Tropical Life Sciences Research, 21(2), 41–50, 2010

Toxicity of *Barringtonia racemosa* (L.) Kernel Extract on *Pomacea canaliculata* (Ampullariidae)

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Abstrak: Beberapa spesies tumbuhan tropika telah dikenalpasti sebagai anti moluska dan *Barringtonia racemosa* (L.) ialah salah satu daripadanya. Kesan racun ekstrak biji benih *B. racemosa* terhadap *Pomacea canaliculata* telah dikaji. *Lethal concentration* pada 50% [LC_{50} *(lower-upper limits)*] dalam ppm/48 jam ialah 70.71 (41.33–120.97), 94.39 (62.48–142.59), 186.84 (129.21–270.17), dan 672.72 (366.57–1234.53) bagi ekstrak diklorometan (DCM), methanol (MeOH), etil asetat (EtOAc), dan heptan (hp) pada 95% *confidence interval* (C. I.) masing-masing. Semua analisis dijalankan menggunakan program *Trimmed Spearman-Karber* (TSK) versi 1.5. Diandaikan bahawa kesan biologi adalah disebabkan oleh saponin dan flavonoid yang terkandung dalam biji benih *B. racemosa*. Ekstrak DCM dan MeOH yang mengandungi saponin dan flavonoid memberikan kesan molusisida yang lebih baik berbanding dengan ekstrak pelarut yang lain. Aktiviti biologi yang diperhatikan mencadangkan bahawa *B. racemosa* berpotensi dalam pengawalan *P. canaliculata*.

Kata kunci: *Barringtonia racemosa*, *Pomacea canaliculata*, Saponin, Flavonoid, Molusisida, LC_{50}

Abstract: A number of tropical plant species have been recognised as molluscicidal plants, and *Barringtonia racemosa* (L.) is one of these. The toxicity effects of *B. racemosa* seed kernel extracts on *Pomacea canaliculata* were evaluated. The lethal concentration at 50% [LC_{50} (lower-upper limits)] values, in ppm/48 hours, were 70.71 (41.33–120.97), 94.39 (62.48–142.59), 186.84 (129.21–270.17), and 672.72 (366.57–1234.53) for the extracts withdrawn using dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc), heptane (hp) solvents, respectively at 95% confidence interval (C. I.). All analyses were conducted using Trimmed Spearman-Karber (TSK) program version 1.5. It is assumed that the observed biological effects of the extracts may be due to the saponins and flavonoids present in the seed. The dichloromethanic and methanolic extracts contain saponin and flavonoid substances. Therefore these extracts have shown more potent molluscicidal activity towards the tested organism compared to the remaining extracts. This observed biological activity suggests a promising role for *B. racemosa* in the control of *P. canaliculata*.

Keywords: *Barringtonia racemosa*, *Pomacea canaliculata*, Saponins, Flavonoids, Molluscicide, LC_{50}

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INTRODUCTION

Barringtonia racemosa (L.) Spreng (Lecythidaceae) is a tropical higher plant that is widely spread in East Africa, Southeast Asia and the Pacific islands (Strey 1976; Chantaranothai 1995). It grows in the moist lower countries where it is exposed to high sunlight intensities and for extended periods. It is locally known as *penteut ie* and its fruits are used as a traditional medicine to treat asthma, cough and diarrhoea. The seed of this plant contains saponins and flavonoids (Ojewole *et al*. 2004; Gowri *et al*. 2009). These compounds are widely distributed amongst plants and show a wide range of biological properties (Sparg *et al.* 2004). Saponins are glycosides with a distinctive foaming characteristic when dissolved in water (Hostettmann & Marston 1995); this is assumed to be due to their amphiphilic nature (Xu *et al*. 1996; Francis *et al*. 2002). Saponins consist of polycyclic aglycone and sugar moieties. The ability of a saponin to foam is caused by the combination of the nonpolar aglycone and the water soluble sugar portion. Saponins are highly toxic to cold-blooded animals due to their ability to lower surface tension. Saponins serve as plant immune systems, acting as a natural antibiotic to protect the plant against microbes and fungi (Estrada *et al*. 1997). The saponin class of natural products induce red blood cell lysis in animal systems (Francis *et al*. 2002). It has been reported that *B. racemosa* seed kernel has a wide range of therapeutic applications (Thomas *et al*. 2002; Gowri *et al*. 2007; Hussin *et al*. 2009). Flavonoids are hydroxylated phenol substances that occur as C6-C3-C6 moieties. These compounds are widely distributed in plants and possess a number of pharmacological properties (Whiting 2001).

