# Molluscicidal Activity of *Aloe vera* and *Chrysanthemum cinerariifolium* and their active ingredients against snail *Lymnaea acuminata*

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**Abstract:** Freshwater snails *Lymnaea acuminata* (Lymnaeidae) are the vectors of the parasite trematode *Fasciola gigantica*, which causes the zoonotic disease fascioliasis in bovine populations and humans as well. The current study sought to ascertain whether the medicinal plants *Chrysenthemum cinerariifolium* and *Aloe vera* may serve as a possible natural molluscicide source to control the intermediate host snail population at the threshold level. The effectiveness of these plants was evaluated through a series of laboratory experiments, where their extracts were tested for toxicity against *Lymnaea acuminata*. To get aqueous extracts, freshly harvested aerial parts of *C. cinerariifolium* and *A. vera* plants were treated. Healthy and acclimated snails were subjected to varying doses of the extract and organic solvents over a period of up to 96 h in order to assess the toxicity of these extracts. The snails were monitored for signs of mortality and the data collected was analyzed to determine the lethal concentration and the effects of the extracts. Both plant extracts' toxicity exhibits a response that varies with time and concentration. During the 96 h exposure period, the toxicity of *C. cinerariifolium* leaf (80.2 mg/l) was more pronounced than that of *A. vera* (406.03 mg/l). The active component Pyrethrum extract from *C. cinerariifolium* compresses (0.20 mg/l) more effective than the 96 h $LC_{50}$ of Aloin (1.11 mg/l). Results indicated that both *C. cinerariifolium* and *A. vera* exhibited significant molluscicidal activity, suggesting their potential use in integrated pest management strategies to reduce the transmission of fascioliasis.

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## 1. Introduction

The eastern region of India's cattle population has an extremely high prevalence of fascioliasis. *Fasciola gingatica* is the cause of this disease, which affects 94% of buffaloes killed at local slaughterhouses (Singh and Agarwal, 1981). *Fasciola* species have intermediate hosts in the snail *Lymnaea acuminata* (Lymnaeidae) (Singh and Agarwal, 1984; Singh et al., 2021). Nearly everywhere in the world, fascioliasis is a leading source of sickness and mortality in both humans and livestock (Mas-Coma et al., 2005, 2014; Singh et al., 2021; Yatoo et al., 2021). The WHO has classified fascioliasis as a Neglected Tropical Disease (NTD). Presently 81 countries facing this disease (Caravedo and Cabada, 2020). Numerous studies that have been published worldwide indicate that fascioliasis causes $302 billion in economic losses annually, both directly and indirectly (Spithill et al., 1999). Gastropods are the weakest link in the trematode life cycle; therefore, managing the population of snails is a strategy for reducing fascioliasis (Singh et al., 2021). Many synthetic molluscicides are extensively used to effectively reduce harmful snails (Safer et al., 2005). However, the use of these synthetic molluscicides is impeded by their deleterious effects; there is a need for alternative safe natural molluscicides. Plants have antimicrobial properties and protect against bacteria, viruses, and fungi. A number of studies were done on *A. vera* for evaluating potential and medical use, namely antiulcer, antihypercholesterolemic, antidiabetic, antioxidative, antimicrobial, antiacne, immunomodulatory, protection of skin from UV-A and UV-B rays, and healing of wounds (Bhuvana et al., 2014). Haller (1990) reported that dose dependent use includes detersive, desiccative, insecticide, antiseptic, vermifuge and cholagogue. Abutaha et al., (2022) noted its larvicidal properties against mosquitoes. Likewise, *Chrysanthemum cinerariifolium* used as a medicinal plant. It is also called pyrethrum. It is native to Yugoslavia and is cultivated on a commercial scale in Kenya, US, Brazil, India and many more countries (The Wealth of India, 1985). In ancient times, it was known for its numerous biological properties, like antibacterial, antifungal, antiviral, antioxidant, anticancerous, and anti-HIV-1 activities (Daniel and Joseph 2019). Earlier, Khallouki et al. (2000) reported the antibacterial and molluscicidal activities of the volatile fraction of *C. viscidehirtum* aerial parts containing limonene and sesquiterpenes against *Bulinus truncatus*. The chemicals present in plant products are less hazardous in the environment, species specific and pose less opportunity for the emergence of resistant strains of pests due to the inherent combination of different chemicals. Although there are a large number of plants that are rich sources of bioactive molluscicides, several more might still be lying unexplored. Plant derived molluscicides are more readily accessible, less costly and biodegradable in nature than their synthetic counterparts. In the present study we identified and evaluated the molluscicidal activity of *Aloe vera* (leaf) and *Chrysanthmum cinerariifolium* (leaf and flower) and their active components against the snail *Lymnaea acuminata*.

