CHARACTERIZATION OF MOLLUSCICIDAL COMPONENT OF *Moringa oleifera* LEAF AND *Momordica charantia* FRUITS AND THEIR MODES OF ACTION IN SNAIL *Lymnaea acuminata*

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**SUMMARY**

The molluscicidal activity of the leaf powder of *Moringa oleifera* and lyophilized fruit powder of *Momordica charantia* against the snail *Lymnaea acuminata* was time and concentration dependent. *M. oleifera* leaf powder (96 h LC₅₀: 197.59 ppm) was more toxic than *M. charantia* lyophilized fruit powder (96 h LC₅₀: 318.29 ppm). The ethanolic extracts of *M. oleifera* leaf powder and *Momordica charantia* lyophilized fruit powder were more toxic than other organic solvent extracts. The 96 h LC₅₀ of the column purified fraction of *M. oleifera* leaf powder was 22.52 ppm, while that of *M. charantia* lyophilized fruit powder was 6.21 ppm. Column, thin layer and high performance liquid chromatography analysis show that the active molluscidal components in *M. oleifera* leaf powder and lyophilized fruit of *M. charantia* are benzylamine (96 h LC₅₀: 2.3 ppm) and momordicine (96 h LC₅₀: 1.2 ppm), respectively. Benzylamine and momordicine significantly inhibited, *in vivo* and *in vitro*, the acetylcholinesterase (AChE), acid and alkaline phosphatase (ACP/ALP) activities in the nervous tissues of *L. acuminata*. Inhibition of AChE, ACP and ALP activity in the nervous tissues of *L. acuminata* by benzylamine and momordicine may be responsible for the molluscicidal activity of *M. oleifera* and *M. charantia* fruits, respectively.

**KEYWORDS:** Acetylcholinesterase; *Lymnaea acuminata*; *Momordica charantia*; *Moringa oleifera*; Phosphatases.

**INTRODUCTION**

Fascioliasis caused by *Fasciola* species is of considerable medical and veterinary importance. It severely affects breeding in cattle, goats, horses, ovines and swine, resulting in serious losses which ultimately affect the economy of live-stock keepers in many countries. The control of the snail population with the help of molluscicides is one of the major tools to reduce the incidence of fascioliasis in cattle as well as in human beings. The development of plant molluscicides, as possible substitutes for synthetic molluscicides, is gaining more attention because they are effective, less expensive and eco-friendly. Many plant products have been found to have a high molluscidal potential.

In the present study, the molluscidal activities of the leaf powder of *Moringa oleifera* Lam. (Moringaceae) and lyophilized fruit powder of *Momordica charantia* Lam. (Cucurbitaceae) against the target snail *Lymnaea acuminata* were evaluated. Active molluscidal components responsible for snail death were isolated, characterized and their effects on acetylcholinesterase (AChE) and acid / alkaline phosphatase (ACP/ALP) activity in the nervous tissue of *L. acuminata* were evaluated.

**MATERIALS AND METHODS**

One afternoon in Gorakhpur (Latitude 26° 46’ N, Longitude 83° 22’ E), U.P. India, fresh *M. oleifera* leaves from the University campus and fresh *M. charantia* fruits from the local agricultural fields were obtained. The specimens of *M. oleifera* leaf (voucher specimen number M-5132) and *M. charantia* fruit (voucher specimen number M-3431) were identified and authenticated by Prof. R.P. Shukla, Taxonomist, Department of Botany, DDU Gorakhpur University, Gorakhpur, U.P. India.

1. **Preparation of crude powder:** Leaves of *M. oleifera* were dried in an incubator at 37 °C for 24 h and then pulverized in an electric grinder (Twister mixer grinder, 410025). The crude powder, thus obtained, was used for the toxicity experiments. The fresh paste of *M. charantia* was obtained by grinding small pieces of fruit with water. This aqueous paste was lyophilized at -40 °C. The lyophilized fruit powder was stored in airtight desiccators and used for toxicity experiments.

