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Original article

Plant-Derived Molluscicides: *Thymus vulgaris* and *Artemisia judaica* Extracts Control the white garden snail, *Thebapisana*

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Abstract
The white garden snail *Theba pisana,* is a significant agricultural pest, and there is growing interest in developing natural molluscicides as alternatives to synthetic chemicals. This study evaluated the efficacy of haxsan and aqueous extracts of *Thymus vulgaris* and *Artemisia judaica* against *Theba pisana* using the leaf-dipping technique. Mortality was assessed after 24, 48, and 72 hours of exposure. Results demonstrated that mortality was significantly influenced by the plant species, extract type, concentration, and exposure time. haxsan extracts consistently outperformed their aqueous counterparts. The haxsan extract of *T. vulgaris* was the most potent, causing 93.3% mortality at a 15% concentration after 72 hours, compared to only 33.3% for its aqueous extract. Probing the data further, probit analysis confirmed the superior toxicity of haxsan extracts, with the haxsan extract of *T. vulgaris* having the lowest LC_{50} value (6.37 %), followed by the haxsan extract of *A. judaica* (LC_{50} = 8.69 %). All treated groups exhibited significantly higher mortality ($p<0.001$) than the control group (0%). This study conclusively shows that haxsan extracts of *T. vulgaris* and *A. judaica* possess strong, time and concentration-dependent molluscicidal activity against *Theba pisana*, highlighting their potential as a source for molluscicide development and integration into pest management tactics.
Keywords. Molluscicidal, White Garden Snail, Haxsan Extracts, Thymus Vulgaris, Artemisia Absinthium.

Introduction
Terrestrial herbivorous gastropods, including snails and slugs, are major agricultural pests worldwide, threatening sustainable farming and causing significant economic damage to a wide range of crops [1]. Their feeding activity directly harms plants by consuming roots, leaves, flowers, and fruits, while also creating entry points for pathogenic microorganisms. Furthermore, contamination from their shells, bodies, and mucus reduces the quality and market value of produce [2]. Many species are highly invasive, and some act as intermediate hosts for pathogens, posing additional risks to human and livestock health [3]. Among these, the white garden snail, *Theba pisana,* is a particularly notorious invasive species. Native to the Mediterranean region, it has spread globally, with its rapid reproduction, gregarious behavior, and tendency to aestivate in large clusters on plants, making it a significant threat. These clusters can also clog and damage harvesting machinery [4]. Control has historically relied on synthetic pesticides, but their overuse raises serious concerns about resistance, environmental contamination, and toxicity to non-target organisms [5]. This underscores an urgent need for effective and sustainable alternatives.
Botanical pesticides present a promising solution. Phytochemicals derived from plants are often highly target-specific, biodegradable, and less harmful to non-target species, thereby helping to preserve biodiversity [6]. Two such plants are *Artemisia judaica* L. and *Thymus vulgaris* L. *A. judaica* is rich in bioactive compounds like piperitone and camphor, and plants from the Artemisia genus have a history of use as natural pesticides . Similarly, *T. vulgaris* contains diverse phytochemicals, and its aqueous extract has shown strong molluscicidal effects against other snail species [7].To our knowledge, no research exists on the insecticidal properties of thyme (*T. vulgaris*)and wormwood (*A. judaica*) against the white garden snail, *Theba pisana.* Therefore, this study aimed to assess the effectiveness of aqueous and alcoholic (hexane) extracts of thyme and wormwood against the white garden snail under laboratory conditions. The findings from this study are expected to provide technical support for developing plant-based molluscicidal strategies and utilizing effective extracts for protection, offering promising alternatives to traditional synthetic pesticides.

Methods
Snail Collection
Adult white garden snails, *Theba pisana* (Müller) (Pulmonata: Helicidae), were collected from farms in the Alqiqab region, Libya, and transported to the plant protection laboratory at Omar Al-Mukhtar University's Faculty of Agriculture. The snails were acclimated for one week in cloth-covered glass aquaria (60 × 40 × 40 cm) containing a 1:1 clay-to-sand soil mixture. They were maintained under laboratory conditions (25±3°C; 60±5% RH) and fed lettuce leaves ad libitum daily. Prior to the bioassay, the snails were starved for 48 hours.