## 2. Material and Methods

**Plant Materials:** Leaves *of Aloe vera* (Family- Asphodelaceae) and flowers/leaf of *Chrysanthmum cinerariifolium* (Family- Asteraceae) were freshly collected by the botanical garden of the University campus and identified by the Department of Botany, Deen Dayal Upadhyaya Gorakhpur University.

**Animal Collection and treatment:** Adult *L. acuminata* (2.35±0.25 cm length) were collected from freshwater ponds inside of the Shaheed Ashfaq Ullah Khan Prani Udyan Gorakhpur Zoo. A Map was attached here with collection site of the snail (Fig-1and 2). Snails were acclimated for 72 h in laboratory conditions. Ten snails of each group were taken in 3 l of dechlorinated tap water at a temperature (23±1°C). Six replicates were taken for each dose. Control groups were not exposed to any molluscicide serve as baseline to compare the effect of the molluscicide. All groups are subjected to the same environmental condition like temperature, DO, $CO_2$, bicarbonate etc. The test animals are similar in age, size etc. The values of pH (7.1-7.3), dissolved oxygen (5.2-6.3), free carbon dioxide (6.6-7.3), bicarbonate alkalinity were (102-104 mg/ L). Dead snails were immediately taken out of the aquarium water to prevent any infection (Singh et. al.1984).

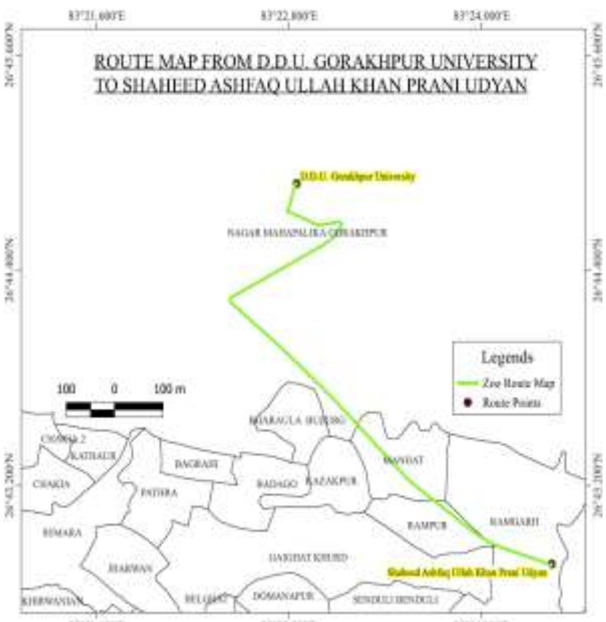


Fig:1 Collection site of test animal

Fig: 2 Test animal *Lymnaea acuminata*

**Plant Extracts:** The leaves of *Aloe vera*, flowers /leaf of *C. cinerariifolium* were taken from the field, leaves cleared of debris, and washes in water (Fig 3 & 4). After roughly chopping 50 gm of fresh *Aloe vera* and C. *cinerariifolium* leaf/ flower were crushed in mortar and pestle separately, the resulting aqueous extract were filtered and used for treatment.