Pomacea canaliculata (Ampullariidae) (Ghesquiere 2007) is a well known golden apple snail, which is originally from South America. It was introduced into Asia through the pet trade and as a potential food source for humans. The rapid invasion of *P. canaliculata* accross Asia during the last decades has been terrifying. The snails have been causing damage to rice production in Indonesia and other countries (Hirai 1988; Halwart 1994; Wada *et al*. 2002; Darby *et al*. 2008; Joshi *et al*. 2008). Some approaches, either mechanical (Teo 2003) or biological (Sumangil 1989) methods to control the snails have been conducted, but with unsatisfactory results due to the widespread distribution of this pest. The chemical approach, using synthetic molluscicides, such as metaldehyde and niclosamide, has been applied (Dela Cruz *et al.* 2000) to control this pest. Unfortunately, the synthetic substances can pollute water bodies, causing the deaths of fishes and livestock (Calumpang *et al*. 1995; Wada 2004).

The advances in the battle against the snails using natural molluscicides must be encouraged in order to minimise the negative side effects to the environment. A number of tropical plants have been investigated for their molluscicidal activity (Kardinan & Iskandar 1997; Arunlertaree *et al*. 2003; Musman 2004, 2006, 2009; Joshi *et al*. 2005; San Martin 2007; Plan *et al*. 2008). The compound groups from plants identified as having active molluscicide activity are saponin, tannin, alkaloid, and flavonoid (Perry 1980; Kloos & McCullough 1987; Huang *et al*. 2003). Literature searches revealed that there have been

scientific studies carried out studying the cytotoxic activity of the seed kernel of *B. racemosa* on *P. canaliculata*. Hence, the present study is focused on evaluating the toxicity potentials of different seed kernel extracts of *B. racemosa* on *P. canaliculata*.

MATERIALS AND METHODS

The experiment was conducted in the Marine Laboratory of the Department of Marine Science, Coordinatorate of Marine Science and Fishery at Syiah Kuala University, Banda Aceh, Indonesia.

Extraction of *B. racemosa* Seed Kernels

B. racemosa fruits were collected from Lam Neuhen village, in the Aceh Besar District on October 2008. The harvested fruits were kept in the Marine Laboratory of the Department of Marine Sciences, Syiah Kuala University. Subsequently, fruits were decorticated to remove the kernels and air dried in the shade for seven days. The seed kernels (1.8 kg, dry) were pulverised with an electric blender and sieved with a 40 mm mesh screen to obtain fine powder. 500 g of the powder was extracted twice sequentially, on each occasion with 2 l of heptane (hp), dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH), at room temperature for 48 hours with shaking. In each case, the filtrates were filtered through Whatman filter paper and were evaporated to dryness by placing the container in a rattling water bath (40ºC). The extracts were further dried by placement in a vacuum desiccator. The extracts were stored in labeled specimen bottles for bioassays.

Snail Collection

The identification and characterisation of *P. canaliculata* was performed based on previously published data (Ghesquiere 2007). *P. canaliculata* with 7–9 in^3 in shell volume (mean±SD was 7.89±0.88) were collected from the rice field at Gla Deyah village, Aceh Besar District, and subsequently acclimated in a glass aquarium for 7 days in the Marine Laboratory of the Department of Marine Sciences, Syiah Kuala University.

Preliminary Phytochemical Analysis

Qualitative phytochemical screening of *B. racemosa* seed kernel extracts was carried out using standard laboratory techniques. The Molisch, Fehling, and Benedict tests were applied for carbohydrates analysis (Evans 1989; Raman 2006), a frothing test was carried out for saponin analysis (Harborne 1984; Evans 1989), Schinoda's test was applied for flavonoid analysis (Evans 1989; Raman 2006), Lieberman-Burchard's test was carried out for triterpenoid analysis (Harborne 1984; Evans 1989), the Mayer's, Wagner's and Dragendorff's tests were applied for alkaloids (Evans 1989), and ferric chloride and alkaline tests were carried out for tannin analysis (Evans 1989). The percentage of yield for

each extraction was calculated according to Kennedy (1990). For each solvent, the yield % was derived following the equation shown below:

$$\% \text{ yield} = \frac{\text{Extract (g)}}{\text{Sample (500g)}} \times 100$$

