Molluscicidal activity of Punica granatum bark and Canna indica root

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Abstract

The molluscicidal activity of Punica granatum Linn. (Punicaceae) and Canna indica Linn. (Cannaceae) against the snail Lymnaea acuminata was studied. The molluscicidal activity of P. granatum bark and C. indica root was found to be both time and dose dependent. The toxicity of P. granatum bark was more pronounced than that of C. indica. The 24 h LC$_{50}$ of the column-purified root of C. indica was 6.54 mg/l whereas that of the column-purified bark of P. granatum was 4.39 mg/l. The ethanol extract of P. granatum (24 h LC$_{50}$: 22.42 mg/l) was more effective than the ethanol extract of C. indica (24 h LC$_{50}$: 55.65 mg/l) in killing the test animals. P. granatum and C. indica may be used as potent molluscicides since the concentrations used to kill the snails were not toxic for the fish Colisa fasciatus, which shares the same habitat with the snail L. acuminata.

Key words
- Punica granatum
- Canna indica
- Plant molluscicide
- Lymnaea acuminata

The causative agents of endemic fascioliasis in the cattle population of eastern Uttar Pradesh are Fasciola hepatica and F. gigantica. These flukes are transmitted by the intermediate host snails Lymnaea acuminata and Indoplanorbis exustus. Use of molluscicides to eradicate the snail vector is considered the method of choice to eliminate fascioliasis (1,2). Synthetic organic molluscicides have been widely used for the effective control of harmful snails (3). Today, it has been realized that these molluscicides are toxic to non-target animals and have a long-term detrimental effect on the aquatic environment (2,4). Molluscicides of plant origin have become the focus of attention since they are less expensive and less hazardous to the environment than their synthetic counterparts (2). A large number of plant products which possess molluscicidal activity have been identified (1,2,5). In the present study we evaluated in the laboratory the molluscicidal activity of different parts of Punica granatum Linn. (Punicaceae) and Canna indica Linn. (Cannaceae) against Lymnaea acuminata snails.

Bark of Punica granatum and root of Canna indica were collected locally. Specimens were identified by the staff of the herbarium of the Botany Department, DDU Gorakhpur University, Gorakhpur, where samples (Nos. 2125 and 2438) have been deposited. Different preparations were obtained from the bark of P. granatum and root of C. indica for the toxicity study. Pieces of P. granatum bark and root of C. indica were taken from the plant and kept in the incubator at 45°C for 48 h. Dried pieces of these parts were pulverized with a grinder and the crude powder thus obtained was used for the toxicity experiment.

Five grams of P. granatum bark and C.
*indic*a root powder were extracted with 100 ml each of 95% ethanol, 99% methanol, 98% ether and 99.7% chloroform at room temperature for 12 h. The solvents were removed under vacuum and the remaining dried parts (*P. granatum* ethanol 0.21 g, methanol 0.24 g, ether 0.06 g, and chloroform 0.04 g and *C. indica* ethanol 0.05 g, methanol 0.04 g, ether 0.04 g, and chloroform 0.03 g) were used for the determination of molluscicidal activity.

One hundred milliliters of the ethanol extract fraction of both *P. granatum* bark and *C. indica* root were subjected to silica gel (60-120 mesh, Qualigens Glass, Precious Electrochemindus Private Limited, Bombay, India) chromatography through a 5 x 45 cm column. Five-milliliter fractions eluted with ethyl alcohol (95%) were collected. Ethyl alcohol was evaporated under vacuum and the remaining solids were used for the determination of molluscicidal activity.

Adult *Lymnaea acuminata* (2.25 ± 0.20 cm in length) snails were collected locally and used as experimental animals. The animals were allowed to acclimatize to laboratory conditions for 72 h. Toxicity experiments were performed by the method of Singh and Agarwal (6). Ten experimental animals were kept in a glass aquarium containing 3 liters of dechlorinated tap water. Snails were exposed to different concentrations of the preparations of *P. granatum* and *C. indica* and toxicity was observed for 24, 48, 72 and 96 h. Six aquaria were set-up for each concentration. Control animals were kept in an equal volume of dechlorinated water under similar conditions but without treatment. The dissolved oxygen in treated and control water was 6.5-7.2 mg/l. The toxicity of these preparations was also tested on *Colisa fasciatus* fish.

Mortality was recorded every 24 h up to 96 h and dead animals were removed immediately so that other test animals would not be contaminated. Snail mortality was established by the contraction of the body within the shell; no response to a needle probe was taken as evidence of death. LC values, upper and lower confidence limits and slope values were calculated using the POLO computer software of Russell et al. (7). The regression coefficient between exposure time and different values of LC$_{50}$ was determined (8).