Fig: 3 *Aloe vera* plant


Fig: 4 *Chrysanthmum cinerariifolium*

**Pure Compound:** Aloin ($C_{12}H_{22}O_{9}$) and Pyrethrum extract ($C_{43}H_{56}O_{8}$) were purchased from Sigma-Aldrich Chemical Co.

**Solvent Extracts:** The 100 gm of the leaf of *Aloe vera and C. cinerariifolium* flower /leaf were extracted separately with 100 ml of each organic solvent viz. ethanol (96%), ether (99.5%), acetone (99%) and chloroform (99%) at room temperature for 48-h.The preparations were separated and filtered individually using sterilized Whatman no. 1 filter paper. The filtered extracts were then freeze dried. The resulting residues were then utilized to measure the molluscicidal activity. The leaf of *Aloe vera* yielded 245 mg ether extract, 315mg acetone extract, 340 mg chloroform extracts, and 400 mg ethanol extract. The flower of *C. cinerariifolium* yielded 145 mg ether extract, 215 mg acetone extract, 240 mg chloroform extract, , and 300 mg ethanol extract and the leaf of *C. cinerariifolium* yielded 122 mg ether extract, 232 mg acetone extract, 280 mg chloroform extract, and 235 mg ethanol extract.

**Column purification:** The ethanol extract of leaf/flower of *C. cinerariifolium* and leaf *Aloe vera* were obtained by dissolving 1.0gm of each in 50ml of ethanol separately. Further, the ethanolic extract of leaf/flower of *C. cinerariifolium* and leaf *Aloe vera* subjected to silica gel (60-120) mesh qualigens glass, (Precious Electro Chemindus Industry Private Limited, Mumbai, Bharat). Chromatography through 95×45cm column. Five milliliters fractions were eluted with 96% ethanol for each column preparation. Ethanol was evaporated under vacuum and the remaining solids obtained from eluents were used for the determination of molluscicidal activity.

**Molluscicidal activity:** Toxicity of different plant preparations and pure compound against snails were done by the Singh and Agarwal (1984). Three liters of dechlorinated water were kept in a glass aquarium with ten healthy snails. Snails were kept from emerging from the aquariums in each setup by placing a fine mesh above the water's surface. Snails were exposed to 96h exposure period. Different concentrations of separate plant extract were used to observe the toxicity (Table 1). Six replicates for each concentration were taken in each experiment. No treatments were given in control groups. During the exposure period, neither food nor disturbance was given to these snails. The mortality rate of snails were noted every 24h up to 96 h. Each experiment was replicated six times and toxicity measure at interval of 24 h to 96 h. Concentration given was the final concentration (w/v) in the glass aquarium water. Dose will be decide after pilot experiment done against test animals.

Table 1: Concentrations of different extract of plant *Aloe vera* and *C. cinerariifolium* plant products used for the toxicity determination against *L. acuminata.*

| **Extract of plants and their active ingredients** | **Concentration used against *L. acuminate* mg/l** |
|---|---|
| ALCE | 300, 500, 700, 900 |
| Ether extract | 30, 50, 70, 90 |
| Acetone extract | 30, 50, 70, 90 |
| Chloroform extract | 30, 50, 70, 90 |
| Ethanol extract | 20, 30, 40, 50 |
| Column purified fraction | 3, 5, 7, 9 |
| Aloin | 1, 3, 5, 7 |
| CLCE | 70, 90, 110, 130 |
| Ether extract | 10, 30, 50, 70 |
| Acetone extract | 10, 30, 50, 70 |
| Chloroform extract | 10, 30, 50, 70 |
| Ethanol extract | 10, 20, 30, 40 |
| Column purified fraction | 7,9,11,13 |
| CFCE | 150, 200, 250, 300 |
| Ether extract | 15, 30, 45, 60 |
| Acetone extract | 15, 30, 45, 60 |
| Chloroform extract | 15, 30, 45, 60 |
| Ethanol extract | 10, 15, 20, 25 |
| Column purified fraction | 1, 3, 5, 7 |
| Pyrethrum extract | 0.5, 0.7, 0.9, 1.2 |

*Abbreviations used: ALCE: *Aloe vera* leaf curde extract, CLCE:*Chrysenthamum* leaf crude extract and CFCE: *Chrysenthamum* flower crude extract.