2. **Preparation of organic solvent extracts:** Five grams each of crude powder of *M. oleifera* leaf and *M. charantia* fruit were extracted separately with 100 mL of each solvent viz. chloroform, ether, acetone and ethanol at room temperature for 24 h. Each preparation was filtered separately through sterilized Whatman no.1 filter paper and the filtered extracts were subsequently evaporated under vacuum at 24 °C. The residues, thus obtained, were used for the determination of molluscidal activity. The leaf powder of *M. oleifera* yielded 230 mg of chloroform residues, thus obtained, were used for the determination of molluscidal activity. The leaf powder of *M. oleifera* yielded 230 mg of chloroform residues, thus obtained, were used for the determination of molluscidal activity. The leaf powder of *M. oleifera* yielded 230 mg of chloroform residues, thus obtained, were used for the determination of molluscidal activity. The leaf powder of *M. oleifera* yielded 230 mg of chloroform residues, thus obtained, were used for the determination of molluscidal activity. The leaf powder of *M. oleifera* yielded 230 mg of chloroform residues, thus obtained, were used for the determination of molluscidal activity. The leaf powder of *M. oleifera* yielded 230 mg of chloroform residues, thus obtained, were used for the determination of molluscidal activity. The leaf powder of *M. oleifera* yielded 230 mg of chloroform residues, thus obtained, were used for the determination of molluscidal activity. The leaf powder of *M. oleifera* yielded 230 mg of chloroform residues, thus obtained, were used for the determination of molluscidal activity.
extract, 355 mg of ether extract, 442 mg of acetone extract and 460 mg of ethanol extract. The lyophilized fruit powder of *M. charantia* yielded 50 mg of chloroform extract, 62 mg of ether extract, 260 mg of acetone extract and 1000 mg of ethanol extract.

3. Column chromatography: Twenty five mL each of the ethanolic extracts of *M. oleifera* leaf powder and *M. charantia* lyophilized fruit powder were subjected to silica gel (60-120 mesh, Qualigens glass, Precious Electrochemical industry, Pvt. Ltd. Mumbai, India) chromatography in a 5 cm × 45 cm column. Five mL fractions of 90 and 60 eluates were eluted with 95% ethanol for each column preparation of *M. oleifera* leaf powder and lyophilized fruit powder of *M. charantia*, respectively. Eluate nos. 20-30 (*M. oleifera* leaf powder) and 30-40 (*M. charantia* lyophilized fruit powder) were used for toxicity studies. Ethanol was evaporated under vacuum at 24 °C and the residues were used for the determination of molluscicidal activity.

4. Pure compounds: Benzylamine (1-Phenylmethanamine) was purchased from Sigma Chemical Co., USA. and momordicine (3,7,23-trihydroxycucurbita-5,24-dien-19-al) from Yian Khonest Bio-Tech Co. Ltd., China.

5. Thin layer chromatography: Thin layer chromatography (TLC) was performed according to the method of JAISWAL & SINGH to identify the active molluscicidal components in *M. oleifera* leaf powder and *M. charantia* lyophilized fruit powder. Thin layer chromatography was carried out on 20 cm × 20 cm precoated silica gel (Precious Electrochemical industry, Pvt. Ltd. Mumbai, India) using benzene/ethyl acetate (9:1, v:v) as the mobile phase. The loading of column purified fractions of *M. oleifera* leaf powder and *M. charantia* lyophilized fruit powder along with their respective active components were applied on TLC plates with a micropipette. TLC plates were developed with I2 vapor. Copies of chromatograms were made by tracing the plates immediately and Rf values were calculated.

6. High performance liquid chromatography: Identification of active compounds present in *M. oleifera* leaf and *M. charantia* fruit were done by HPLC.

6.1. Sample preparation: *M. oleifera* leaf and *M. charantia* fruit samples were prepared by dissolving separately 50 mg each of their column chromatographed extracts in 20 mL of acetonitrile. The samples were properly vortexed to ensure dissolution. Prior to sample injection, the solutions were passed through a Millipore filter (ultra filter disc 3K 43 mm 10 pk, Cole Parmer, Germany) to remove any undissolved particles.

