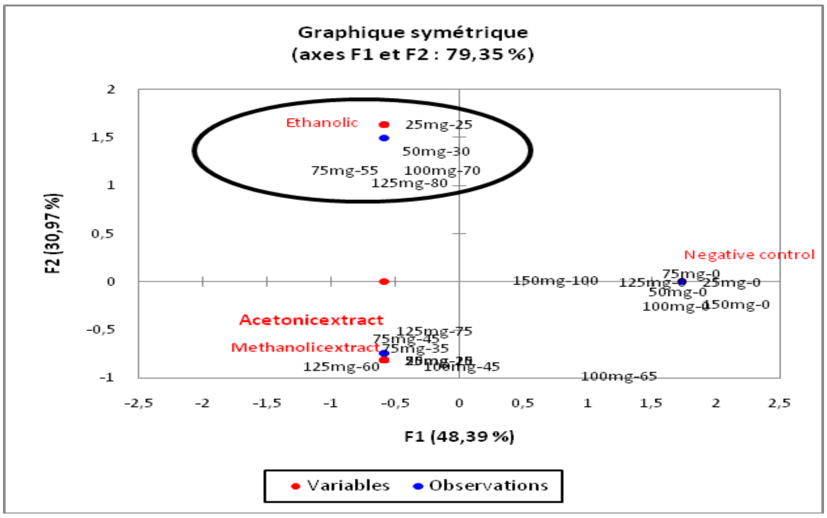


Figure 3: ACM of tick mortality rate as a function of the extraction solvent and *Retama raetam* extract concentration Table 1 shows the percentage of tick mortality for each immersion period and solvent. The mortality rate generally increases with increasing immersion time. In addition, mortality is higher with ethanol, followed by methanol and then acetone.

Table 1: *Effect of Extracts on Mortality Rates of Hyalomma aegyptium Ticks*

		1st			3rd			7th			9th			12th			15th		
Immersion time (min.)		30	60	120	30	60	120	30	60	120	30	60	120	30	60	120	30	60	120
Mortality (%)	**Ethanol**	7	12	20	25	32	35	45	50	55	65	70	74	83	87	90	96	99	100
Mortality (%)	**Methanol**	2	3	6	7	9	11	12	13	14	16	18	26	30	32	36	37	40	42
Mortality (%)	**Acetone**	1	3	3	4	5	6	7	9	11	12	13	15	15	17	20	25	29	33
Control (%)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0

Table 1 shows that the mortality rate of the *Hyalomma aegyptium* tick reaching 20%, 35%, 55%, 74%, 90%, and 100%. The 1st, 3rd, 7th, 9th, 12th, and 15th days, in the case of 70mg/mL concentration of ethanolic extract, will be 30 minutes to 120 minutes. In the case of 70 *mg/mL* concentration of the methanolic extract, the tick mortality rate reached 6%, 11%, 14%, 26%, 36%, and 42% on the 1st, 3rd, 7th, 9th, 12th and 15th days at 30 minutes to 120 minutes.In the case of the acetone extract, the tick mortality rates reached 3%, 6%, 11%, 15%, 20%, and 33% on the 1st, 3rd, 7th, 9th, 12th, and 15th days at 70 *mg/mL* concentration for 30 minutes to 120 minutes. In the control group, the mortality rate was 6% at the end of the treatment experience. The low level of mortality for all periods of immersion indicates that the mortality observed in the other groups is due to the extraction of solvents and their effects. The results suggest that the effectiveness of solvents in tick mortality depends on the time of immersion. Thus, with the increase in immersion time, the mortality rate is also subjected to increase.

