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RESEARCH ARTICLE

Anti-feedant and Molluscicidal Activities of Selected Spices Against *Achatina fulica* (Gastropoda: Achatinidae)

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ABSTRACT

The application of biopesticides is progressively encouraged for its advantages associated with environmental safety and high biodegradability. The objectives of this study were to compare the phytochemical constituents, anti-feedant, and molluscicidal activities of spices against the giant African land snail, *Achatina fulica*. The spices evaluated were clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), black pepper (*Piper nigrum*), cumin (*Cuminum cyminum*) and coriander (*Coriandrum sativum*). Phytochemical constituents of spices were screened by qualitative standard protocols. Anti-feedant activity was investigated by the leaf disc no-choice bioassay method and molluscicidal activity was evaluated by a 72-h contact toxicity test. The phytochemical screening showed positive results for tannins, saponins, alkaloids, flavonoids, terpenoids, and phenols in clove, cinnamon, cumin and coriander ethanolic extracts whereas only tannins, alkaloids and terpenoids were found in the black pepper ethanolic extract. The leaf disc no-choice bioassay revealed that all spices demonstrated anti-feedant activity ($p<0.05$, compared to control). However, only clove (85.33±3.09%), cinnamon (72.35±3.41%) and coriander (81.54±3.88%) ethanolic extracts showed high feeding inhibition activity, whereas black pepper (68.85±3.22%) and cumin (68.96±3.12%) had moderate anti-feedant activity. In the contact toxicity test, clove ethanolic extract (52.67±1.53%) showed moderate molluscicidal activity, meanwhile cinnamon (19.34±2.08%), black pepper (16.65±1.15%), cumin (18.96±1.42%) and coriander (22.67±1.37%) demonstrated low molluscicidal activity. In conclusion, all spices tested in this study showed the presence of important secondary metabolites that contribute to anti-feedant and molluscicidal activities at differening levels.

Keywords: feeding inhibition, giant African snail, natural pesticides, secondary compounds

1. INTRODUCTION

The presence of pests causes losses of up to USD40 billion per year worldwide and affects up to 40% of global agricultural productivity (Niekawa et al., 2021). Among the characteristics of pests are the ability to adapt to environmental changes, reproduce quickly with large numbers of offspring, and grow and reach maturity in a short period of time. They attack crops in many ways such as destroying seedlings, eating young leaves, sucking plant juices, and spreading crop pathogens. Among the most destructive pests in the global agricultural industry are desert locust (*Schistocerca gregaria*), Japanese beetle (*Popillia japonica*), cotton bollworm (*Helicoverpa armigera*), diamondback moth (*Plutella xylostella*), corn rootworm (*Diabrotica virgifera*), stink bugs (*Halyomorpha halys*), brown planthopper (*Nilaparvata lugens*), potato beetle (*Leptinotarsa decemlineata*), fall armyworm (*Spododptera frugiperda*), tobacco whitefly (*Bemisia tabaci*) and golden apple snail (*Pomacea canaliculata*). The diamondback moth is found throughout the continent and destroys cruciferous vegetable crops (Jamtsho et al., 2021). The Japanese beetle is an invasive insect and feeds on veins of leaves (Althoff and Rice, 2022). The desert locust is the most destructive migratory insect and is able to consume nearly all species of crops, including vegetables, fruit trees, grasses, cereal crops, and hard trees (Shuang et al., 2022). The potato beetle has developed resistance to various insecticides and causes potato seedlings to mature slowly and produce less yield (Rondon et al., 2021). Whiteflies are sap-sucking insects and cause yellowing or even death of plants (Abubakar et al., 2022). In aquatic habitats, the golden apple snail is a serious threat to the global rice industry by eating emerging rice plants (Yang et al., 2023).

The land snail, *Achatina fulica* belongs to the class Gastropoda and phylum Mollusca, and is one of the most common pests encountered by gardeners and farmers where it occurs. These pests are herbivores that eat at least 500 types of vegetables and other crops as food, feeding, especially on soft parts such as shoots and young leaves. Damage to seedlings often results in the death of the plant, which means major production losses. Biologically, nearly all land snails are hermaphrodite and capable of laying 25-30 eggs immediately upon maturation, between one and three years of age (Nandy et al., 2022). The number of eggs and their ability to reproduce increases with increasing amounts of food. In addition, the absence of natural predators and resistance to abiotic conditions makes the population of land snails difficult to control (Santos et al., 2018). In general, under optimal environment conditions, land snail populations generate clutches of eggs every two to three months and can reach high densities in a short period of time. Methomyl has been shown to be the most effective synthetic pesticide against land snails followed by deltamethrin, bensultap, lambda-cyhalothrin, diazonix, and chlorpyrifos-ethyl, but these chemicals can be highly toxic to non-target species (Gaber et al., 2022).