6.2. Preparation of standard solution: Pure standard solutions of benzylamine (0.01 M) and momordicine (0.001 M) were prepared by diluting 0.01 mL of benzylamine in 20 mL of acetonitrile and dissolving 10 mg of momordicine in 20 mL of acetonitrile. The mixtures were vortexed to ensure proper dissolution of pure compounds. The solutions, thus obtained, were passed through Millipore filter (ultra filter disc 3K 43 mm 10 pk, Cole Parmer, Germany).

6.3. Instrumentation: The HPLC system was equipped with two LC-10 AT VP pumps, a Cecil CE 4201 UV-variable detector and a Microliter® #702 (Hamilton-Bonaduz, Schweiz) syringe with a loop size of 20 μL. Reverse-phase chromatographic analysis was carried out under isocratic conditions using a reverse-phase Luna 5 µ C18 Phenomenex column (250 mm × 4.6 mm) at 27 °C. Acetonitrile (HPLC grade) was used as the mobile phase solvent under a pressure of 260-270 Kgf/cm2 and run time of 15 min. The analysis was carried out at a flow rate of one mL/min., the column effluent being monitored at 260 nm. Data acquisition were done with Power Stream software.

7. Collection of snails: Adult freshwater snails (*L. acuminata*, 2.25 cm ± 0.20 cm in shell length) were collected locally from different ponds in Gorakhpur, India. They were acclimatized for 72 h to laboratory conditions in a glass aquarium containing dechlorinated tap water (Temp., 22-24 °C; pH, 7.1-7.3; dissolved oxygen, 6.5-7.2 ppm; free carbon dioxide, 5.2-6.3 ppm; bicarbonate alkalinity, 102-105 ppm).

8. Treatment protocol for concentration-response relationship: The toxicity experiments were performed according to the method of SINGH & AGARWAL. Ten experimental snails were kept in a glass aquarium containing 3 L of dechlorinated tap water. Snails were continuously exposed for 96 h to different concentrations of plant products, separately. Six aquaria were set up for each concentration. Control snails were kept in the equal volumes of dechlorinated tap water under similar conditions without treatment. Snail mortality was assessed at 24 hourly intervals up to 96 h. Dead animals were promptly removed to avoid contamination of aquarium water. Mortality was indicated by the contraction of body within the shell; lack of response to a needle probe was taken as evidence of death. The LC50 values, lower and upper confidence limits (LCL and UCL), slope values, t-ratio, g-values and heterogeneity factors were calculated using the POLO computer software of ROBERTSON et al. The regression coefficient between exposure time and different LC50 values was determined by the method of SOKAL & ROHLF.

9. Bioassays: Each set of experimental snails were exposed to sublethal concentrations; 40% and 80% of 24 h LC50 and 40% and 80% of 48 h LC50 of different molluscsicides for 24 and 96 h, respectively. The sublethal concentrations were based on 24 and 96 h LC50 values obtained from section 8. After 24 and 96 h of exposure, snails were removed from aquaria and rinsed with water. The nervous tissues of snails in experimental and control groups were taken out for the measurement of acetylcholinesterase (AChE), acid phosphatase (ACP) and alkaline phosphatase (ALP) activities.

The in vitro experiments were performed by dissolving benzylamine (0.3, 0.5, 0.7 and 0.8 mM) and momordicine (0.06, 0.11, 0.15 and 0.19 mM) in ether, and 4 mL of each was added separately to 10 mm path length cuvette. Ether was then allowed to evaporate. Molluscicides were pre-incubated with an enzyme source for 15 min at 25 °C, following which enzyme activity was determined. The control cuvette contained ether only. The Michaelis-Menten constant (Km) and maximum velocity (Vmax) were calculated by plotting Lineweaver-Burk plots for the hydrolysis of different concentrations of substrate by treated (0.7 mM of benzylamine and 0.15 mM of momordicine) and untreated enzyme.