3. Discussion

In recent decades, the field of tick control has faced significant hurdles. Extensive research has been conducted to analyze the acaricide and insecticidal properties of various plant species to manage phytophagous pests, mosquitoes, mites, and ticks (Calmasur et al., 2006); (Mukandiwa et al.,2014); (Kim et al., 2004); (Nong et al., 2013a); (Lori et al., 2005). Prior research has primarily examined the impact of chemical acaricides on different insects and mites, both in controlled laboratory settings and in real-life conditions, along with the level of toxicity (Wilson, 1948); (Guilhon, 1950); (Arthur, 1951); (Hadani et al., 1969).

Ticks are obligatory ectoparasites that feed on blood and infest approximately 80% of livestock on a global level. *Hyalomma aegyptium* tick is well studied with respect to external parasites that consume animals as their source of nutrition. These parasites transmit illnesses that result in economic losses in the livestock industry (Sajid, 2017). Chemical miticides are used as conventional approaches to control ticks. These applications are limited to environmental considerations. The invasive species and the development of insecticides and acaricides produce unintended impacts on the health of humans and the environment. Thus, this also raises doubts about the utilization of insecticides (Cafarchia et al., 2011).

This study assesses the extent to which the extract of *Retama raetam* plant can be implement to get rid of the ticks. As pointed out by the researcher of this study, this is the first time that attempts the research the impacts of the extracts of *Retama raetam* plant and come up with positive results towards acaricidal activities. But these plant extracts have in the recent past been applied in controlling horticultural pests such as the plant eating insects and Mosquitoes (Balandrinet al., 1995). [Finding/Solution]: It is apparent; the outcome of this study indicates that extract of *Retama raetam* possesses toxicological impacts on *Hyalomma aegyptiu*m tick from a range of extracting solvents. These findings also suggest that the plant leaves have certain bioactive compounds with acaricidal activity so that the plant could be used for controlling ticks. This research indicates the difference in effectiveness between the extracting solvents used to extract significant compounds from the plant material. As per the analysis results, ethanol has the highest efficacy rate, followed by methanol, which acquires mild efficacy, and acetone, which has the most negligible efficacy. This assumption is underlined by LC50 and LC90, which point to the fact that lower concentrations of ethanol extract are needed to obtain the toxic effect as compared to methanol and acetone extracts are needed for it.

This study's output has the potential to generate advanced biological control strategies for parasites, especially the ticks. The combination and application of plant extracts can be effective and efficient in protecting animals from being infected by bacteria and parasites whether through creams or food supplements while it is environmentally friendly and safer than using synthetic chemical products.

Recently, several plant products, raw extracts, and essential oils have been evaluated. It is to ascertain their efficacy in repelling and eliminating economically important tick species at their life cycle stages, including adult, pupa, larva, and egg. These assessment results have indicated a high potential (Chungsamarnyart et al., 1988); (Chungsamarnyart et al., 1990); (Chungsamarnyart et al., 1991a);(Mehlhorn et al., 2005), (Coskun et al., 2008), (Demon et al., 2009), (Magadum et al., 2009), (Monteiro et al., 2009); (Monteiro et al., 2012); (Clémente et al., 2010); (Kamaraj et al., 2010); (Zorloni et al., 2010)(Ghosh et al., 2011); (Koc et al., 2012); (Singh et al., 2014). Moreover, numerous periodic assessments are published on the aspects and characteristics of anti-tick and acaricidal effects (Kaaya, 2000); (Copping and Menn, 2000); (Flamini, 2003); (Nerio et al., 2010); (garEbadollahi, 2011); (Zoubiri and Baaliouamer, 2011); (Maïa and Moore, 2011); (Borges et al., 2011); (Andreotti et al., 2014); (Georges et al., 2014); (Ghosh and Ravindran, 2014).

Plant extracts have been used to control pests since they are cost-effective and environmentally friendly (Reverter et al., 2014). Many plant species are evaluated for their efficacy against ticks and bacterial infections (Rodriguez-Vivas et al., 2018). Most plant extracts are analyzed to cure different species of *Hyalomma aegyptium* tick (Singh et al., 2017).

