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Assessment of the molluscicidal activity of wormwood (*Artemisia dubia*, Wallich) leaves ethanolic extract on *Oncomelania hupensis quadrasi*, Möllendorff

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Abstract: This study assessed the in vitro molluscicidal activity of *Artemisia dubia* (wormwood) leaf ethanolic extract against adult and juvenile *Oncomelania hupensis quadrasi* and profiled its qualitative phytochemical content. The plants and snails were collected locally. There were 8 concentrations used for adult and juvenile snails: distilled water and 1% ethanol as negative controls; niclosamide (2 mg/L) as a positive control; and different concentrations of wormwood ethanolic extracts for adult snails including 3.98% (T1), 4.46% (T2), 5% (T3), 5.61% (T4), and 6.30% (T5) and for juvenile snails including 7.94% (T1), 8.91% (T2), 10% (T3), 11.22% (T4), and 12.59% (T5). Each treatment was replicated 5 times with 20 snails per replicate. Results showed that the concentrations that were statistically comparable with niclosamide (2 mg/L) were 6.30% (T5) with 92% mortality for adults and 10% (T3), 11.22% (T4), and 12.59% (T5) for juveniles with 94%, 95%, and 98% mortality, respectively ($P > 0.05$). The qualitative phytochemical tests for secondary metabolites revealed the presence of tannins, saponins, and terpenoids. Independently or in combination, these secondary metabolites may be responsible for the mortality of the snails. This study indicates the possibility of using wormwood ethanolic extract as a potent and a possible alternative for synthetic molluscicides.

Key words: In vitro, mortality, schistosomiasis, snails

1. Introduction

Eliminating *Oncomelania hupensis quadrasi* (family Pomatiopsidae; Möllendorff, 1895) is important in schistosomiasis control. The control of these mollusks through the use of molluscicides will eliminate them and interrupt the life cycle of *Schistosoma japonicum* (family Schistosomatidae; Katsurada, 1904), and it keeps the prevalence of schistosomiasis in both animals and humans at a low level [1]. In developing countries where schistosomiasis is endemic, snail control is difficult owing to the high cost of synthetic compounds. This has resulted in frequent and increased studies on plants as alternative source of molluscicides [2,3].

Artemisia dubia var. *orientalis*, known as wormwood (family Compositae; Pamp., 1930), is considered one of the herbal plants that have potential molluscicidal activity. Species of plants of the genus *Artemisia* have been used for centuries as moth repellants, general pesticides, and as tea or spray to repel slugs and snails [4,5]. Thus, this study investigated the use of wormwood leaf ethanolic extract as molluscicides, specifically on *O. hupensis quadrasi*, and determined its potential as a molluscicide. It may be able to control the *O. hupensis quadrasi* populations where schistosomiasis is prevalent.

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2. Material and methods

2.1 Collection of wormwood leaves

The plants were collected from Barangay Gabas, Baybay City, Leyte, Philippines during the growth stage between in April and May and were identified by a botanist, Dr. Beatriz S. Belonias of the Department of Biological Sciences, College of Arts and Sciences, Visayas State University. The identification of plants was based on morphological keys as formerly described by previous studies [6,7]. The dirt and debris from the leaves were washed with running tap water. After washing, the leaves were air-dried at room temperature for 3 days [8]. Dried leaves were chopped finely into manageable pieces, ground finely, and air-dried again at room temperature for 7 days to remove the remaining moisture content of the leaves [9].

2.2. Extraction of bioactive compounds

Ground dried leaves were completely submerged with ethanol for infusion for 48 h. After infusion, the preparation was filtered using a funnel topped with filter paper. The filtrate was placed in a beaker and the plant leaf residue was discarded. The filtrate was concentrated in a rotary evaporator at 40 °C and recovered 1/3 of its original volume. The recovered concentrated extract of wormwood

leaves ethanolic extract (wormwood EE) was stored in a properly labeled and sealed amber bottle. It was kept in the refrigerator until needed, but not more than 7 days to preclude possible contamination.

2.3. Phytochemical analysis

Qualitative phytochemical analysis of the wormwood EE was conducted by determining the following secondary metabolites: for alkaloids (Mayer's test and Wagner's test), a 2% HCl was mixed in 5 mL of extract and placed in a steam bath for warming; 2 different preparations were made and were added with 2 drops of Mayer's and Wagner's solutions, respectively. For tannins (tannins test), 2 drops of 5% $FeCl_3$ were added to 2 mL of extract; for saponins (Froth test), the extract in a test tube was simply shaken; for flavonoid tests, both alkaline reagent and lead acetate tests were performed, wherein 2–3 drops of 20% sodium hydroxide solution and 10% acetate solution were added to 2 mL of the prepared extracts; and for terpenoids (chloroform and sulfuric acid test), 2 mL of the extract was added and shaken into 2 mL of chloroform and 5 mL of concentrated sulfuric acid was added carefully. A qualitative grading system of the phytochemicals was applied as follows: strongly positive (+++), moderately positive (++), weakly positive (+), traces (-), and undetected (0) [10].