**Analytical statistics:** Using the polo software program (PoLoPlusLeOra software version 2.0), the $LC_{50,}$ lower confidence limits (LCL), upper confidence limits (UCL), t-ratio heterogeneity factor, slope values, and g-values were determined.

## 3. Results

The toxicity of leaf compound of *Aloe vera* and leaf/flower of *Chrysanthmum cinerariifolium* against *L. acuminata* shows concentration and time-dependent response. $LC_{50}$ of extracted and pure leaf compound of *Aloe vera and* leaf/flower of *Chrysanthmum cinerariifolium* in laboratory condition at 24 h $LC_{50}$ were 961.64 mg/l, 154.06 mg/l and 363.46 mg/l, respectively. The toxicity trend was pronounced at 96 h $LC_{50}$ i.e., 406.03 mg/l, 80.20mg/l and 175.02mg/l (Table 2, 3 and 4). $LC_{50}$ of extracted column purified leaf extract of *Aloe vera* and leaf/flower of *Chrysanthmum cinerariifolium* at 24h $LC_{50}$ (10.05 mg/l), (1.97 mg/l and 0.96 mg/l) respectively, was lower than the toxicity of all organic solvents (Table 2, 3 and 4).

Among different organic solvent extracts of *Aloe vera* leaf (ethanol extract of leaf at 24h $LC_{50}$ was 60.67mg/l) more toxic against *L. acuminata* than other organic solvents. The toxicity of Leaf/flower ethanol extract of *C. cinerariifolium* (24h $LC_{50}$ 43.09mg/l and 38.41mg/l) respectively, is more pronounced than other organic solvent extract (Table 2, 3 and 4). The toxicity of 24h $LC_{50}$ of *C. cinerariifolium* active ingredient pyrethrum extract was (24h 0.96 mg/l) against *L. acuminate*, while the active ingredient aloin in *Aloe* leaf (24h $LC_{50}$ was 7.88 mg/l) (Table 2 and 4). At 96h $LC_{50}$ of pyrethrum extract of *C. cinerariifolium* and aloin of *Aloe* were (0.20 mg/l) and (1.11 mg/l), respectively. (Table 2 and 4). The order of toxicity of these two plants against *L. acuminata* was *Aloe* leaf > *C. cinerariifolium* leaf > *C. cinerariifolium* flower. In control group of animal there was no mortality up to 96 h exposure periods.

The individual estimate of LC based on the six repetitions was found to be within the 95% confidence limits of $LC_{50}$ .The heterogeneity factor was less than 1.0 and the regression analysis t-ratio was greater than 1.96. The slope values were steep and separate estimation of LC based on each of six replicate were found to be within 95% confidence limits of $LC_{50}$. The t-ratio was greater than 1.96 and the heterogeneity factor is less than 1.0 at all probability levels (90, 95 and 99).

Table-2: Toxicity of *Aloe vera* crude extract, different organic extract, and column purified fraction and its active ingredient against *L. acuminata* at different exposure period.