Bioassay Technique

The extracts of *B. racemosa* seed kernel were evaluated at 50, 100, 200, 400 and 800 ppm concentrations in an aqueous solution. A sample size of 480 individual *P. canaliculata* snails were collected based on the specified size (Ghesquiere 2007) and screened for molluscicidal bioassay with a protocol adapted from the Food and Agriculture Organisation (FAO) method (Reish & Oshida 1987). Twenty four glass aquariums measuring 45 x 28 x 35 cm (l x w x h) were arranged in 4 groups (in order to apply the hp, DCM, EtOAc, and MeOH extracts) consisting of 6 aquariums (to apply solutions at 0, 50, 100, 200, 400, and 800 ppm). The aquariums were filled up with paddy-field water (pH 6.4 and temperature 31^{o}C) taken from the location where the *P. canaliculata* were collected. The water was filled into the aquarium to a depth of 10 cm, as measured from the bottom. Each aquarium was then filled with as many as 20 individual *P. canaliculata*. The tested organisms were allowed a free moving period of 30 minutes. The aquaria groups were arranged in order first for hp, second for DCM, third for EtOAc, and fourth for MeOH extract applications, in which each group consisted of 6 rows of aquariums. The 6 rows of aquariums consisted of a first row for the control, second row for 50 ppm, third row for 100 ppm, fourth row for 200 ppm, fifth row for 400 ppm, and sixth row for 800 ppm concentrations. The aqueous extracts of *B. racemosa* were poured into the aquariums in the first, second, third, and fourth groups, according to the row position, in volumes of up to 100 ml. The mixture had a pH of 6.1 at the end of the bioassay. *P. canaliculata* mortality was assessed after 48 hours of exposure by probing the snail secreted mucus through an operculum gap. The toxicity of the compound towards the snails is reflected in the release of mucus from the tested snails. The signs of mortality were taken to be rigidity and lack of movement. Lethal concentration at 50% (LC_{50}) calculations were used for the determination of lethal concentration endpoints in the acute toxicity bioassay tests. Mortality data were used to calculate LC_{50} values using Trimmed Spearman-Karber (TSK) program (Hamilton *et al.* 1997) version 1.5 software downloaded from the US Environmental Protection Agency.

RESULTS AND DISCUSSION

The phytochemical analysis results are show in Table 1. Application of the extract to the tested organisms showed a significant effect on the mortality rate of *P. canaliculata* (Table 2). The molluscicidal character, in this case is toxicity of

the extract to apple snails. The extract was predicted to demonstrate toxicity towards snails due to the presence of saponins (Hostettmann 1984; Sparg *et al*. 2004; Ojewole *et al*. 2004) and flavonoids (Macheix *et al*. 1990) in the extracts (Table 1). The molluscicidal activity of saponin was due to its ability to effect the cell membrane and its ability to lower the surface tension of water. The extract caused various responses in the tested subjects as a result of direct contact between the tested organisms and the saponin (Hostettmann *et al*. 1982; Mahato & Nandy 1991; Hutchings *et al*. 1996). The tested *P. canaliculata* demonstrated a response to the molluscicidal action of saponin. This response was initiated by the production and secretion of mucus in order to reduce further contact of their body surfaces with the molluscicide (Brain *et al*. 1990). During a period of 48 hours, the data revealed that the mortality rate of the tested *P. canaliculata* was higher in extracts containing both saponins and flavonoids compared to the extracts containing only flavonoids (Fig. 1). It was expected that both substances could block the process of breathing. This probably is due to diffusion of oxygen through the gills of the snails, which is subsequently obstructed by the mucus.

Table 1: Phytochemical constituents of the *B. racemosa* seed kernel extract.

Solvent	Yield (%)	Car	Sap	Fla	Trt	Alk	Tan
hp	3.72	+	−	+	−	−	−
DCM	2.10	+	+	+	+	−	−
EtOAc	4.27	+	−	+	+	−	−
MeOH	6.64	+	+	+	+	−	−

Notes: hp = heptane, DCM = dichloromethane, EtOAc = ethyl acetate, MeOH = methanol, Car = carbohydrates, Sap = saponins, Fla = flavonoids, Trt = triterpenoids, Alk = alkaloids, Tan = tannins.