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Plant material
Two plant species were utilized in this study. *Thymus vulgaris* L. (Lamiaceae) and *Artemisia judaica* L. (Compositae) were collected from the Al-Jabal al-Akhdar (Green Mountain) region, Libya. These plants were kindly identified by the Botany Department, Faculty of Sciences, Omar Al-Mukhtar University, Libya. They kindly provided taxonomic identification, which was verified using the Libyan Flora Encyclopedia (Flora of Libya).

Preparation of powders and extracts of plants
The plant materials were shade-dried at room temperature (26 ± 3°C) and ground into a fine powder using a mechanical grinder. An aqueous extract was prepared by soaking 100 g of the powder in 1 L of distilled water for 24 hours, according to the method of [8]. The resulting suspension was filtered and centrifuged (3000 rpm, 10 min) to obtain a clear, homogeneous extract. An alcoholic extract was also prepared by soaking 25 g of powder in 100 mL of hexane. This mixture was shaken for 2 hours and then left to stand for 24 hours before being filtered and centrifuged. For both extracts, working concentrations of 5%, 10%, and 15% (w/v) were prepared in distilled water. All extracts were stored in dark glass containers at 5°C and used shortly after preparation to preserve their efficacy.

Molluscicidal assay of plant extracts on adult snails
Uniform pieces of green lettuce leaves were immersed in one of three plant extract concentrations (5%, 10%, and 15% w/v) for five minutes. Each treatment was replicated three times using 100 mL of solution per jar. After air-drying, the treated leaves were placed in plastic cups, each containing five adult snails of uniform size. The cups were sealed with muslin cloth held in place with rubber bands. An untreated control group of lettuce disks was also included. Snail mortality was recorded at 24, 48, and 72 hours after treatment.

Statistical analysis
Data were subjected to analysis of variance (ANOVA) using CoStat, version 6.303 (VSN International Ltd, Hemel Hempstead, UK). Where significant effects were found, means were compared using the Least Significant Difference (LSD) test at the 5% probability level. The lethal concentration (LC) was calculated with the software program Ldp Line® model "Ehabsoft." [9], which was used to perform a probit analysis of concentration-mortality data

Results
Efficiency of haxsan and aqueous plant extracts on *Theba pisana* snails using the leaf dipping technique**.** The mortality percentages of some aqueous and haxsan plant extracts against *Theba pisana* using the leaf dipping technique after 24, 48, and 72 hours are presented in Table 1. The results showed that the mortality of the land snail *T. pisana* was influenced by the type of *T. vulgaris* extract, its concentration, and the exposure time. The haxsan extract demonstrated superior efficacy, with mortality reaching 93.3% at 15% concentration after 72 hours. In contrast, the aqueous extract at the same concentration and duration caused only 33.3% mortality. A strong time-dependent effect was observed; for instance, the 10% haxsan extract caused 26.7% mortality at 24 h, which increased significantly to 80.6% by 72 h. All treatments resulted in significantly higher mortality ($p<0.001$) compared to the control group.

Table 1. Effect of Thymus vulgaris haxsan and aqueous extracts on the mortality of Theba pisana.

Plant Extract	Type	Conc.	Mortality (%± SE)		
			24 h	**48h**	**72h**
T. vulgaris	Aqueous	5 %	0.00±0.00^{e}	0.00±0.00^{e}	13.30±0.58^{cde}
		10 %	0.00±0.00^{e}	6.70±0.58d^{e}	26.70±0.58^{bcd} ^{e}
		15 %	6.70±0.58d^{e}	20.00±1.00^{bcd} ^{e}	33.30±1.53^{bcd}
	Haxsan	5 %	0.00±0.00^{e}	0.00±0.00^{e}	33.30±1.15^{bcd}
		10 %	26.70±0.58^{bcd} ^{e}	40.00±1.00^{bc}	80.60±2.00^{a}
		15 %	33.30±1.15^{bcd}	46.70±1.53^{b}	93.30±1.53a
Control	-	-	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}
L.S.D			1.58***		

Values within a column followed by the same letter are not significantly different according to the Least Significant Difference (LSD) test.

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The insecticidal efficacy of *A. judaica* extracts was concentration and time-dependent, with the haxsan extract demonstrating superior potency over the aqueous extract (Table 2). After 72 hours, mortality rates for the haxsan extract ranged from 26.7% to 73.3% across the 5-15% concentration gradient, while the aqueous extract resulted in 13.3% to 53.3% mortality. Notably, at 48 hours, the 10% and 15% haxsan extracts already caused considerable mortality (46.7%), whereas the aqueous extract at 15% reached 33.3%. The control group consistently showed 0% mortality, confirming the bioactivity of the plant extracts

Table 2. Effect of Artemisia judaica haxsan and aqueous extracts on the mortality of Theba pisana .