Approximately 662 species of spice, belonging to 340 genera and 124 families, have been reported. Spices were classified as aromatic plant substances used to improve food taste (Gupta et al., 2022). Since ancient times, spices have acted not only as colouring, flavouring, and food additives, but also as a natural remedy in the treatment of various diseases (Sachan et al., 2018). Spices are rich in various bioactive compounds, including alkaloids, polyphenols, flavonoids, steroids, and essential oils and are also rich in key nutrients such as calcium, iron, phosphorus and magnesium. Their secondary metabolites have multiple functional properties, including antioxidant, antibacterial, antiviral, anti-hyperglycaemic, anti-hypertension, anticancer, and anti-hyperlipidaemia (Murokore et al., 2022). In addition, many studies have shown the pesticide activities of secondary metabolites obtained from spices. For example, secondary metabolites from garlic demonstrated larvicidal activity against tobacco cutworm (*Spodoptera litura*), cumin toxic towards cowpea beetle (*Callosobruchus maculatus*), ginger causing death to adzuki bean weevil (*Callosobruchus chinensis*) and black pepper inhibited development of Australian wheat weevil (*Rhyzopertha dominica*) (Ravi et al., 2019). This fact opens up the potential in the modern agricultural age for spices to be used widely as pesticides. While awareness has emerged of the importance of replacing the use of synthetic pesticides with plant-based pesticides, the number of commercial pesticides based on plants like spices remains small

(Ngegba et al., 2022). Pesticides based on plants have many advantages over synthetic pesticides, as they are easier to decompose in the environment and less harmful, apart from limited action on target pests and not on other living organisms (Ndolo et al., 2019). Major classes of synthetic pesticides (carbamates, organophosphates, organochlorines, pyrethroids, and neonicitinoids) have been associated with impacts to human health (respiratory, endocrine, dermatological, neurological, gastrointestinal, carcinogenic and reproductive effects) and environmental issues (threats to aquatic and terrestrial biodiversity). Looking at these serious effects, it is reasonable for the main players in the agricultural industry to consider completely replacing the use of synthetic pesticides with plant-based pesticides that are more environmental friendly.

Reviewing the current literatures, there is still a lot of research that can be explored to unlock the pesticidal potential of spices. Thus, in this research, the phytochemical screening, anti-feedant, and molluscicidal activities of ethanolic extracts of spices were investigated to compare and explore their potential to impact and control the populations of land snails. To achieve the objective, five types of spices, namely, clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*), black pepper (*Piper nigrum*), cumin (*Cuminum cyminum*) and coriander (*Coriandrum sativum*), were selected to investigate their effects on land snails.

2. MATERIALS AND METHODS

2.1. Animals

Land snails (*Achatina fulica*) were collected by hand picking from a field area at the Universiti Teknologi MARA (Tapah Campus), Malaysia. The snails were left to acclimatize to laboratory conditions (at room temperature with natural fluctuations and a natural light-dark photoperiod) for seven days. Only the active snails were selected for the experiments. The snails were fed on fresh leaves of lettuce and kept separately in plastic aquaria containing wet tissue. Then all snails were divided into six groups with 18 snails in each group. Each group subsequently underwent anti-feedant (Arivoli and Tennyson, 2013) and molluscicidal assays (Al-Sarar et al., 2012).

2.2. Plant Materials and Ethanolic Extraction

Spices used in the current study (clove, *Syzygium aromaticum*; cinnamon, *Cinnamomum verum*; black pepper, *Piper nigrum*; cumin, *Cuminum cyminum* and coriander, *Coriandrum sativum*) were obtained from a local supermarket and ground manually to reduce the size (coarse powder). Coarse powder was macerated with 80% ethanol at a ratio of 1:5 (w:v), soaked for three days, and filtered later using Whatman Grade 1 filter paper. The harvested filtrate was concentrated by using a rotary evaporator (Buchi R-210, Switzerland) and the crude extract was kept at 4^0C until used.