10. Enzyme assay

10.1. Acetylcholinesterase: Acetylcholinesterase activity was measured according to the method of ELLMAN et al. as modified by SINGH & AGARWAL. Fifty milligrams of the nervous tissue of
L. acuminata taken around the buccal mass was homogenized in 1.0 mL of 0.1 M phosphate buffer pH 8.0 for five min in an ice bath and centrifuged at 1000g for 30 min at 4 °C. The supernatant was used as the enzyme source. Enzyme activity was measured in a 10 mm path-length cuvette using an incubation mixture consisting of 0.1 mL of enzyme source, 2.9 mL of 0.1 M phosphate buffer pH 8, 0.1 mL of chromogenic agent DTNB (5,5'-dithio-bis-2-nitrobenzoic acid) and 0.02 mL of freshly prepared ATChl (acetylthiocholine iodide) solution in distilled water. The change in optical density at 412 nm was recorded every 30 secs for three min at 25 °C. Enzyme activity was expressed as µmol ‘SH’ hydrolyzed/min/mg protein.

For the estimation of the kinetic constants of AChE, in vitro inhibition of the enzyme was carried out at different concentrations (3.0 × 10⁻⁴, 5.0 × 10⁻⁴, 7.0 × 10⁻⁴ and 1.0 × 10⁻³ M) of the substrate acetylthiocholine iodide.

10.2. Acid and alkaline phosphatase: Acid and alkaline phosphatase activities in the nervous tissue of L. acuminata were measured according to the method of BERGMeyer and SINGH & AGARWAL using p-Nitrophenyl phosphate as the substrate. Tissue homogenate (2%, w/v) was prepared in ice cold 0.9% NaCl and centrifuged at 5000g for 15 min at 4 °C. The supernatant was used as the enzyme source. Standard curves were drawn with p-nitrophenol. The yellow color developed due to the formation of p-nitrophenol was determined colorimetrically at 420 nm. Enzyme activities of acid and alkaline phosphatases (ACP/ALP) were expressed as µmol substrate hydrolyzed/30 min/mg protein.

For the determination of the kinetic constants of acid and alkaline phosphatases, in vitro inhibition of the enzymes was carried out at different concentrations (1.25 × 10⁻⁴, 1.8 × 10⁻⁴, 3.0 × 10⁻⁴ and 5.4 × 10⁻⁵ M) of the substrate p-nitrophenyl phosphate.

11. Protein: Protein estimation was carried out according to the method of LOWRY et al. using bovine serum as a standard. 1.0 mL of enzyme supernatant was mixed in 5.0 mL of 5% trichloroacetic acid TCA and centrifuged at 6000 g for 20 min. The precipitate was washed with 5.0 mL of 5% TCA and again centrifuged at the same speed for 20 min. The precipitate was dissolved in 4.0 mL of 1 N NaOH. One mL of dissolved solution was mixed with 5.0 mL of reagent C ( 50 mL of 2% sodium carbonate in 0.1 N NaOH (reagent A) mixed with 1 mL of copper sulphate in 1% sodium potassium tartrate (reagent B)), and the mixture was left standing for 10 min at room temperature. In the reaction mixture, 0.5 mL of reagent D (freshly prepared phenol reagent and distilled water in 1:2 ratio) was added and mixed thoroughly. Resulting blue color was monitored in absorbance at 600 nm after 10 min. Standard curves were prepared with different concentrations of bovine serum albumin.

12. Statistical analysis: Each experiment was replicated at least six times and the results expressed as mean ± SE of six replicates. The Student’s t-test was used to test for any significant variation (p < 0.05) between control and treated groups.

RESULTS

1. Molluscicidal activity: The toxicity of different extracts of the leaf powder of M. oleifera and lyophilized fruit powder of M. charantia were time and concentration dependent. The 24 h and 96 h LC₅₀ values of the leaf powder of M. oleifera were 602.75 ppm and 197.6 ppm, respectively (Fig. 3) while the corresponding values for lyophilized M. charantia fruit powder were 1249.12 and 318.29 ppm, respectively (Fig. 4). Maximum toxicities were recorded with the ethanolic extracts (Fig. 3-4). The column-purified fractions of M. oleifera and M. charantia were highly toxic. The 24 h and 96 h LC₅₀ values of the column-purified fraction of M. oleifera leaf powder were 53.16 and 22.52 ppm, respectively while those of the column-purified fraction of lyophilized fruit powder of M. charantia were 12.33 and 6.21 ppm, respectively. The 24 h LC₅₀ values of benzylamine and momordicine were 14.4 and 10.0 ppm, respectively (Fig. 5-6).