| Exposure periods | Tested Materials | $LC_{50}$ | Limits | | Slope Value | t-ratio | Heterogeneity |
|---|---|---|---|---|---|---|---|
| | | | LCL | UCL | | | |
| 24-h. | ALCE | 961.64 | 816.65 | 1295.32 | 3.16±0.59 | 5.7 | 0.25 |
| | Ether extract | 90.28 | 76.75 | 246.99 | 2.91±0.56 | 5.15 | 0.22 |
| | Acetone extract | 125.18 | 95.59 | 156.65 | 2.42±0.58 | 4.12 | 0.20 |
| | Chloroform extract | 104.48 | 85.86 | 80.89 | 2.83±0.59 | 4.7 | 0.42 |
| | Ethanol extract | 60.67 | 47.20 | 11.25 | 2.16±0.56 | 3.2 | 0.25 |
| | Column purified | 10.05 | 7.90 | 09.33 | 1.11±0.34 | 3.5 | 0.23 |
| | Aloin | 7.88 | 6.56 | 10.65 | 1.06±0.31 | 3.2 | 0.20 |
| 48-h. | ALCE | 779.78 | 679.38 | 91.73 | 2.53±0.51 | 5.8 | 0.30 |
| | Ether extract | 73.08 | 62.84 | 151.96 | 2.59±0.50 | 5.12 | 0.19 |
| | Acetone extract | 92.83 | 74.50 | 109.49 | 2.06±0.50 | 4.09 | 0.16 |
| | Chloroform extract | 81.08 | 68.36 | 82.06 | 2.83±0.59 | 4.7 | 0.42 |
| | Ethanol extract | 47.48 | 25.71 | 12.47 | 1.43±0.50 | 3.8 | 0.17 |
| | Column purified | 8.22 | 6.55 | 11.34 | 1.41±0.31 | 3.4 | 0.15 |
| | Aloin | 5.87 | 3.21 | 7.34 | 1.40±0.30 | 3.2 | 0.15 |
| 72-h. | ALCE | 555.13 | 456.05 | 676.92 | 2.11±0.47 | 5.9 | 0.27 |
| | Ether extract | 56.82 | 49.15 | 65.85 | 2.78±0.49 | 5.6 | 0.22 |
| | Acetone extract | 71.30 | 57.41 | 106.78 | 1.72±0.47 | 3.6 | 0.20 |
| | Chloroform extract | 64.47 | 53.82 | 82.37 | 2.09±0.47 | 4.3 | 0.27 |
| | Ethanol extract | 31.24 | 21.23 | 65.97 | 1.16±0.46 | 3.5 | 0.12 |
| | Column purified | 4.41 | 3.22 | 5.98 | 1.15±0.45 | 3.1 | 0.12 |
| | Aloin | 3.65 | 1.69 | 4.75 | 1.16±0.43 | 3.2 | 0.11 |
| 96-h. | ALCE | 406.03 | 323.30 | 471.82 | 2.69±0.49 | 6.3 | 0.42 |
| | Ether extract | 44.20 | 37.62 | 50.02 | 3.22±0.50 | 6.4 | 0.58 |
| | Acetone extract | 34.43 | 53.91 | 54.91 | 2.11±0.47 | 3.9 | 0.55 |
| | Chloroform extract | 45.09 | 34.43 | 53.91 | 2.11±0.47 | 4.4 | 0.76 |
| | Ethanol extract | 23.38 | 11.49 | 50.03 | 1.96±0.56 | 3.6 | 0.25 |
| | Column purified | 2.88 | 1.14 | 4.36 | 1.11±0.41 | 3.5 | 0.23 |
| | Aloin | 1.11 | 0.65 | 1.84 | 1.10±0.40 | 3.4 | 0.12 |

Mortality was determined every 24-h up to 96-hrs, each set of experiment was replicate six times, LCL- Lower confidence limit, UCL: Upper confidence limit.