The toxicity of the bark powder of *P. granatum* and of the root powder of *C. indica* and their organic solvent-extracted fractions against *L. acuminata* was time and dose dependent. Twenty-four-hour toxicity of *P. granatum* bark powder (LC$_{50}$ - 138.08 mg/l) against *L. acuminata* was higher than that of *C. indica* root powder (773.58 mg/l) (Tables 1 and 2). Ninety-six-hour LC$_{50}$ of *P. granatum* bark powder and *C. indica* root powder against *L. acuminata* was 62.62 mg/l and 359.02 mg/l, respectively (Tables 1 and 2).

Among the organic solvent-extracted fractions, the ethanol extract of bark was the most toxic. The 24-h LC$_{50}$ of ethanol extracts of *P. granatum* bark and *C. indica* root against *L. acuminata* was 22.42 mg/l and 55.65 mg/l, respectively (Tables 1 and 2). The column-purified fractions of both plants were highly toxic. The 24-h LC$_{50}$ of the purified fraction of *P. granatum* bark was 4.39 mg/l, whereas the 24-h LC$_{50}$ of the purified fraction of *C. indica* root was 6.54 mg/l. At 96-h exposure the toxicity of the purified fractions was in the range of 0.94 to 1.84 mg/l (Tables 1 and 2). No mortality was observed in *C. fasciatus* fish exposed to the same concentrations as used in treatments against the snail *L. acuminata*.

The slopes were steep and the separate estimates of LC based on each of the six replicates were found to be within the 95% confidence limits of LC$_{50}$. The t-ratio was greater than 1.96 and the heterogeneity factor less than 1.0. The g value was less than 0.5 at all probability levels (Tables 1 and 2).

It was evident from the above results that *P. granatum* (pomegranate) bark and *C. in-
dica root are potential sources of botanical molluscicides. Their toxic effects are time as well as dose dependent. The toxicity study revealed that the toxic components of P. granatum bark and C. indica root are water soluble. Punica granatum plants have been used for different therapeutic treatments as antidiarrhea, antiviral, antibacterial and anti-diabetic agents (9-17). The dried bark of root and stem has long been used for the tape-worm treatment (18,19). The bark and stem of P. granatum contain a number of alkaloids belonging to the pyridine group, namely pelletierine, pseudopelletierine, iso-pelletierine and methyl iso-pelletierine (18,19). Among them, iso-pelletierine is the most potent taenicide (18,19). Hukkeri et al. (18) reported that the aqueous extract of P. granatum fruit rind was more active against tape-worms than against earthworms and roundworms. It has been reported that iso-pelletierine causes muscular weakness followed by paralysis and death in treated animals. Production of an insecticidal preparation from P. granatum bark including the alkaloids and their tannates has been patented in

| Exposure time | Root extracts   | Lethal concentration (LC₅₀) (w/v) | 95% Confidence limits | Slope value |
|---------------|-----------------|----------------------------------|-----------------------|-------------|
|               |                 | LCL (mg/l)                        | UCL (mg/l)            |             |
| 24 h          | Root powder     | 773.58                           | 649.60                | 1123.09     | 3.31 ± 0.71 |
|               | Methanol extract| 75.57                            | 58.25                 | 133.32      | 1.90 ± 0.43 |
|               | Ethanol extract | 55.65                            | 42.29                 | 95.60       | 1.94 ± 0.39 |
|               | Chloroform extract| 101.82                       | 73.29                 | 231.29      | 1.99 ± 0.48 |
|               | Ether extract   | 111.43                           | 70.81                 | 629.85      | 1.36 ± 0.43 |
|               | Column purified | 6.54                             | 5.67                  | 7.87        | 2.65 ± 0.49 |
| 48 h          | Root powder     | 579.73                           | 509.67                | 719.47      | 3.10 ± 0.64 |
|               | Methanol extract| 48.23                            | 38.90                 | 66.92       | 1.74 ± 0.04 |
|               | Ethanol extract | 36.06                            | 28.26                 | 53.97       | 1.60 ± 0.34 |
|               | Chloroform extract| 71.27                        | 54.60                 | 130.56      | 1.73 ± 0.42 |
|               | Ether extract   | 60.65                            | 46.33                 | 113.07      | 1.48 ± 0.40 |
|               | Column purified | 5.04                             | 4.35                  | 5.72        | 3.05 ± 0.49 |
| 72 h          | Root powder     | 463.90                           | 408.94                | 530.60      | 3.16 ± 0.63 |
|               | Methanol extract| 33.25                            | 27.53                 | 39.01       | 2.41 ± 0.41 |
|               | Ethanol extract | 23.47                            | 18.04                 | 30.49       | 1.57 ± 0.33 |
|               | Chloroform extract| 47.17                        | 38.34                 | 63.65       | 1.81 ± 0.40 |
|               | Ether extract   | 33.76                            | 25.15                 | 42.47       | 1.67 ± 0.40 |
|               | Column purified | 4.07                             | 3.07                  | 5.80        | 1.35 ± 0.27 |
| 96 h          | Root powder     | 359.02                           | 304.84                | 400.26      | 3.71 ± 0.67 |
|               | Methanol extract| 23.46                            | 17.73                 | 27.95       | 2.52 ± 0.44 |
|               | Ethanol extract | 15.47                            | 11.69                 | 18.81       | 2.07 ± 0.35 |
|               | Chloroform extract| 29.90                        | 23.10                 | 35.90       | 2.08 ± 0.41 |
|               | Ether extract   | 23.12                            | 16.63                 | 28.05       | 2.27 ± 0.43 |
|               | Column purified | 1.84                             | 1.27                  | 2.37        | 1.63 ± 0.27 |
Japan (19). It seems that the higher molluscicidal activities of the column-purified fractions of *P. granatum* against *L. acuminata* are due to the number of alkaloids present in them.

