



## Potential Extracts of *Pangium edule* Reinw and *Derris elliptica* Wallich as Botanical Molluscicides for Management of Golden Apple Snail *Pomacea canaliculata* Lamarck

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### Abstract

The research purposed was compared of two extracts as molluscicidal activities from root of *Derris elliptica* Wallich. and *Pangium edule* Reinw seed, that assessed to 3-month old snails *Pomacea canaliculata* L. The Golden apple snails is widely regarded as worst invasive pest species in the rice growing area. It normally destroys the young stems and leaves paddy and could consume 7 – 24 rice seedlings per day. The experiment research started with the mortality test of the golden apple snail, meanwhile hexane extract fraction and etanol extract fraction have completely jumble mode; using the lethal concentration (LC) have mean to describe short term potency of poisonous (toxicity) from materials and can gave little effect or impact for environment; processing phytochemical test from n-hexane extract and etanol extract of root *D.elliptica* and *P.edule* showed positive to contain tannin, saponin and phenol. The data of LC<sub>50</sub> from n-hexane fraction measure with probit analyze (9,905 mg L<sup>-1</sup>) by *D.elliptica* L. with high toxic category, and n-hexane fraction (11,574 mg L<sup>-1</sup>) by *P.edule* Reinw. with toxic category are more effective for golden apple snail control. The golden apple snail mortality was highest using 5000 ppm n-hexane fraction showed 93,3% from *D.elliptica* and using 5000 ppm n-hexane fraction from *P.edule* showed 63,3%. In conclusion, both of extracts from *D.elliptica* roots and *P.edule* seeds was showed potency as botanical molluscicides and it can be apply in the field.

Keywords: *Botanical molluscicides, Derris elliptica, Pangium edule, Phytochemical screening, Pomacea canaliculata*

## A. Introduction

The Golden Apple Snail (GAS) *Pomacea canaliculata* Lamarck (Gastropoda: Ampulariidae), GAS is one of the world's 100 worst invasive pest species and potential. The invasiveness is related to its inherent characteristic; a high reproductive rate, adaptability to harsh environmental conditions, ability to invade diverse habitats through multiple pathways (Arunlertaree *et al.*, 2012).

Paddy is important food substance that have nutrient and energy for growth, also it has contents many substances can change as energy. The result of the people increased, can made many areas for farming more increase. The government with many programmes all of the time trying for reserve rices production with wide areal farming, but one of inhibitor factor is destroyer organism can attack paddy.

*P.canaliculata* is a highly voracious nocturnal herbivore, it can destroy newly seedling rice as long as there is water in field. It cuts the base of young seedlings with its layered tooth (radula) and munches if the succulent tender sheath of rice. The Golden apple snails is widely regarded as worst invasive pest species in the rice growing area. It normally destroys the young stems and leaves paddy and could consume 7 – 24 rice seedlings per day (Joshi 2005; Manoppo 2003; Manoppo, 2015).

GAS is difficult to manage once it invades new areas because of its biological and morphological characteristics. A female GAS can lay 50-500 eggs at one time, and GAS has a gill (ctenidium) and a lung like organ enabling it to survive in and out water individual snails can live more than 4 year. The population of GAS approximately 8 GAS/m<sup>2</sup> can cause significant yield loss productions, the extent of damage to the rice crop depends on snails size and density and the growth stage of the rice plant, that assessed to 3 month old snails and that are 20-40 mm are the most destructive, regardless of rice establishment method. Gas also feeds on wide variety of live host plants, its other hosts and food include livestock feed, decaying matter, animal flesh and other crops (Musman, 2010; Manoppo, 2003; Manoppo, 2015).

Many ways that already used for controlling the pest, in other hands to control the snails, we must know about the behaviour and live circles (Kertoseputro *et al.*, 2007). The controlling of GAS most of the time can using way mechanic, biology and chemistry. However controlling with synthetic molluscicides can causes poisonous for farmer, animals especially yield of farming can containing residu from synthetic molluscicides (Soenaryo *et al.*, 1989)

The advances in the battle againsts the snails using natural molluscicides must be encourage in order to minimize the negative side effect to the environment. A number of tropical plants have been investigated for their molluscicides activity such as crown flower (*Calotropis gigantea*), sambong leaf (*Blumea balsamifera* L.), *Euphorbia tirucalli*, *Derris elliptica* Wallich, *Pangium edule* Reinw. (Suharto., 2005; Wijayakusuma *et al.*, 1992; Manoppo, 2003; Manoppo, 2015). The compound groups from plants identified as having molluscicides activity are saponin, tanin, alkaloid and flavonoid also fenol.