Table 2: The effect of the *B. racemosa*'s extract on the mortality of *P. canaliculata.*

Conc. (ppm)	Number (ind) of *P. canaliculata* per application	Mortality (ind) of *P. canaliculata* after a fraction of *B. racemosa* kernel extract was applied			
		hp	DCM	EtOAc	MeOH
50	20	0	8	3	10
100	20	0	12	6	15
200	20	5	17	11	20
400	20	7	20	14	20
800	20	11	20	18	20
LC_{50} (L-U limits)/48 hours at 95% C. I.		672.72 (366.57–1234.53)	70.71 (41.33–120.97)	186.84 (129.21–270.17)	94.39 (62.48–142.59)

Notes: hp = heptane, DCM = dichloromethane, EtOAc = ethyl acetate, MeOH = methanol, L-U limits = lower-upper limits, C. I. = confidence intervals.

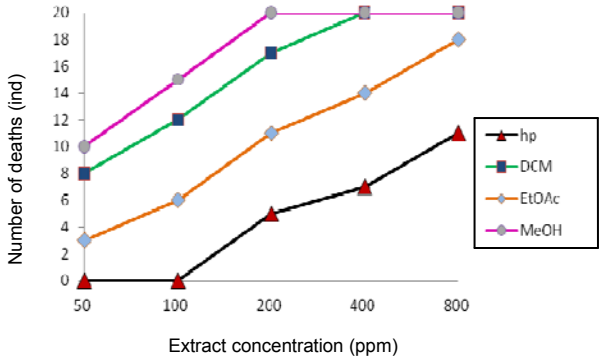

Figure 1: Number of mortalities of *P. canaliculata* in solutions of *B. racemosa*.

The study showed that a 50% mortality rate of *P. canaliculata* was reached within 48 hours of exposing the *P. canaliculata* to 50 ppm of the aqueous solution of the MeOH extract. A 100% *P. canaliculata* mortality rate was observed within 48 hours of incubation of the snails in 200, 400, and 800 ppm of the aqueous solution of DCM and MeOH extracts (Fig. 1). It is predicted that a variety of active compounds dissolved in the same solvents are responsible for the different effects observed in the mortality of the tested species. This experiment also demonstrated that the mortality of the tested *P. canaliculata* was different depending on the time (48 hours after exposure) at which the extract was applied (Table 2). This is such as saponin will break down into sugar and aglycone whereas flavanoid will undergo dissociation when both compounds are dissolved in water leading to inactiveness. The inactiveness in the biological properties depends on time, as shown in Table 2, where the higher is the concentration used, the higher is the number of mortality, at 48 hours after exposure. This suggested that the active compounds of the extract were unstable in water.

CONCLUSION

In this study, we determined that the LC_{50} values (lower-upper limits) of the *B. racemosa* seed kernel extract in the hp, DCM, EtOAc, and MeOH solvents were 672.72 (366.57–1234.53), 70.71 (41.33–120.97), 186.84 (129.21–270.17), and 94.39 (62.48–142.59) (ppm/48 hours), respectively, at 95% C.I. Furthermore, we found that the dichloromethanic extract showed the strongest cytotoxic activity (LC_{50} = 70.71 ppm) and that the heptanic extract had the least cytotoxic activity (LC_{50} = 672.72 ppm). It is assumed that the observed biological effects of the extracts are largely due to the saponins and flavonoids present in the seed. Thus, these results support that the *B. racemosa* seed kernel extract is an attractive compound for further studies leading to molluscicidal development.

REFERENCES

Arunlertaree C, Meeposom C, Navanugraha C and Hutacharoen R. (2003). The utilization of cashew nut shell crude extract for golden apple snail *(Pomacea canaliculata)* control. *Environment and Natural Resources Journal* 1(2): 77–84.

Brain K R, Hadgraft J and Al-Shatalebi M. (1990). Membrane modification in activity of plant molluscicides. *Planta Medica* 56: 663–664.

Calumpang S M F, Medina M J B, Tejada A W and Medina J R. (1995). Environmental impact of two molluscicides: Niclosamide and metaldehyde in a rice paddy ecosystem. *Bulletin of Environmental Contamination and Toxicology* 55(4): 494–501.