Table3: Toxicity of *Chrysanthemum cinerariifolium* leaf crude extract, different organic extract, and column purified fraction against *L. acuminata* at different exposure period.

| **Exposure periods** | **Tested Materials** | **$LC_{50}$** | **limits** | | **Slope Value** | **t-ratio** | **Heterogeneity** |
|---|---|---|---|---|---|---|---|
| | | | **LCL** | **UCL** | | | |
| 24-h. | CLCE | 154.06 | 129.11 | 259.28 | 3.38±0.93 | 3.87 | 0.30 |
| | Ether extract | 110.39 | 72.20 | 307.34 | 1.35±0.32 | 3.52 | 0.33 |
| | Acetone extract | 93.18 | 59.75 | 290.71 | 1.09±0.28 | 4.42 | 0.27 |
| | Chloroform extract | 90.79 | 58.61 | 274.98 | 1.09±0.29 | 5.45 | 0.24 |
| | Ethanol extract | 43.09 | 31.35 | 50.55 | 1.19±0.29 | 4.47 | 0.24 |
| | Column purified | 1.97 | 1.32 | 2.65 | 2.11±0.24 | 3.22 | 0.24 |
| 48-h. | CLCE | 121.40 | 106.97 | 161.45 | 3.14±0.85 | 4.70 | 0.25 |
| | Ether extract | 56.68 | 41.58 | 96.64 | 1.27±0.28 | 4.44 | 0.30 |
| | Acetone extract | 60.27 | 37.49 | 231.59 | 0.79±0.26 | 4.41 | 0.36 |
| | Chloroform extract | 62.44 | 42.18 | 148.43 | 1.00±0.27 | 5.42 | 0.33 |
| | Ethanol extract | 31.59 | 25.80 | 50.49 | 1.00±0.27 | 3.41 | 0.24 |
| | Column purified | 1.46 | 1.15 | 2.54 | 1.03±0.26 | 3.42 | 0.26 |
| 72-h. | CLCE | 93.84 | 84.08 | 103.26 | 2.03±0.23 | 4.41 | 0.41 |
| | Ether extract | 25.23 | 17.72 | 33.24 | 4.07±0.84 | 5.39 | 0.25 |
| | Acetone extract | 28.33 | 15.44 | 36.20 | 1.38±0.26 | 6.45 | 0.43 |
| | Chloroform extract | 30.67 | 20.04 | 45.41 | 1.06±0.26 | 4.42 | 0.36 |
| | Ethanol extract | 18.14 | 14.00 | 30.06 | 1.04±0.26 | 3.22 | 0.38 |
| | Column purified | 1.26 | 0.72 | 2.23 | 1.25±0.26 | 3.21 | 0.36 |
| 96-h. | CLCE | 80.20 | 71.01 | 86.92 | 2.22±0.25 | 3.43 | 0.46 |
| | Ether extract | 15.16 | 10.25 | 19.61 | 1.19±0.90 | 3.38 | 0.58 |
| | Acetone extract | 13.06 | 7.66 | 17.81 | 1.76±0.28 | 5.46 | 0.48 |
| | Chloroform extract | 16.43 | 9.79 | 22.32 | 1.52±0.27 | 4.41 | 0.80 |
| | Ethanol extract | 9.21 | 07.25 | 22.03 | 1.35±0.26 | 3.20 | 0.63 |
| | Column purified | 1.05 | 0.44 | 2.10 | 2.07±0.23 | 3.22 | 0.42 |

Mortality was determined every 24-h up to 96-hrs, each set of experiment was replicate six times, LCL- Lower confidence limit, UCL: Upper confidence limit.

Table 4: Toxicity of *Chrysanthamum cinerariifolium* flower crude extract, different organic extract, and column purified fraction and its active component against *L. acuminata* at different exposure period.