Plant	Extract Type	Conce	Mortality (%± SE)		
			24 h	48h	72h
A. judaica	Aqueous	5 %	$0.00±0.00^{f}$	$0.00±0.00^{f}$	$13.30±0.58^{ef}$
		10 %	$0.00±0.00^{f}$	$6.70±0.58^{ef}$	$26.70±1.15^{cdef}$
		15 %	$6.70±0.58^{ef}$	$33.30±0.58^{bcde}$	$53.30±1.53^{abc}$
	Haxsan	5 %	$0.00±0.00^{f}$	$0.00±0.00^{f}$	$26.70±0.58^{cdef}$
		10 %	$20.00±1.00^{def}$	$46.70±0.58^{abcd}$	$56.00±0.71^{ab}$
		15 %	$26.70±0.58^{cdef}$	$46.70±1.53^{abcd}$	$73.30±1.53^{a}$
Control	-	-	$0.00±0.00^{f}$	$0.00±0.00^{f}$	$0.00±0.00^{f}$
L.S.D			1.34***		

Lethal concentrations of the tested haxsan and aqueous plant extracts

The results in Table 3 and Figure 1 show the lethal effects of various *haxsan* and aqueous plant extracts on the land snail species *T. pisana* over exposure durations ranging from 24h to 72h. The probit analysis was used to determine the lethal concentrations (LC_{50} and LC_{95}) of the plant extracts. The haxsanic extract of *T. vulgaris* proved to be the most potent, with an LC_{50} of 6.37 % (95% CL: 5.26 - 7.34). This was significantly more effective than its aqueous form (LC_{50} = 28.92 %). Similarly, for *A. judaica*, the haxsan extract (LC_{50} = 8.69 %) demonstrated higher potency than the aqueous extract (LC_{50} = 15.17 %). All probit models showed a good fit to the data, as indicated by the probability values, which indicated the statistical significance of the results, with higher values reflecting greater confidence in the findings. All extracts showed high probabilities (0.71 for Aqueous *T. vulgaris*, 0.16 for Alcoholic *T. vulgaris,* 0.11 for Aqueous *A. judaica,* and 0.94 for haxsan *A. judaica*), indicating that their effects were statistically significant.

Table 3. Summary of probit analysis on the mortality data of two different haxsan and aqueous plant extracts on Theba pisana after 72 h

Plant Extract	Type	LC_{50} % (CL)	LC_{95}% (CL)	Slope ± SE	x2	P
T. vulgaris	Aqueous	28.92 (20.81-44.53)	406.83 (352.10-577.90)	1.43±0.42	0.13	0.71
	Haxsan	6.37 (5.26-7.34)	17.91 (14.22-27.07)	3.66±0.45	1.97	0.16
A. judaica	Aqueous	15.17 (12.30-23.03)	69.21 (37.30-338.16)	2.49±0.43	2.47	0.11
	Haxsan	8.69 (7.12-10.40)	37.30 (24.46-94.34)	2.60±0.39	0.005	0.94

χ2: Chi-square of goodness of fit test; P: The probability of chi-square of a goodness-of-fit test. CL: Confidence limit

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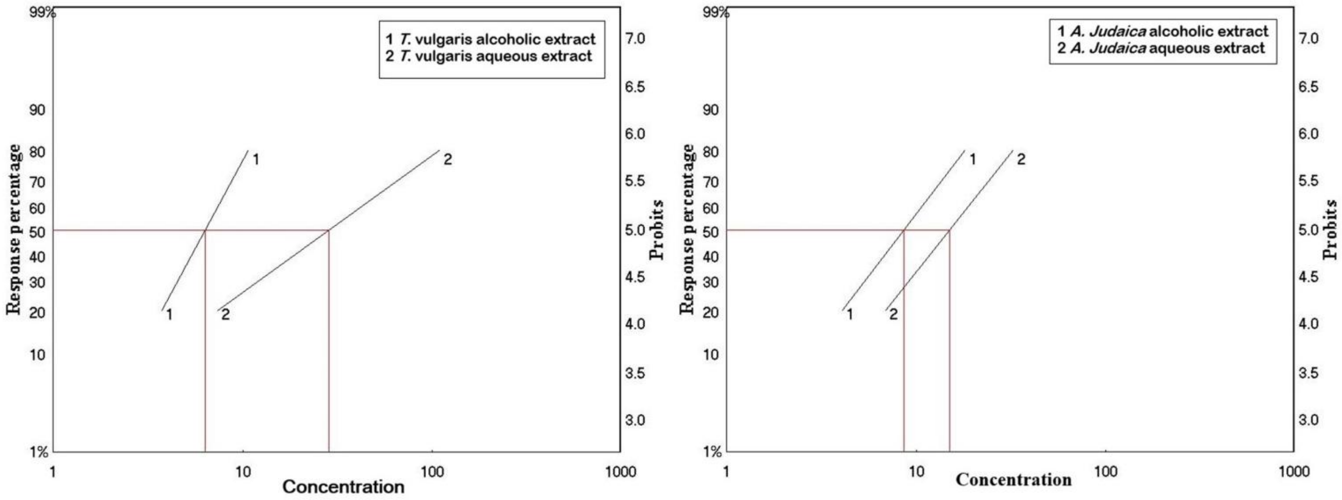