Chantaranothai P. (1995). *Barringtonia* (Lecythidaceae) in Thailand. *Kew Bulletin* 50(4): 677–694.

Darby P C, Bennetts R E and Percival H F. (2008). Dry down impacts on apple snail (*Pomacea paludosa*) demography: Implications for wetland water management. *Wetlands* 28(1): 204–214.

Dela Cruz M S, Joshi R C and Martin E C. (2000). Potential effects of commercial molluscicides used in controlling golden apple snalts on the native snail *Vivipara costata* (Quoy and Gaimard). *Philippine Entomologist* 14(2): 149–157.

Estrada A, Redmond M J and Laarveld B. (1997). Quinoa saponin composition and methods of use edition. *United States Patent no. 5,597,807.*

Evans W C. (1989). *Trease and Evans' pharmacognosy*, 13th ed. London: Cambridge University Press.

Francis G, Kerem Z, Makkar H P S and Becker K. (2002). The biological action of saponins in animal systems: A review. *The British Journal of Nutrition* 88(6): 587–605.

Ghesquiere S A I. (2007). *Apple snail (Ampullariidae).* http://www.applesnail.net (accessed 28 April 2007).

Gowri P M, Radhakrishnan S V, Basha S J, Sarma A V and Rao J M. (2009). Oleanane-type isomeric triterpenoids from *Barringtonia racemosa*. *Journal of Natural Products* 72(4): 791–795.

Gowri P M, Tiwari A K, Ali A Z and Rao J M. (2007). Inhibition of α-glucosidase and amylase by bartogenic acid isolated from *Barringtonia racemosa* Robx. seeds. *Phytotherapy Research* 21(8): 796–799.

Halwart M. (1994). The golden apple snail *Pomacea canaliculata* in Asean rice farming systems: Present impact and future threat. *International Journal of Pest Management* 40: 199–206.

Hamilton M A, Russo R C and Thurston R V. (1997). Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science and Technology* 11(7): 714–719.

Harborne J B. (1984). *Phytochemical methods*, 2nd ed. London: Chapman & Hall.

Hirai Y. (1988). Apple snail in Japan: The present status and management. *Japan Agricultural Research Quarterly* 22(3): 161–165.

Hostettmann K. (1984). On the use of plants and plant-derived compounds for the control of schistosomiasis. *Die Naturwissenschaften* 71(5): 247–251.

Hostettmann K and Marston A. (1995). *Chemistry and pharmacology of natural products: Saponins*. London: Cambridge University Press.

Hostettmann K, Kizu H and Tomimori T. (1982). Molluscicidal properties of various saponins. *Planta Medica* 44: 34–35.

Huang H, Liao S, Chang F, Kuo Y and Wu Y. (2003). Moluscicidal saponins from *Spindus mukorossi,* inhibitory agents of golden apple snails, *Pomacea canaliculata. Journal of Agricultural and Food Chemistry* 51(17): 4916–4919.

Hussin N M, Muse R, Ahmad S, Ramli J, Mahmood M, Sulaiman M R, Shukor M Y A, Rahman M F A and Aziz K N K. (2009). Antifungal activity of extracts and phenolic compounds from *Barringtonia racemosa* L. (Lecythidaceae). *African Journal of Biotechnology* 8(12): 2835–2842.

Hutchings A, Scott A H, Lewis G and Cunningham A. (1996). *Zulu medicinal plants: An inventory*. Pietermaritzburg, South Africa: University of Natal Press.

Joshi R C, Martin R, Saez-Navarrete C, Alarcon J, Sainz J, Antolin M M, Martin A R and Sebastian L S. (2008). Efficacy of quinoa *(chenopodium quinoa)* saponins against golden apple snail *(Pomacea canaliculata)* in the Philippines under laboratory conditions. *Crop Protection* 27(3–5): 553–557.

Joshi R, Meepagala K, Sturtz G, Cagauan A, Mendoza C, Dayan F and Duke S. (2005). Molluscicidal activity of vulgarone B from *Artemisia douglasiana* (Besser) against the invasive, alien, mollusk pest, *Pomacea canaliculata* (Lamarck). *International Journal of Pest Management* 51(3): 175–180.

Kardinan I and Iskandar M. (1997). Efficacy of some plants as botanical molluscicides on glden snail. *Journal Penelitian Pertanian* 16(3): 141–146.