The extracts from two botanicals, *D.elliptica* root and *P.edule* seed were evaluated against golden apple-snails inhibitor respiratory system and make a slow of desible of heart also inhibitor for catch oxygen. The research purposed was focused on potential extract of *D.elliptica* root and *P.edule* seed to manage the GAS as botanical molluscicides that friendly for environment. The research purposed was compared of two extracts as molluscicidal activities from root of *Derris elliptica* Wallich. and *Pangium edule* Reinw seed, that assessed to 3-month old snails *Pomacea canaliculata* L. The experiment research started with the mortality test of the golden apple snail, meanwhile hexane extract fraction and etanol extract fraction have completely jumble mode; using the lethal concentration (LC) have mean to describe short term potency of poisonous (toxicity) from materials and can gave little effect or impact for environment.

## B. Methodology

### 1. Plant Material and Research Equipment

Specimen plants of *D.elliptica* Wallich roots and *P.edule* Reinw seeds were collected from Tonsealama village, in the North Tondano District, Northern Sulawesi. The *P.edule* Reinw. seeds were collected from trees at a height of about 3.5 m – 6.5 m, and placed in green house (28 ± 5°C) to dry, after which they were then crushed into a crude material and stored in an airtight container until use.

The type of solvent that has been using was ethanol and n-hexane pro-Analyze (PA), a number of extraction kit rotavapor (Buchi R-250), blender, vacuum desicator, oven, digital ohaus, vacuum pump.

## 2. Snail collection

The identification and characteristic of *P.canaliculata* was performed based on data. *P.canaliculata* with 7-9 in<sup>3</sup> or 3 month old snails were collected from the rice field at Tonsealama village, North Tondano District and subsequently acclimated in glass aquarium.

## 3. Early procedure

Seed of *P.edule* Reinw have to separate from egg fruit, washing the seed *P.edule* can helping next step for taken flesh of seed after broken the seed, after then seed of *P.edule* must be dried, with temperature room without got sunlight directly (two weeks) or 14 days (Sakul *et al*, 2012). Meanwhile, roots of *D.elliptica* must be got the same point or same treatment, whereas we were used dry roots of *D.elliptica* (Manoppo, 2003).

## 4. Water content measure

After seed of *P.edule* and root of *D.elliptica* were dried, taken the sample both of plant approximately 3 gram with digital ohaus per sample and put in into electric oven that have temperatures average 105°C, for 5 hours, after then taken the sample and let it cooling into vacuum desicator. We still controlling for temperate in order to get maximum result. The water content measure was purpose to get less 10% of water content of seed *P.edule* and root *D.elliptica* and one each case for more better solvent etanol and n-hexane will working analyze.

## 5. Extraction process of *P.edule* seed and *D.elliptica* root with n-hexane fraction (solvent)

The water content measure with result 9,009 % for *P.edule* seed and 9,0 % for *D.elliptica* root, and we were used 512,3 gram seed of pangi and 500 gram *D.elliptica*, that shows both of plant ready to go in the next stage of extract.

First stage is maceration early with n-hexane 1000 ml, whereas root and seed have to separates kit or glasses, let the solvent 24 hours and we can got 2 layers as waste and result filtrat (maceration first made). The waste of first maceration mix with 800 ml n-heksan and let it solvent 24 hours after that if there is 2 layers of solvent took the waste result of second maceration (second filtrat).

Collected the result of maceration I and II, and refine with Whatmann Paper with vaccum pump for helping and got filtrat has been clearly yellow colour. In this case both of plant have the same treatment. After that put the filtrat into evaporator with temperate 40°C as long as 1 hour, the patch of evaporator is 1000 ml.

The result of extract were put into minies bottle, dont forget for to take the measuring for bottle weight with empty condition of full substance, its mean for got substance measuring after all.

## 6. Extraction process of *P.edule* seed and *D.elliptica* root with using ethanol fraction (solvent).

The extraction with ethanol solvent has same point procedure with that process of *P.edule* seed extract usin n-hexane solvent. If result of macerate still though, its mean many the filtrat can collected. When both of extract already got, with the same point ethanol and n-hexane into the next step are LC<sub>50</sub> test to aimed where is potential extract more an active for increase GAS mortality.

## 7. Statistical analysis

LC<sub>50-48h</sub> data values were determined following probit analysis and experimental data were subjected to one way ANOVA at 0.05 significance level using SPSS IBM-Software Ver.20. Means were then compared by Least Significance Different (LSD/BNT).