The slope values were steep and separate estimation of LC₅₀ based on each of the six replicates, were found to be within 95% confidence limits of LC₅₀. The t-ratio was higher than 1.96 and heterogeneity factor was less than 1.0. The g-value was less than 0.5 at all the probability levels i.e. 0.95, 0.99. There was significant negative regression (p < 0.05) between exposure time and LC₅₀ values (Fig. 1-2).

The thin layer chromatography analysis showed that the RI values of benzylamine (0.06) and momordicine (0.13) were equivalent to the RI values of column-purified fractions of M. oleifera (0.06) and M. charantia (0.13).

Identification of active components was done by comparing the retention time (Rt) and chromatographic peaks of M. oleifera leaf and M. charantia fruit samples with their respective active components,
benzylamine and momordicine (Fig. 3–6). The HPLC fingerprint profiles of *M. oleifera* leaf and *M. charantia* fruit samples showed major peaks at the retention time of 2.59 min and 8.45 min, respectively, whereas, the pure standard solutions of benzylamine and momordicine showed major peaks at the retention time of 2.58 min and 8.49 min, respectively.

2. *In vivo* inhibition of enzymes

2.1. Acetylcholinesterase: Table 1 shows that acetylcholinesterase activity in the nervous tissue of control *L. acuminata* was 0.74 µmol ‘SH’ hydrolyzed/min/mg protein. *In vivo* exposure to 40% and 80% of 24 and 96 h LC_{50} of benzylamine and momordicine caused significant inhibition of AChE activity in the nervous tissue of *L. acuminata*. AChE activity decreased to 85.27% and 64.32% of control values after exposure to 80% of the 24 h LC_{50} of benzylamine and momordicine, respectively. Maximum inhibition of AChE activity was observed when snails were exposed to 80% of the 96 h LC_{50} values of momordicine (48.91% of control) and benzylamine (57.02% of control) (Table 1).

2.2. Acid phosphatase: Acid phosphatase activity in the nervous tissue of control *L. acuminata* of control group was 35.44 µmol substrate hydrolyzed /30 min/mg protein (Table 1). *In vivo* exposure to 40% and 80% respectively, of the 24 and 96 h LC_{50} values of benzylamine and momordicine caused significant inhibition of ACP activity in the nervous tissue of *L. acuminata*. ACP activity decreased to 55.30% and
53.52% of control values after exposure to 80% of the 24 h LC₉₀ values of benzylamine and momordicine, respectively. Maximum inhibition of ACP activity was observed when snails were exposed to 80% of the 96 h LC₉₀ of benzylamine (40.74% of control) and momordicine (42.40% of control) (Table 1).

### 2.3. Alkaline phosphatase: Alkaline phosphatase activity in the nervous tissue of control L. acuminata was 32.44 µmol substrate hydrolyzed /30 min/mg protein (Table 1). In vivo exposure to 40% and 80% respectively of the 24 and 96 h LC₉₀ values of benzylamine and momordicine caused significant inhibition of ACP activity in the nervous tissue of L. acuminata. ALP activity decreased to 75.30% and 59.55% of control values after exposure to 80% of the 24 h LC₉₀ values of benzylamine and momordicine, respectively. Maximum inhibition of ALP activity was observed when snails were exposed to 80% of the 96 h LC₉₀ values of momordicine (53.20% of control) and benzylamine (63.62% of control) (Table 1).

### 3. In vitro inhibition of enzymes: In vitro pre-incubation with 0.3, 0.5, 0.7 and 0.8 mM benzylamine and 0.06, 0.11, 0.15 and 0.19 mM momordicine caused significant dose dependent inhibition of AChE, ACP and ALP activities (Table 2). In vitro exposure to 0.7 mM of benzylamine and 0.15 mM of momordicine reduced AChE, ACP and ALP activity in the nervous tissue of L. acuminata (Table 2).