It is clear from the present results that *C. indica* root has sufficient molluscicidal activity. It has been previously reported that aqueous and ether extracts of the whole plant and methanol extracts of root and leaves have molluscicidal activity (20,21). Mahran et al. (22,23) and Motawe (24) have reported the molluscicidal potency of petroleum ether extracts of different parts of *C. indica* against *Biomphalaria alexandrina* snails, with root extracts being more toxic than other extracts. These investigators isolated a bioactive molluscicidal compound called cannagenin. It seems that the molluscicidal activity of column-purified fractions of *C. indica* root against *L. acuminata* was also due to cannagenins.

The steep slope indicates that even a small increase in the concentrations causes

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| Exposure time | Root extracts | Lethal concentration (LC50) | 95% Confidence limits | Slope value |
|--------------|--------------|----------------------------|----------------------|------------|
|              |              | (mg/l)                     | LCL (mg/l)           | UCL (mg/l) |
| 24 h         | Bark powder  | 138.08                     | 115.54               | 202.72     | 3.35 ± 0.72 |
|              | Methanol extract | 29.52    | 23.99               | 48.55     | 2.67 ± 0.64 |
|              | Ethanol extract    | 22.42    | 17.81               | 35.18     | 2.20 ± 0.45 |
|              | Chloroform extract | 30.77    | 21.93               | 72.80     | 1.93 ± 0.47 |
|              | Ether extract    | 31.98    | 22.69               | 77.40     | 2.01 ± 0.49 |
|              | Column purified  | 4.39     | 3.54                | 5.72      | 1.80 ± 0.29 |
| 48 h         | Bark powder  | 114.32                     | 97.66                | 156.93     | 2.84 ± 0.63 |
|              | Methanol extract | 21.46    | 18.61               | 27.23     | 2.76 ± 0.59 |
|              | Ethanol extract    | 11.03    | 9.41                | 12.89     | 2.59 ± 0.39 |
|              | Chloroform extract | 18.62    | 14.75               | 28.96     | 1.81 ± 0.40 |
|              | Ether extract    | 20.23    | 15.66               | 34.42     | 1.73 ± 0.40 |
|              | Column purified  | 2.58     | 1.96                | 3.24      | 1.69 ± 0.27 |
| 72 h         | Bark powder  | 84.79                      | 73.28                | 101.64     | 2.64 ± 0.60 |
|              | Methanol extract | 16.05    | 14.10               | 18.10     | 3.27 ± 0.58 |
|              | Ethanol extract    | 7.60     | 5.99                | 8.99      | 2.44 ± 0.39 |
|              | Chloroform extract | 11.88    | 9.75                | 14.72     | 1.99 ± 0.38 |
|              | Ether extract    | 12.08    | 9.83                | 15.19     | 1.90 ± 0.38 |
|              | Column purified  | 1.53     | 1.26                | 1.94      | 2.00 ± 0.35 |
| 96 h         | Bark powder  | 62.62                      | 52.09                | 70.33      | 3.32 ± 0.63 |
|              | Methanol extract | 12.12    | 10.27               | 13.56     | 3.88 ± 0.61 |
|              | Ethanol extract    | 8.47     | 6.87                | 9.94      | 2.46 ± 0.39 |
|              | Chloroform extract | 8.67     | 6.97                | 10.25     | 2.32 ± 0.38 |
|              | Ether extract    | 7.63     | 5.90                | 9.12      | 2.28 ± 0.38 |
|              | Column purified  | 0.94     | 0.79                | 1.08      | 2.87 ± 0.41 |
higher snail mortality. Values of the t-ratio higher than 1.96 indicate that the regressions are significant. Values of the heterogeneity factor of less than 1.0 denote that in the replicate test of the random sample the concentration-response curves would fall within the 95% confidence limits and thus the model fits the data adequately. The index of significance of potency estimation, g value, indicates that the value of the mean is within the limits at all probabilities (90, 95, 99) since it is less than 0.5.

On the basis of the present study, we conclude that *P. granatum* and *C. indica* may be used as potent molluscicides since the concentrations used to kill snails were not toxic for *C. fasciatus* fish. The mechanism by which these preparations cause snail death is not exactly known and will require further studies for elucidation.

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