## C. Result and Discussion

The phytochemical analysis of *P.edule* seed extract and *D.elliptica* root extract, have the purpose to prove, there is tanin, as we know tanin have natural polifenol and carboxil cluster with result "browning enzymatic" that cause colouring seed change from white to brown. This reaction is catalized by polifenolase enzyme. Tanin has a strong characteristic especially interaction with protein. Tanin consist of katekin, leukoantosianin and hidroxy acid (galat, kafeat and khlorogenat acid). In this case using 1 gram extract plant result and NaCl 10% also Fe<sub>3</sub>Cl, if this solvent has tanin the result must have gradation blueblack colouring with settled at bottom.

Saponin analysis have to result constant of bubbles, whereas extract of *P.edule* and *D.elliptica* with etanol as positive and extract with n-hexane negatif result. In each case with fenol screening, there is different result. For fenol test, can usage 1% Fe<sub>3</sub>Cl, and the solvent with n-hexane shows positif fenol with blue colour stabil and bubble will form.

The extraction of root *D.elliptica* and seed *P.edule* doing stuff with the maceration process, and after that continue with the evaporation technique with using n-hexane fraction and ethanol fraction. The result of treatment to measure the mortality of *P.canaliculata*, the table shows below :

Table 1. The test results of mortality *P.canaliculata* based on activity from *D.elliptica* plant root extract fraction of n-hexane after 48 hours

| Treatment          | 50 ppm | 1000 ppm | 2000 ppm | 3000 ppm | 4000 ppm | 5000 ppm |
|--------------------|--------|----------|----------|----------|----------|----------|
| 1                  | 3      | 3        | 4        | 5        | 8        | 10       |
| 2                  | 1      | 2        | 3        | 5        | 7        | 9        |
| 3                  | 2      | 8        | 4        | 6        | 9        | 9        |
| Total of mortality | 6      | 8        | 11       | 16       | 24       | 28       |
| Average            | 2      | 2,67     | 3,67     | 5,33     | 8        | 9,33     |
| Percentage         | 20%    | 26,6%    | 36,7%    | 53,3%    | 80%      | 93,3%    |

Table 2. Test of Homogeneity of Variances and Analysis Of Varians From *D.elliptica* plant root extract fraction of n-hexane after 48 hours

#### Test of Homogeneity of Variances

VAR00001

| Levene Statistic | df1 | df2 | Sig. |
|------------------|-----|-----|------|
| ,031             | 2   | 15  | ,969 |

#### ANOVA

VAR00001

|                | Sum of Squares | df | Mean Square | F    | Sig. |
|----------------|----------------|----|-------------|------|------|
| Between Groups | 4,000          | 2  | 2,000       | ,223 | ,803 |
| Within Groups  | 134,500        | 15 | 8,967       |      |      |
| Total          | 138,500        | 17 |             |      |      |

According to result of dependent variable test with multiple comparison shows that treatment can gave effect that was real in 5%. For mortality of Golden Apple-Snails or number of GAS where as got the best result after test 48 hours. How ever that concentrate 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm shows different result and the high result is 5000 ppm, whereas a number of snails was died in 48 hours for *D.elliptica* n-hexane fraction. The golden apple snail mortality was highest using 5000 ppm n-hexane fraction showed 93,3% from *D.elliptica* and using 5000 ppm n-hexane fraction from *P.edule* showed 63,3%.

Table 3. The test results of mortality *P.canaliculata* based on activity from *P.edule* plant seed extract fraction of n-hexane after 48 hours

| Treatment          | 50 ppm | 1000 ppm | 2000 ppm | 3000 ppm | 4000 ppm | 5000 ppm |
|--------------------|--------|----------|----------|----------|----------|----------|
| 1                  | 2      | 2        | 4        | 5        | 4        | 8        |
| 2                  | 1      | 3        | 2        | 4        | 4        | 6        |
| 3                  | 2      | 1        | 3        | 4        | 7        | 5        |
| Total of mortality | 5      | 6        | 9        | 13       | 15       | 19       |
| Average            | 1,67   | 2,00     | 3,00     | 4,33     | 5        | 6,33     |
| Percentage         | 16,7%  | 20%      | 30%      | 43,3%    | 50%      | 63,3%    |

Table 4. Test of Homogeneity of Variances and Analysis Of Varians *From P.edule* plant seed extract fraction of n-hexane after 48 hours**Test of Homogeneity of Variances**

VAR00001

| Levene Statistic | df1 | df2 | Sig. |
|------------------|-----|-----|------|
| ,120             | 2   | 15  | ,888 |

**ANOVA**

VAR00001

|                | Sum of Squares | df | Mean Square | F    | Sig. |
|----------------|----------------|----|-------------|------|------|
| Between Groups | 2,111          | 2  | 1,056       | ,249 | ,782 |
| Within Groups  | 63,500         | 15 | 4,233       |      |      |
| Total          | 65,611         | 17 |             |      |      |