Fig. 7 shows Lineweaver-Burk plots of benzylamine and momordicine inhibited and uninhibited AChE activities at different substrate concentrations. This plot shows that the Kₘ and Vₘₐₓ values of uninhibited AChE were 7.69×10⁻⁴ M and 1.67 µmol ‘SH’ hydrolyzed/ min/mg protein, respectively (Table 3). Kₘ of benzylamine (Fig. 7A) and momordicine (Fig. 7B) inhibited AChE were 10×10⁻⁴ and 2.85×10⁻⁴ M, respectively. Vₘₐₓ of benzylamine and momordicine-inhibited AChE were 1.67 and 0.48 µmol ‘SH’ hydrolyzed/min/mg protein. Acid phosphatase activity, µmol substrate hydrolyzed/30 min/mg protein. Alkaline phosphatase activity, µmol substrate hydrolyzed/30 min/mg protein. A, benzylamine; B, momordicine. *Significant (p < 0.05) when Student’s t-test was used for locating difference between treated and control group of animals.
A comparison of the molluscicidal activity of column-purified fractions of *M. oleifera* leaf powder and *M. charantia* lyophilized fruit powder with synthetic molluscicides clearly demonstrates that the former are more potent. The 96 h LC₅₀ value of the column purified fraction of *Momordica charantia* lyophilized fruit powder (6.21 ppm) against *Lymnaea acuminata* is lower than those of synthetic molluscicides-carbaryl (14.40 ppm), phorate (15.0 ppm), formothion (8.56 ppm) and niclosamide (11.8 ppm). The 96 LC₅₀ values of the crude powder of *M. oleifera* leaf (197.59 ppm) and *M. charantia* lyophilized fruit (318.29 ppm) against *Lymnaea acuminata* are lower than the crude powders of *Zingiber officinale* rhizome (273.80 ppm), *Allium cepa* bulb (253.27 ppm), *Canna indica* root (359.02 ppm), *Cinnamomum tamala* leaf powder (830.90 ppm).

It is evident from the steep slope values that a small increase in the concentration of different treatments causes a marked mortality in snails. A t-ratio value greater than 1.96 indicates that the regression is significant. Values of heterogeneity factor less than 1.0 denote that in the replicate tests of random samples, the concentration response lines would fall within 95% confidence limits, and thus the model fits the data adequately. The index of significance of potency estimation g-values (less than 0.5) indicates that the values of the mean are within the limits at all probability levels (90, 95, 99) as it is less than 0.5.

Table 2

*In vitro* effect of different concentrations (mM) of benzylamine and momordicine on the acetylcholinesterase (AChE), acid phosphatase (ACP) and alkaline phosphatase (ALP) activity in the nervous tissue of *Lymnaea acuminata*

<table>
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<tr>
<th>Enzyme</th>
<th>Treatment</th>
<th>Enzyme activity</th>
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<td>Control</td>
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<td></td>
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<td>0.73±0.02</td>
<td>0.65±0.01*</td>
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<td>(100)</td>
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<td>AChE</td>
<td>Momordicine</td>
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<td></td>
<td>Control</td>
<td>0.06 mM</td>
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<td></td>
<td>0.73±0.02</td>
<td>0.54±0.01*</td>
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<td>Benzylamine</td>
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<td></td>
<td>Control</td>
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<td>35.07±0.01</td>
<td>30.08±0.03*</td>
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<td>(100)</td>
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<td>ACP</td>
<td>Momordicine</td>
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<td></td>
<td>Control</td>
<td>0.06 mM</td>
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<td>35.07±0.01</td>
<td>22.36±0.01*</td>
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<td>(100)</td>
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<td>Benzylamine</td>
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<td>30.71±0.27</td>
<td>21.13±0.03*</td>
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<td>(100)</td>
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<td>ALP</td>
<td>Momordicine</td>
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<td>30.71±0.27</td>
<td>24.15±0.03*</td>
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Values are mean ± SE of six replicates. Values in parentheses indicate percent enzyme activity with control taken as 100%. Concentrations (w/v) have been expressed as final concentration in the incubation mixture present in the cuvette. Acetylcholinesterase activity, µmol ‘SH’ hydrolyzed/min/mg protein. Acid phosphatase activity, µmol substrate hydrolyzed/30 min/mg protein. Alkaline phosphatase activity, µmol substrate hydrolyzed/30 min/mg protein. *Significant (p < 0.05) when Student’s t-test was applied between treated and control groups.