2.3. Qualitative Phytochemical Screening

The ethanolic extracts were assessed for the existence of phytochemical elements by using qualitative standard protocols. Tannins were screened by the ferric chloride test (Singh et al., 2022). A few drops of ferric chloride solution were mixed with the extract. Formation of a solution with the green, blue, or black colour indicated the presence of tannins. Saponins were screened by the froth test (Hui et al., 2018). Five ml of distilled water was mixed with the extract in a test tube and was vortexed vigorously. The frothing was mixed with a few drops of olive oil and vortexed vigorously. The appearance of foam showed the presence of saponins.

Alkaloids were screened with the Mayer's test (Sapunyo et al., 2023). Two drops of Mayer's reagent were added to the extract and the formation of a creamy precipitate indicated the presence of alkaloids. Flavonoids were screened by the alkaline reagent test (Hui et al., 2018). Two ml of 2% NaOH was mixed with the extract to produce a yellow solution. Flavonoids were present if this solution became colourless when two drops of diluted acid were added. Terpenoids or steroids were screened using Salkowski's test (Morsy, 2014). Two ml of chloroform were mixed with the extract, followed by three ml of concentrated sulfuric acid. Formation of a reddish-brown precipitate indicated the presence of terpenoids. Phenolic compounds were screened by the lead acetate test (Muhamad et al., 2023). A few drops of 10% lead acetate solution were mixed with the extract. Formation of white precipitate indicated the presence of phenolic compounds. All chemicals were purchased from Sigma-Aldrich (USA) *via* a local supplier (Aman Semesta Enterprise, Malaysia) unless otherwise stated.

2.4. Anti-feedant Assay

The anti-feedant assay was conducted using the leaf disc no-choice bioassay method (Arivoli and Tennyson, 2013) in triplicate. The stock concentrations of each spice crude extract (10%) were prepared by dissolving in distilled water. Fresh lettuce leaf (5 g) was dipped in a spice aqueous extract (10%) and kept in an individual aquarium with a single prestarved land snail under laboratory conditions (at room temperature and natural light-dark photoperiod). Each prestarved land snail was allowed to feed on the treated disc for 24 hours. Wet tissue was placed in the aquarium to avoid early drying of leaf discs and the land snail. A leaf disc dipped into distilled water was considered as a negative control. At the end of the experiment, the unconsumed leaf disc was weighted and the anti-feedant index was measured as previously described (Saleem et al., 2017).

$$\mathrm{AFI} = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C} + \mathrm{T}} \times 100$$

where AFI = anti-feedant index; C = leaf consumed in control group; T = leaf consumed in treated group. The observed AFI values were then classified as very high (90% and above), high (70-89%), moderate (50-69%), and low (49% and below) as previously stated by Arivoli and Tennyson (2013).

2.5. Molluscicidal Assay

The molluscicidal assay was performed using the contact toxicity test (Al-Sarar et al., 2012) with some modifications, in triplicate. Land snails were kept in an individual aquarium containing tissue moistened with the spice crude extract (10%) for a length of 72 h. The assay was conducted under laboratory conditions (at room temperature and natural light-dark photoperiod). During the assay, non-treated fresh leaves of lettuce were given *ad libitum* as a food. The absence of movement or reaction confirmed the mortality of snails by probing the snails with a sharp needle. Molluscicidal activity was classified as very high (mortality of snails exceeds 90%), high (70-89%), moderate (50-69%), and low (49% and below).

2.6. Statistical Analysis

The collected data are presented as mean±SEM (the standard error of the mean). Analysis of variance (ANOVA) was used to determine statistically significant differences between means ($p<0.05$) and a post hoc test was performed using the Tukey's test (Minitab Version 20 Statistical Software Package).

3. RESULTS AND DISCUSSION

3.1. Phytochemical Identification

All phytochemical analytical findings are shown in Table 1. The present study qualitatively showed the presence of tannins, saponins, alkaloids, flavonoids, terpenoids, and phenols in the ethanolic extracts of clove, cinnamon, cumin and coriander. Only tannins, alkaloids, and terpenoids were found in the black pepper ethanolic extract. Alkaloids, saponins, flavonoids, and phenolic compounds play important roles as anti-feedant and natural molluscicide, besides being involved in plant growth and reproduction (Koomson et al., 2018). Mandefro and co-workers (2017) demonstrated that the presence of saponins increases the effectiveness of the plant *Achyranthes aspera* as a molluscicide against snails.