Table 5. The test results of mortality *P.canaliculata* based on activity from *D.elliptica* plant root extract fraction of etanol after 48 hours

| Treatment          | 50 ppm | 1000 ppm | 2000 ppm | 3000 ppm | 4000 ppm | 5000 ppm |
|--------------------|--------|----------|----------|----------|----------|----------|
| 1                  | 1      | 1        | 4        | 4        | 4        | 6        |
| 2                  | 1      | 2        | 3        | 3        | 5        | 8        |
| 3                  | 2      | 2        | 3        | 4        | 6        | 7        |
| Total of mortality | 4      | 5        | 10       | 11       | 15       | 21       |
| Average            | 1,33   | 1,67     | 3,33     | 3,67     | 5        | 7        |
| Percentage         | 13,3%  | 16,6%    | 33,3%    | 36,7%    | 50%      | 70%      |

Table 6. Test of Homogeneity of Variances and Analysis Of Varians *From D.elliptica* plant root extract fraction of etanol after 48 hours**Test of Homogeneity of Variances**

VAR00001

| Levene Statistic | df1 | df2 | Sig. |
|------------------|-----|-----|------|
| ,129             | 2   | 15  | ,880 |

**ANOVA**

VAR00001

|                | Sum of Squares | df | Mean Square | F    | Sig. |
|----------------|----------------|----|-------------|------|------|
| Between Groups | 1,333          | 2  | ,667        | ,138 | ,873 |
| Within Groups  | 72,667         | 15 | 4,844       |      |      |
| Total          | 74,000         | 17 |             |      |      |

According to table 5 and 6, it mean that etanol fraction from species *D.elliptica* root extract have possibilities become a bioinsecticide, and the best concentration only 5000 ppm, because the total mortality of *P.canaliculata* can reach 21 species or 70% of lethal death.

Table 7. The test results of mortality *P.canaliculata* based on activity from *P.edule* plant seed extract fraction of etanol after 48 hours

| Treatment          | 50 ppm | 1000 ppm | 2000 ppm | 3000 ppm | 4000 ppm | 5000 ppm |
|--------------------|--------|----------|----------|----------|----------|----------|
| 1                  | 2      | 2        | 4        | 5        | 4        | 7        |
| 2                  | 1      | 3        | 2        | 3        | 4        | 5        |
| 3                  | 2      | 1        | 3        | 2        | 6        | 5        |
| Total of mortality | 5      | 6        | 9        | 10       | 14       | 17       |
| Average            | 1,67   | 2,00     | 3,00     | 3,33     | 4,67     | 5,67     |
| Percentage         | 16,6%  | 20%      | 30%      | 33,3%    | 46,7%    | 56,7%    |

Based of data, according to table 7 and 8, it mean that etanol fraction from species *P.edule seed* extract have possibilities become a bioinsecticide too, but if we compare with the table 5 and 6, this differencies of total mortality from GAS (*P.canaliculata*) it very wide 56,7% < 70%, and the best concentration still in 5000 ppm, because the total mortality of *P.canaliculata* can reach 17 species or 56,7% of lethal death.

Table 9. Toxicity classification LC<sub>50</sub> and Toxicity Rating (ISO, 1982)

| LC <sub>50</sub> (mg/L) | Toxicity Rating |
|-------------------------|-----------------|
| >10000                  | Non Toxic       |
| 1000 – 10000            | Very low toxic  |
| 100 – 1000              | Low toxic       |
| 10 – 100                | Toxic           |
| 1 – 10                  | High Toxic      |
| 0,1 – 1                 | Very High Toxic |
| < 0,1                   | Extreme Toxic   |

Results showed that n-hexane fraction is the most effective againts Golden Apple-Snails mortality (LC<sub>50-48h</sub> = 9,905 mg/L) from *D.elliptica* root extract, (LC<sub>50-48h</sub> = 11,574 mg/L) from *P.edule* Reinw extract. According to the table 9, it's show the toxicity rating, *D.elliptica* root extract has a High Toxic category, it is same with *P.edule* Reinw extract, toxic too.

#### D. Conclusion

In this research, we determined that LC<sub>50</sub> values of *D.elliptica* root extract and *P.edule* seed extract, with probit analyze shows that *D.elliptica* root extract had been highest effect (toxic) for Golden Apple-Snails mortality in 48 hours after treatment. In each case of *P.edule* seed extract shows effected for GAS mortality in 36 hours after treatment. It's assumed for screening phytochemistry that the observed biology effects of largely due to Tanin, Saponin, Fenol present in the root and seed extract. Thus, these results support that both of extracts from *D.elliptica* roots and *P.edule* seeds was showed potency as botanical molluscicides and an attractive compound for further studies leading to molluscicidal development.

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