powder compared to other organic solvent extracts indicates that the molluscicidal components in the leaves and fruits are more soluble in ethanol than other organic solvents. Thin layer chromatography studies indicate that benzylamine and momordicine are probably the active components in *M. oleifera* leaf powder and *M. charantia* lyophilized fruit powder, respectively. HPLC fingerprinting is the best way for chemical characterization. In the present study, similar retention times were recorded for column purified *M. oleifera* leaf extract (2.59 min) and benzylamine (2.58 min). Benzylamine is identical to the alkaloid moringine. However, there is one major peak at the retention time of 8.42 min which indicates that although benzylamine is the molluscicidal component in *M. oleifera* leaf, there could be some other chemical components in the column-purified fraction that may be responsible for *M. oleifera* leaf molluscicidal activity. Chronic administration of benzylamine in the drinking water improves glucose tolerance, reduces body weight gain and circulating cholesterol in high-fat diet-fed mice. *M. oleifera* leaf extract has a protective effect against lipid peroxidation. Extract of *M. oleifera* leaf is also reported to have anti-carcinogenic and anti-bacterial activity.

The molluscicidal activity of lyophilized fruit powder of *M. charantia* is due to the presence of momordicine, as evidenced from individual toxicity, thin layer chromatography, and is confirmed by the HPLC retention value (8.4 min; same with that of pure standard compound). Momordicine, a bitter glucoside, is an alkaloid, concentrated in the fruits of *M. charantia*, the extracts of which possess anti-diabetic activity.
Characterization of molluscicidal component of Moringa oleifera leaf and Momordica charantia fruits and their modes of action in snail Lymnaea acuminata.


Fig. 7 - Lineweaver-Burk plots showing the effects of active molluscicidal components benzylamine (0.7 mM) (a) and momordicine (0.15 mM) (b) on the inhibition of acetylcholinesterase (AChE) activity in the nervous tissues of snail Lymnaea acuminata.

Fig. 8 - Lineweaver-Burk plots showing the effects of active molluscicidal components benzylamine (0.7 mM) (a) and momordicine (0.15 mM) (b) on the inhibition of acid phosphatase (ACP) activity in the nervous tissues of snail Lymnaea acuminata.

Fig. 9 - Lineweaver-Burk plots showing the effects of active molluscicidal components benzylamine (0.7 mM) (a) and momordicine (0.15 mM) (b) on the inhibition of alkaline phosphatase (ALP) activity in the nervous tissues of snail Lymnaea acuminata.

tissue of L. acuminata. Inhibition of AChE activity inhibition results in accumulation of acetylcholine at the nerve synapses, such that the postsynaptic membrane is in a state of permanent stimulation, resulting in producing paralysis, ataxia, general lack of coordination in neuromuscular system and eventual death. The methanolic extract of M. oleifera leaf significantly inhibited AChE activity at a concentration of 100 µg/mL. Untreated pulp extract of M. charantia (IC₅₀ - 7.2 mg/mL) similarly inhibited AChE activity.

Acid phosphatase, a lysosomal enzyme, which plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis, and alkaline phosphatase, which plays a critical role in protein synthesis, shell formation and other secretory activities, and transport of metabolites in gastropods. M. oleifera fruit seeds inhibits ACP and ALP activities in arsenic exposed mice, while M. charantia fruit extract decreased ALP levels in ammonium chloride-induced hyperammonemic rats.