2.4. Collection of *Oncomelania hupensis* quadrasi

Adult and juvenile *O. hupensis quadrasi* snails were collected from the municipality of Palo, Leyte, Philippines. The collected snails were identified by a zoologist, Emelda R. Legaspi of the Center for Health Development, Department of Health - Eastern Visayas, using a previously described identification key [11]. Protective gears were used during the collection to avoid contamination and possible infection with *Schistosoma japonicum*. The snails were placed in a plastic container layered with moistened filter paper during transport and were transferred to an aquarium with an aerator in the laboratory until used for assays. Prior to assays, snails were sorted into juvenile and adult groups. Juvenile snails measure up to a maximum of 3 mm and snails that reached more than 3 mm were considered adult [12].

2.5. Molluscicidal assay

The different treatments and corresponding concentrations of wormwood EE used were as follows: $T0_{(-)1}$ - distilled water and $T0_{(-)2}$ - 1% ethanol as negative controls; $T0_{(+)}$ - 0.0002% of niclosamide as a positive control; for adult snails T1 (3.98%), T2 (4.46%), T3 (5%), T4 (5.61%), and T5 (6.30%); and for juvenile snails T1 (7.94%), T2 (8.91%), T3 (10%), T4 (11.22%), and T5 (12.59%). All treatments were replicated 5 times with 20 snails per replicate. The different concentrations were computed based on preliminary trials conducted, wherein the LC_{50} values of wormwood EE for both adult and juvenile *O. hupensis quadrasi* were determined. The LC_{50} values for adult and juvenile snails were 4.66 and 4.69, respectively. The LC_{50} was based on a probit analysis at 95% confidence level. After the establishment of the LC_{50}, 2 higher and lower concentrations were computed through logarithmic methods.

The adult and juvenile snails were sorted in different petri dishes and tested separately. For the assay, a flooding technique [9] was carried out with viable snails to determine the molluscicidal efficacy of wormwood EE. The snails were observed and viewed under a 10× scanning objective; they were considered viable when their head-foot part protruded from the shell and their tentacles were extended as if attempting to crawl. Each snail was placed in a petri dish with individual circles (5 mm diameter) drawn on the plate and 10 mL of different concentrations of the extract was added. The assay was conducted for 24 h, after which the snails were transferred to another petri dish with distilled water and left for another 24 h to observe further whether they were dead or alive.

For the motility test, snails that moved out from the circles were considered alive, while those that remained immobile and retained their positions after 3 h were further tested for sensitivity.

For the sensitivity test, snails were observed under a microscope (10×) and it was checked whether the snails were sensitive to mechanical stimulation through needle prodding. Live snails usually retract their operculum when prodded. Immobile snails that were sensitive to prodding were considered alive.

2.6. Data evaluation

The experiment was laid out in completely randomized design. Analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) test were used to analyze the variation among groups and to determine the comparisons among groups, respectively. The differences among means were considered statistically significant at $P < 0.05$. The statistical analysis was carried out with SPSS (IBM Corp., Armonk, NY, USA).

3. Results

For the adult snails, variation in the number of dead snails was observed and was concentration-dependent. The % efficacy of wormwood EE was based on the percent mortality of the dead snails [13]. For 6.30% (T5) and 5.61% (T4), both treatments were highly effective with a mortality rate of 92% and 84%, respectively. This result was statistically comparable with niclosamide ($P > 0.05$). On the other hand, 5% (T3) had a mortality rate of 63%, 4.46% (T2) a rate of 38%, and 3.98% (T1) a rate of 23%; these were considered ineffective and not comparable among treatments including niclosamide ($P < 0.05$). Based on the percent efficacy of the European Agency for the Evaluation of Medicinal Products, treatments T1, T2, T3, and T4 were

considered not effective since the mortality rate was <90%, while T5 was considered effective as its mortality rate was >91%.

For juveniles, variation in the number of dead snails was also concentration-dependent. The efficacy of wormwood EE based on the percent mortality for 7.94% (T1) was 86%, for 8.91% (T2) was 90%, for 10% (T3) was 94%, for 11.22% (T4) was 95%, and for 12.59% (T5) was 98%, and these were all considered to be highly effective [13]. Based on the percent efficacy of the European Agency for the evaluation of Medicinal Products, T1 and T2 were considered not effective and not comparable with niclosamide ($P < 0.05$). On the other hand, T3, T4, and T5 were considered effective as the mortality rates were >91%. Overall, T3, T4, and T5 are statistically comparable with commercially available molluscicide niclosamide ($P > 0.05$) (Table 1).