These remedies are in the form of chemicals obtained from plant extracts that is used to treat illnesses. These chemical substances listed above CCO enable Inhibition of the mating of viral cells, prevent the formation of the Exoskeleton, slow down the multiplication of the bacterial cells, and also the eye-laying capability. Researchers also do ongoing studies about the effectiveness and efficacy of ethanol-based extracts on different ticks (Pereira and Famadas, 2004). The substances present in the stems of *Retama raetam* plants that can be extracted are Polyphenols, Piscidic acid, Quinic acid and Proanthocyanidin Pinocembrin (Touati et al. , 2017). The solvent with a high content of flavonoids, tannins, and alkaloids involves ethyl acetate (Edziriet al. , 2007). Few plant extracts were soluble in water and had innate antibacterial solid qualities, and polar activity (Mariem et al., 2014). Employee engagement: a study for Boussahel et al. (2017).

Plant extracts based on methanol have been used to study the fruits of the *Retama raetam* plant. These fruits are anti- inflammatory and antioxidant-rich. Plant extracts based on methanol exhibit mild acaricidal properties. They have larval mortality and can also inhibit the hatching of eggs on ticks. (Balan and colleagues, 2017) has researched various plant extracts with the help of extracting solvents. In comparison with methanol-based extracts and acetone-based extracts, ethanol-based extracts are highly effective in controlling ticks.

Solvents, such as hexane, acetone, and ethanol, are used as extraction agents or solvents. However, ethanol has been a solvent that has shown promising results in controlling ticks. In a study conducted by (Gonçalves et al., 2007), the impact of solvents and surfactants on adult female and cow tick larvae was assessed. The results indicated that acetone and methanol exhibited the highest level of toxicity among the solvents, while ethanol demonstrated a moderate level of toxicity. Nevertheless, (Ravindran and colleagues, 2011a);

(Ravindran and colleagues, 2011b) observed that methanol can dissolve plant extracts to assess acaricidal activities. It is worth noting that although aqueous solvents are frequently employed in ethnoveterinary medicine, organic solvents may be more effective in acaricidal bioassays. The tick cuticle, made primarily of exterior waxes and inside proteins, allows for more effective penetration by non-polar chemical substances (Balashov, 1972); (Chagas et al., 2002). The tick also represents a significant economic issue, responsible for severe losses related to various aspects such as tick anxiety, blood loss, skin damage, injection of toxins and transmission of diseases. Worldwide, extracts from nearly 55 plants belonging to 26 different families have been evaluated for their efficacy against ticks (Borges et al., 2011).

Nevertheless, despite the comprehensive research conducted on assessing plants' anti-tick and acaricidal characteristics, certain constraints have been recognized. One challenge in comparing different investigations is the absence of defined testing procedures or protocols for making extraction agents. These challenges disrupt the ability of plant extracts to establish a relationship between the research findings and their practical implementation in animals who contract ticks and bacterial infections.

4.Conclusion

This research evaluates the acaricidal efficacy of *Retama raetam* extract against *Hyalomma aegyptium* ticks in turtles, based on LC50 and LC90 test results. The findings demonstrate that ethanol is an effective extracting solvent for isolating bioactive compounds from *R. raetam*, which exhibit significant acaricidal activity even at low concentrations. These results highlight its potential as a natural alternative to synthetic chemicals for controlling tick infestations. The study further suggests that *R. raetam* extract could play a crucial role in reducing tick populations and mitigating ectoparasite-related threats to turtle populations, thereby contributing to their conservation. Additionally, its traditional use in folk medicine underscores its dual role in treating bacterial infections and ectoparasitic infestations. To ensure long-term efficacy, researchers emphasize the need to analyze the performance of *R. raetam* extract under varying environmental conditions and against different tick species. This approach would help determine its broader applicability in managing tick-borne diseases across diverse host species. Future studies should also explore potential variations in tick-host interactions and evaluate its impact on other animals susceptible to bacterial and viral infections.

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