Results of the kinetic study clearly indicate that inhibition of AChE by benzylamine is competitive as the Kₘ values of uninhibited and inhibited enzymes were different while the Vₘₐₓ were the same (same intercept (1/Vₘₐₓ) on the Y axis of LineWeaver-Burk plots). Inhibition of AChE by momordicine is uncompetitive; the slopes of momordicine inhibited and uninhibited AChE

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Kinetic constant (K_m and V_max) of different enzyme inhibition by benzylamine (0.7 mM) and momordicine (0.15 mM) in snail Lymnaea acuminata

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Untreated (control)</th>
<th>Treated (benzylamine)</th>
<th>Treated (momordicine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K_m</td>
<td>V_max</td>
<td>K_m</td>
</tr>
<tr>
<td>AChE</td>
<td>7.69 × 10^{-4}</td>
<td>1.67</td>
<td>10.4 × 10^{-4}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.85 × 10^{-4}</td>
</tr>
<tr>
<td>ACP</td>
<td>1.89 × 10^{-5}</td>
<td>58.82</td>
<td>1.40 × 10^{-5}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.85 × 10^{-5}</td>
</tr>
<tr>
<td>ALP</td>
<td>2.12 × 10^{-5}</td>
<td>66.67</td>
<td>3.22 × 10^{-3}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.12 × 10^{-5}</td>
</tr>
</tbody>
</table>

Michaelis-Menten constant K_m and V_max of different enzymes were calculated from Lineweaver-Burk Plots (1/V versus 1/S) were parallel to each other, whereas the intercepts were changed. The K_m and V_max of uninhibited and inhibited enzymes were different. Inhibition of ALP by benzylamine is competitive non-competitive. It is a mixed type of inhibition. In this case, K_m and V_max values of uninhibited and inhibited enzymes were different and slopes were also changed.

Inhibition of ACP and ALP by momordicine is non-competitive, as the K_m values of uninhibited and inhibited enzymes were the same while their V_max were different, as evident from different intercepts (1/V_max) on the Y axis of Lineweaver-Burk plots. Inhibition of ACP by benzylamine is also non-competitive.

In conclusion, it can be stated that the molluscicidal activity of the leaf powder of M. oleifera and lyophilized fruit powder of M. charantia is due to benzylamine and momordicine, respectively. Inhibition of AChE, ACP and ALP in the nervous tissue of L. acuminata by benzylamine and momordicine may be responsible for the molluscicidal activity of M. oleifera and M. charantia. Therefore, purified ethanolic extracts can be used as potent molluscicides as they are easily available, eco-friendly and culturally more acceptable.

RESUMO
Caracterização do componente moluscicida das folhas da Moringa oleifera e das frutas da Momordica charantia e seus modos de ação sobre o caramujo Lymnaea acuminata

A atividade moluscicida do pó das folhas de Moringa oleifera e do pó liofilizado das frutas da Momordica charantia contra o caramujo Lymnaea acuminata é dependente do tempo e da sua concentração. O pó da folha da M. oleifera (96 h LC_{50} 197.59 ppm) foi mais tóxico do que o pó liofilizado da fruta da M. charantia (96 h LC_{50} 318.29 ppm). Os extratos etanólicos do pó de folha de M. oleifera e do pó liofilizado da fruta da M. charantia foram mais tóxicos do que outros extratos orgânicos solvantes. O 96 h LC_{50} de fração purificada por coluna do pó das folhas da M. oleifera foi 22.52 ppm enquanto que o pó liofilizado do fruto da M. charantia foi 6.21 ppm. Coluna, camada fina e a alta performance da análise da cromatografia líquida mostram que os componentes ativos moluscídicos do pó da folha da M. oleifera e do liofilizado da fruta da M. charantia são a benzilamina (96 h LC_{50} 22.3 ppm) e a momordicina (96 h LC_{50} 1.2 ppm), respectivamente. A benzilamina e a momordicina inibiram de maneira significante in vivo e in vitro a acetilcolinesterase (AChE), as atividades das fosfatases alcalina e ácida (ACP/ALP) nos tecidos nervosos da L. acuminata. A inibição da atividade da AChE, ACP e ALP nos tecidos nervosos da L. acuminata pela benzilamina e momordicina podem ser responsáveis pela atividade moluscicida da M. oleifera e dos frutos da M. charantia, respectivamente.

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