| Exposure periods | Tested Materials | $LC_{50}$ | limits | | Slope Value | t-ratio | Heterogeneity |
|---|---|---|---|---|---|---|---|
| | | | LCL | UCL | | | |
| 24-h. | CFCE | 363.46 | 208.43 | 649.71 | 3.02±0.83 | 3.63 | 0.33 |
| | Ether extract | 121.56 | 79.35 | 351.70 | 1.37±0.33 | 4/08 | 0.39 |
| | Acetone extract | 90.02 | 63.06 | 223.10 | 1.23±0.30 | 3.27 | 0.19 |
| | Chloroform extract | 92.28 | 61.66 | 235.47 | 1.23±0.31 | 3.94 | 0.21 |
| | Ethanol extract | 38.41 | 56.48 | 94.4 | 1.07±0.21 | 4.01 | 0.29 |
| | Column purified | 6.8 | 5.5 | 12.5 | 1.15±0.24 | 3.80 | 0.25 |
| | Pyrethrum extract | 0.96 | 0.79 | 2.6 | 2.22±0.23 | 3.21 | 0.42 |
| 48-h. | CFCE | 278.71 | 241.8 | 383.2 | 2.81±0.76 | 3.69 | 0.30 |
| | Ether extract | 61.71 | 46.27 | 102.5 | 1.37±0.36 | 4.40 | 0.40 |
| | Acetone extract | 65.43 | 42.29 | 245.57 | 1.87±0.29 | 3.31 | 0.35 |
| | Chloroform extract | 64.97 | 46.41 | 130.51 | 1.17±0.30 | 3.20 | 0.19 |
| | Ethanol extract | 52.12 | 24.04 | 100.02 | 1.10±0.29 | 4.22 | 0.27 |
| | Column purified | 4.7 | 3.7 | 10.87 | 2.21±0.34 | 4.8 | 0.42 |
| | Pyrethrum extract | 0.84 | 0.62 | 5.26 | 2.22±0.26 | 5.14 | 0.52 |
| 72-h. | CFCE | 208.85 | 184.65 | 232.5 | 3.63±0.75 | 4.1 | 0.25 |
| | Ether extract | 29.56 | 21.76 | 37.64 | 1.57±0.32 | 4.3 | 0.44 |
| | Acetone extract | 26.03 | 19.87 | 40.58 | 1.25±0.29 | 3.22 | 0.33 |
| | Chloroform extract | 35.25 | 25.14 | 48.52 | 1.28±0.30 | 3.20 | 0.28 |
| | Ethanol extract | 20.71 | 12.52 | 36.99 | 1.46±0.30 | 5.7 | 0.28 |
| | Column purified | 2.35 | 1.27 | 8.90 | 2.23±0.43 | 6.2 | 0.43 |
| | Pyrethrum extract | 0.62 | 0.41 | 3.40 | 2.21±0.41 | 5.4 | 0.41 |
| 96-h. | CFCE | 175.02 | 152.50 | 191.64 | 4.60±0.82 | 5.5 | 0.47 |
| | Ether extract | 19.53 | 14.27 | 24.02 | 2.16±0.34 | 6.3 | 0.57 |
| | Acetone extract | 17.50 | 11.62 | 22.36 | 1.91±0.31 | 4.2 | 0.39 |
| | Chloroform extract | 21.69 | 15.32 | 27.22 | 1.81±0.32 | 4.1 | 0.56 |
| | Ethanol extract | 12.47 | 08.27 | 23.78 | 1.28±0.22 | 3.2 | 0.41 |
| | Column purified | 0.52 | 0.75 | 3.87 | 1.22±0.21 | 5.4 | 0.42 |
| | Pyrethrum extract | 0.20 | 0.11 | 5.24 | 1.11±0.25 | 3.4 | 0.42 |

Mortality as determined every 24 up to 96hts. Each set of experiment was replicating six times, LCL- Lower confidence limit, UCL: Upper confidence limit.