Kennedy J H. (1990). *Analytical chemistry: Principles,* 2nd ed. New York: Saunders College Publishing.

Kloos H and McCullough F S. (1987). Plant molluscicides. *Journal of Medicinal Plant Research* 46: 195–209.

Macheix J-J, Fleuriet A and Billot J. (1990). *Fruit phenolics*. Florida: CRC Press Inc.

Mahato S B and Nandy A K. (1991). Triterpenoid saponins discovered between 1987 and 1989. *Phytochemistry* 30(5): 1357–1390.

Musman M. (2004). Effect of methanol extract of fruit of penteut (*Barringtonia asiatica*) to mortality of golden snail (*Pomacea canaliculata*). *Jurnal Natural* 4(2): 9–11.

_____. (2006). Laboratory and field evaluation of molluscicide of golden snail *(Pomacea canaliculata)*. *Jurnal Bionatura* 8(1): 39–46.

_____. (2009). The potency of *Penteut Ie* [Achehnese, *Barringtonia racemosa* (L.) Spreng] as molluscicide of *Pomacea* species (Ampullariidae). *Proceeding of The International Conference on Natural and Environmental Sciences 2009 (ICONES '09)*. *Understanding Disaster and Environmental Issues with Science and Engineering towards Sustainable Development.* Banda Aceh, Indonesia, 6–8 May 2009.

Ojewole J A O, Nundkumar N and Adewunmi C O. (2004). Molluscicidal, cercariacidal, larvacidal and antiplasmodial properties of *Barringtonia racemosa* fruit and seed extracts. *Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas* (*BLACPMA)* 3(5): 88–92.

Perry L M. (1980). *Medicinal plants of East and Southeast Asia: Attributed properties and uses*. London: MIT Press.

Plan M R R, Saska I, Cagauan A G and Craik D J. (2008). Backbone cyclised peptides from plants show molluscicidal activity against the rice pest *Pomacea canaliculata* (golden apple snail). *Journal of Agricultural and Food Chemistry* 56(13): 5237–5241.

Raman N. (2006). *Phytochemical technique*. New Delhi: New Indian Publishing Agencies.

Reish D L and Oshida P S. (1987). Manual of methods in aquatic environment research. Part 10: Shorterm static bioassay. *FAO Fisheries Technical Paper* no. 247.

San Martin R. (2007). Recent development in the use botanical molluscicides against golden apple snails, (*Pomacea canaliculata*). In R C Joshi and L S Sebastian (eds.). *Global advances in ecology and management of golden apple snails.* Neur Ecija, Philippines: PhilRice, 393–403.

Sparg S G, Light M E and van Staden J. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology* 94(2–3): 219–243.

Strey R G. (1976). *Barringtonia racemosa. The Flowering Plants of Africa* 43: 1706.

Sumangil J P. (1989). *Biological control.* In B O Acosta and R S V Pullin (eds.). *Environmental impact of the golden snail (Pomacea* sp*.) on rice farming system in the Philippines.* Manila: International Center for Living Aquatic Resources Management, 26–27.

Teo S S. (2003). Damage potential of the golden apple snail *Pomacea canaliculata* (Lamarck) in irrigated rice and its control by cultural approaches. *International Journal of Pest Management* 49(1): 49–55.

Thomas T J, Panikkar B, Subramoniam A, Nair M K and Panikkar K R. (2002). Antitumour property and toxicity of *Barringtonia racemosa* Roxb seed extract in mice. *Journal of Ethnopharmacology* 82(2–3): 219–243.

Wada T. (2004). Strategies for controlling the apple snail *Pomacea canaliculata* (Lamarck) (Gastropoda: Ampullariidae) in Japanese direct-sown paddy fields. *Japan Agricultural Research Quarterly* 38(2): 75–80.

Wada T, Yusa Y and Joshi R C. (2002). *Proceedings of the Special Working Group on the 2002 Golden Apple Snail* (Pomacea spp.), *7th ICMAM*. Los Banos, Phillippines, 21–24 October 2002.

Whiting D A. (2001). Natural phenolic compounds 1900–2000: A bird's eye view of a century's chemistry. *Natural Products Reports* 18(6): 583–606.

Xu R, Zhao W, Xu J, Shao B and Qin G. (1996). Studies on bioactive saponins from Chinese medicinal plants. *Advances in Experimental Medicine and Biology* 404: 371–382.