Fig. 1. Log-concentration probit-mortality lines of T. vulgaris haxsan, aqueous extract, and A. judaica haxsan, aqueous extract against T. pisana after 72 h

Discussion

Plant extracts are environmentally safe alternatives to the use of pesticides. They are used as one of the recent trends in integrated control programs. In our present study clearly demonstrates the potent molluscicidal activity of both *T. vulgaris* and *A. judaica* extracts against the land snail *T. pisana*, with efficacy being significantly influenced by the plant species, the type of solvent used for extraction, the concentration of the extract, and the duration of exposure. Aprimary finding is the superior performance of the alcoholic extracts over their aqueous counterparts for both plant species. For instance, the alcoholic extract of *T. vulgaris* at 15% concentration achieved a remarkable mortality rate of 93.3% after 72 hours, significantly higher than the 33.3% mortality induced by the aqueous extract at the same concentration and time. Similarly, the alcoholic extract of *A. judaica* (11%) resulted in 73.3% mortality, compared to 53.3% for its aqueous equivalent. This pattern can be attributed to the enhanced extraction efficiency of ethanol for a broader range of bioactive compounds, including non-polar secondary metabolites such as terpenoids, phenolic compounds, and essential oil constituents. Alcohol is known to break down plant cell walls more effectively than water, facilitating the dissolution of a wider spectrum of lipophilic and hydrophilic molluscicidal agents [10] The aqueous extracts, while less potent, still showed significant time- and concentration-dependent activity, suggesting that water-soluble compounds in these plants also possess bioactive properties.

The results also reveal a distinct difference in potency between the two plant species. *T. vulgaris* consistently elicited higher mortality rates than *A. judaica*, particularly at the higher concentrations and with the *haxsan* extracts. The 93.3% mortality from *T. vulgaris* (15% *haxsan*) is notably higher than the 73.3% from *A. judaica* at the same parameters. This suggests that *T. vulgaris* may contain a higher concentration or more potent suite of molluscicidal compounds, likely its well-characterized monoterpenoids such as thymol and carvacrol, which are renowned for their insecticidal and molluscicidal properties [11]. The activity of *A. judaica*, though comparatively lower, is still substantial and aligns with previous reports on the biocidal properties of Artemisia species, often attributed to compounds like camphor, 1,8-cineole, and various sesquiterpene lactones. A critical factor underpinning the efficacy of both extracts is the clear time- and concentration-dependent mortality response. In all active treatments, mortality increased progressively from 24 to 72 hours. For example, the 10% *haxsan* extract of *T. vulgaris* caused 26.7% mortality at 24 h, which increased to 40.0% at 48 h, and peaked at 80.6% by 72 h. This indicates that the toxic compounds may act through a cumulative or delayed mode of action, possibly involving the gradual disruption of neurological function, inhibition of essential enzymes, or damage to epithelial tissues in the snail's foot and mantle [12]. Furthermore, the significant jump in mortality between concentrations (from 5% to 10% *haxsan* extracts) underscores the importance of applying a sufficient dosage to achieve effective control in field conditions. The statistical significance of the results, as confirmed by the low Least Significant Difference (LSD) values at $p < 0.001$, reinforces the reliability of these observations. The clear separation of means into distinct statistical groupings (denoted by superscript letters in the data) allows for a robust comparison of the treatments. It confirms that the observed effects are not due to random chance.