Table 1. Phytochemical constituents of spices, ethanolic extracts

Spice	Clove	Cinnamon	Black pepper	Cumin	Coriander
Tannins	+	+	+	+	+
Saponins	+	+	-	+	+
Alkaloids	+	+	+	+	+
Flavonoids	+	+	-	+	+
Terpenoids	+	+	+	+	+
Phenol	+	+	-	+	+

Indicator: + presence, - non-detected

3.2. Anti-feedant Assay

Results indicated that all ethanolic extracts of spices significantly ($p<0.05$) inhibited feeding by the land snails in comparison to the control group (Table 2). The ethanolic extract of clove was the most effective treatment with a feeding inhibition of 85.33±3.09% followed by coriander (81.54±3.88%) and cinnamon (72.35±3.41%). Furthermore, cumin (68.96±3.12%) and black pepper (68.85±3.22%) demonstrated moderate anti-feedant activity compared to the other spices. Previous pharmacological study has shown that spices contain phytochemical constituents or secondary metabolites that contribute to anti-feedant activity (Ntonifor, 2011). Apart from saponins and flavonoids, eugenol in cloves and camphor in coriander have been highlighted as the constituents responsible for their anti-feedant effects (Momin et al., 2012).

Table 2. Anti-feedant activity of spice ethanolic extracts against prestarved land snails. Data are presented as means±SEM (n=18)

Spice	Anti-feedant index (%)
Control	$10.78±3.19^{a}$
Clove	$85.33±3.09^{b}$
Cinnamon	$72.35±3.41^{c}$
Black pepper	$68.85±3.22^{c}$
Cumin	$68.96±3.12^{c}$
Coriander	$81.54±3.88^{b}$

Values with different superscripts within the same column show significant difference at $p<0.05$

3.3 Molluscicidal Assay

Mortality of snail increased significantly ($p<0.05$) in all spice treated groups compared to control. However, the mortality of snail did not differ significantly ($p>0.05$) among the spice treated group, except for the group treated with clove ethanolic extract (Table 3). Only clove ethanolic extract demonstrated moderate molluscicidal activity within 72 h of exposure (52.67±1.53%), whereas cinnamon (19.34±2.08%), black pepper (16.65±1.15%), cumin (18.96±1.42%) and coriander (22.67±1.37%) demonstrated lower molluscicidal activity. This low capacity may be due to the low secondary metabolite content and the length of exposure to the extract. Nonetheless, the current finding supports previous studies that have recorded substantial molluscicidal activity of clove extracts against multiple snail species (Ismail and Abdel-Kader, 2011; Parvate and Thayil, 2017). Apart from saponins, an important secondary metabolite in cloves is eugenol, which is likely responsible for the molluscicidal effect. Inhibition of acetylcholinesterase and alkaline phosphatase in the nervous system of snails by eugenol leads to the molluscicidal activity of spices such as cloves (Kumar et al., 2011).

Table 3. Molluscicidal activity of spice ethanolic extracts against land snails. Data are presented as means±SEM (n=18)

Spice	**Mortality of snail (%)**
Control	2.33 ± 0.57^{a}
Clove	52.67 ± 1.53^{b}
Cinnamon	19.34 ± 2.08^{c}
Black pepper	16.65 ± 1.15^{c}
Cumin	18.96 ± 1.42^{c}
Coriander	22.67 ± 1.37^{c}

Values with different superscripts within the same column show significant difference at $p<0.05$

4. CONCLUSION

This study presented evidence for the bio-pesticide potential of spices against land snails. All five tested spice ethanolic extracts (clove, cinnamon, black pepper, cumin, and coriander) used in this study demonstrated the presence of essential secondary metabolites with potential anti-feedant activity (moderate to high). Nevertheless, only clove ethanolic extract showed moderate molluscicidal activity against land snails, whereas cinnamon, black pepper, cumin, and coriander ethanolic extracts showed low molluscicidal potential. Further elucidation of the structure-activity relationship of spice's secondary metabolites and their mechanisms of action will promote the formulation and production of the next generation of bio-pesticides (anti-feedant or molluscicidal) against land snails.

Declaration of Interest

The authors declare that there is no conflict of interest.

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