Qualitative phytochemical tests were conducted in order to determine the possible bioactive components that might have contributed to the molluscicidal activity of the wormwood EE. Based on the phytochemical tests conducted, the secondary metabolites that were found to be positive were tannin, saponin, and terpenoids (Table 2). A condensed tannin was indicated with blue-black and brownish-green discoloration of the test solutions; for saponin, a copious lather formation indicates a positive result; a reddish-brown coloration of the test solution was positive for terpenoids. Mayer's test and Wagner's test for alkaloids both showed negative results, as did the alkaline test and ferric chloride test for flavonoids.

4. Discussion

The wormwood EE exhibited potential molluscicidal activity against adult and juvenile *O. hupensis quadrasi* at 24 h of exposure. One of the bioactive components that is found in *Artemisia* spp. is vulgarone B, and this bioactive component, although not isolated and quantitatively determined in this study, is known to have molluscicidal activity [4,14]. Phytochemical tests of wormwood EE revealed the presence of tannins, saponins, and terpenoids. Previous studies indicated that these compounds were also present in concentrated extracts of other *Artemisia* spp. [15] and in different solvent extracts of *Artemisia dubia* [5,16]. The presence of these secondary metabolites as bioactive components that were identified during the qualitative phytochemical tests may be responsible for the molluscicidal activity of wormwood EE. These classes of compounds independently or in combination may be responsible for the mortality of snails [9,13].

It is well known that tannin-bearing plants have molluscicidal activity and these plants are generally avoided by mollusks [17,18]. Previous studies indicated that plants containing hydrolysable and condensed tannins exhibited strong molluscicidal activity against *Biomphalaria glabrata*, an intermediate host of *S. japonicum* [19]. In a different study, a bark extract of *Stryphnodendron polyphyllum* contained rich tannins and demonstrated promising molluscicide action against *Biomphalaria glabrata* [20]. Saponins have also been reported as potent molluscicides [20,21,22] with hemolytic properties and have toxic effects against cold-blooded animals including snails [23]. In addition, some effects of saponins on animal cells are the formation of a complex reaction with plasma and membrane cholesterols causing cell membrane damage [24]; the molluscicidal activity of terpenoids could be due to the reaction of the compound with thiol-containing enzymes [25,26]. Terpenoids can diffuse easily across cell membranes and induce biological reactions [27]. Most

Table 1. Mortality rates of wormwood leaf ethanolic extract against adult and juvenile *Oncomelania hupensis quadrasi* (mean ± SEM).

Treatment	Mean no. of snails	Mean no. of dead adult snails	% Mortality	Mean no. of snails	Mean no. of dead juvenile snails	% Mortality
$T0_{(-)1}$	20	0 ± 0	0^{a} ± 0	20	0 ± 0	0^{a} ± 0
$T0_{(-)2}$	20	0 ± 0	0^{a} ± 0	20	0 ± 0	0^{a} ± 0
$T0_{(+)}$	20	20 ± 0	100^{f} ± 0	20	20 ± 0	100^{d} ± 0
T1	20	4.6 ± 0.55	23^{b} ± 2.74	20	17.2± 1.30	86^{b} ± 6.52
T2	20	7.6 ± 0.89	38^{c} ± 4.47	20	18 ± 1.22	90^{bc}± 6.12
T3	20	12.6 ± 1.82	63^{d} ± 9.08	20	18.8 ± 1.0	94^{bcd}± 6.52
T4	20	16.8 ± 0.84	84^{e} ± 4.14	20	19 ± 1.0	95^{bcd} ± 5.0
T5	20	18.4 ± 0.55	92^{ef} ± 2.74	20	19.6 ± 0.55	98^{cd} ± 2.74

Mean % mortalities within a column with different superscripts are significantly different ($P < 0.05$).
No. of replicates per treatment = 5; no. of snails per replicate = 20; N = 100.

Table 2. Secondary metabolites of wormwood leaves ethanolic extract.

Phytochemical	Wormwood ethanolic extract
Alkaloids	0
Tannins	++
Terpenoids	+++
Flavonoids	0
Saponins	++

+++ Strongly positive, ++ moderately positive, + weakly positive, - traces, 0 undetected [10].

likely their action involves the alkylation(s) of the essential enzymes of the snail's metabolism, resulting in death [28,29].

Two separate assays were performed for juvenile and adult snails. Juvenile snails are more susceptible and sensitive to molluscicides than adults [30]. Morphological, physiological, and biochemical characteristics may be completely different between life stages [31]. The susceptibility of adults to chemical agents is less compared to younger or neonatal organisms. This mechanism could be attributed to the capability of an adult snail of excreting toxic chemicals and is related to age-dependent toxicokinetics and the influence of body size [32].

The efficacy of wormwood EE may vary according the level of bioactive compounds present [33]. Other factors such as the type of soil, temperature, altitude, radiation, atmosphere, light exposure, and nutrition can influence the variability in a plant's secondary compounds that could affect the safety and reliability of the plant's efficacy [34]. The use wormwood EE as a molluscicide in actual field trials is highly recommended to determine if the efficacy is consistent and would not contradict results under laboratory conditions.

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