The probit analysis further quantifies this observation. A clear variation in toxicity based on both the plant species and the solvent used for extraction, with *haxsan* extracts consistently exhibiting superior potency. The most striking finding is the significantly higher toxicity of the *haxsan* extracts compared to their aqueous counterparts for both plant species. This is evidenced by the considerably lower LC_{50} values for the alcoholic extracts (*T. vulgaris* The probit analysis further quantifies this observation. A clear variation in toxicity

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based on both the plant species and the solvent used for extraction, with *haxsan* extracts consistently exhibiting superior potency. The most striking finding is the significantly higher toxicity of the *haxsan* extracts compared to their aqueous counterparts for both plant species. This is evidenced by the considerably lower LC_{50} values for the alcoholic extracts (*T. vulgaris* alcoholic: 6.37%; *A. judaica haxsan*: 8.69%) versus the aqueous extracts (*T. vulgaris* aqueous: 28.92%; *A. judaica* aqueous: 15.17%). This pattern strongly suggests that the active molluscicidal compounds in both plants are more efficiently extracted by organic solvents like ethanol or methanol. *haxsan* solvents are proficient at extracting a broader range of secondary metabolites, including terpenoids, phenolic compounds, alkaloids, and essential oil components, which are often implicated in plant defense mechanisms and possess known biocidal activities [13]. The aqueous extraction, being more polar, likely captured a different, less potent, or more limited profile of compounds, such as polar glycosides and water-soluble tannins.: 6.37%; *A. judaica haxsan*: 8.69%) versus the aqueous extracts (*T. vulgaris* aqueous: 28.92%; *A. judaica* aqueous: 15.17%). This pattern strongly suggests that the active molluscicidal compounds in both plants are more efficiently extracted by organic solvents like ethanol or methanol. *haxsan* solvents are proficient at extracting a broader range of secondary metabolites, including terpenoids, phenolic compounds, alkaloids, and essential oil components, which are often implicated in plant defense mechanisms and possess known biocidal activities [14]. The aqueous extraction, being more polar, likely captured a different, less potent, or more limited profile of compounds, such as polar glycosides and water-soluble tannins.

Between the two plants, *T. vulgaris haxsan* extract emerged as the most potent, with the lowest LC_{50} value of 6.37%. This was followed closely by the *haxsan* extract of *A. judaica* (LC_{50} = 8.69%). The high potency of *T. vulgaris* is likely attributable to its high content of thymol, carvacrol, and other monoterpenoids, which are well-documented for their insecticidal, antimicrobial, and molluscicidal properties [15]. These compounds are known to disrupt cellular membranes and interfere with vital physiological processes. The steep slope of the probit line for *T. vulgaris haxsan* extract (3.66 ± 0.45) indicates a relatively homogeneous response within the test population and a narrow range between the concentration required to kill 50% and 95% of the snails. This is a desirable trait for a molluscicide, as it suggests a more predictable and rapid increase in mortality with concentration.

The aqueous extract of *T. vulgaris* showed remarkably low toxicity (LC_{50} = 28.92%), and the fact that confidence limits (CL) for both LC_{50} and LC_{95} could not be calculated suggests a shallow dose-response curve and a high degree of variability in the snail population's response. This further reinforces the conclusion that water is a suboptimal solvent for extracting the primary toxic principles from thyme.For *A. judaica*, both extracts were more active than the aqueous extract of *T. vulgaris*, but followed the same solvent-dependent trend. Artemisia species are known to contain a complex mixture of bioactive compounds, including sesquiterpene lactones, flavonoids, and volatile oils, which could be responsible for the observed molluscicidal activity [16]**.** The *haxsan* extract, with its lower LC_{50}, successfully extracted a more potent blend of these compounds. The probit regression analysis for all treatments showed a non-significant Chi-square (x2) value (P > 0.05), indicating that the mortality data adequately fit the probit model and that the results are reliable for estimating the lethal concentrations.

Conclusion

Our work highlights the crucial role of solvent choice in developing plant-based molluscicides. In this study, we found that haxsan extracts from both *T. vulgaris* and *A. judaica* served as potent, eco-friendly alternatives to synthetic chemicals, with the *T. vulgaris* extract standing out as the most effective candidate for integrated pest control. The simplicity of preparing and applying these extracts further supports their practical use. To build on these results, future research should employ bioassay-guided fractionation to identify and characterize the active compounds responsible for the molluscicidal effect. Determining their mode of action and environmental safety will be the final critical steps in developing these extracts into sustainable, standardized alternatives.

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