## 4. Discussion

The present study clearly demonstrates that the plants of *Aloe vera* and *Chrysanthemum cinerariifolium* have potent molluscicidal properties. Their molluscicidal effect is time- and concentration dependent. Both plants, *Aloe vera* and *Chrysanthemum cinerariifolium*, are reported earlier as having antimicrobial and insecticidal properties (Haller, 1990; Bhuvana et al., 2014). It has been used in homeopathic, ayurvedic, and allopathic streams of medicine (Haller, 1990). *Aloe vera* contains secondary metabolites such as alkaloids, tannins, flavonoids, reducing compound metabolites, triterpenes, coumarins, and anthraquinones, etc. (Benzidia et al., 1999). Alkaloids were considered a digestive toxin. Additionally, it has the ability to stop animals from growing; glycoside and saponin act as stomach poison (Kumar et al., 2005). Tannin prevents animals from breaking down food, thereby serving as a plant defense. As a result, the animal may die due to a reduction in growth and disruption of water absorption (Subramaniam et al., 2012). Flavonoids are poisonous, plant-defensive substances that might impede an animal's ability to digest food. Both a respiratory toxin and a respiratory inhibitor are produced by flavonoids. When flavonoids enter an animal body through its respiratory system, they damage the spiral and create withering on the nerves (Verma et al., 2013).

A comparison of molluscicidal activity of column purified fraction of *Aloe vera and C. cinerariifolium* leaf/flower with synthetic molluscicidal clearly demonstrated that the 96 h $LC_{50}$ of column purified extract of *Aloe vera* leaf is (2.88mg/l) and *C. cinerariifolium* leaf (1.05mg/l) and flower is (0.52mg/l) more potent than those of synthetic molluscicide Carbaryl (14.4mg/l), phorate (15.5 mg/l), formothion (8.5 mg/l) (Singh and Agrawal,1983). While the 96 h $LC_{50}$ of crude extract of *C. cinerariifolium* leaf/flower (80.20mg/l) and (175.02mg/l) was lower than the plant molluscicides reported earlier in our laboratory. The 96 h $LC_{50}$ of latex powder *Ferula asafoetida* (82.71 mg/l) and seed powder of *Carum carvi* (140.58 mg/l) (Kumar and Singh, 2006), *Thuja orientalis* leaf powder against the snail *L. acuminata* the 96h $LC_{50}$ was (250.5 mg/l) (Singh & Singh, 2009). The 96h $LC_{50}$ *Sapindus mukorossi* fruit (119.57 mg/l), *Bauhinia variegata* leaf (238.17 mg/l) and *Momordica charantia* fruit (318.29 mg/l) (Upadhyay and Singh, 2011; Upadhyay et al., 2013; Singh et al., 2012). Pyrethrum extract from *C. cinerariifolium* is already in most lists of approved organic insecticides. It has been already reported that pyrethrum is a contact poison and contain pyrethrin I and II (The wealth of India, 1995). In addition higher phenols are present up to 0.7%. It may be possible that the toxicity is either individually or it may be collectively of the mixture secondary metabolites, it is a matter of further research to identify the exact ingredient to explore the full potential of the toxic compound. The *C. cinerariifolium* and *Aloe vera* plant is easily found in India and its application as plant molluscicides could complement existing snail control techniques and perhaps lead to the development of a new class of molluscicides that is both safe and effective. The discovery of the *C. cinerariifolium* and *Aloe vera* plant as a possible source of new molluscicides will provide an affordable substitute for synthetic molluscicides, which are costly to acquire and highly hazardous to macrofauna that is not their intended target.

Evidence from the steep slope shows values indicate 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 lives would fall within 95% confidence and thus the model fits the data adequately. The index of significance of potency estimation g-value of the mean is within the limits at all probability levels (90, 95, 99) less than 0.5.

## 5. Conclusion

Present study clearly demonstrated that *Chrysanthemum cinerariifolium* and *Aloe vera* are effective molluscicides. These indigenous plants have the potential to be an important, safe and environmentally sound source of molluscicidal agents without having an adverse effect on the population of non-target biota. Further, research is necessary to clarify the mechanism of action in the snail's body.

**Author's Contributions:**

VKS: Regulation, contemplation, analyse, software, assessments, evaluate and emend. SS: writing—including the production of the first draft— and analysed data, Polo and drafted the manuscript. Data curation, investigation and visualization.

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