INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
11–368/AWB/2012/14–1–111–115
http://www.fspublishers.org

Full Length Article

Antibacterial Activities of *Solanum stramonifolium* Seed Extract

RAKRUDEE SARNTHIMA[1] AND SARANYU KHAMMUANG[1]
Protein and Enzyme Technology Research Unit and Center of Excellence for Innovation in Chemistry, Department of Chemistry, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand
[1]Corresponding author's e-mails: rsarnthima@yahoo.com; skhammuang@yahoo.com

ABSTRACT

Solanum stramonifolium Jacq. fruit is a commonly found vegetable and herb in Thailand. Its seed aqueous extract was investigated for antibacterial activity by disc diffusion method, protein profile and protein purification. Its seed aqueous extract showed very good inhibition against pathogenic bacteria both gram positive including *Stapyllococcus aureus, Bacillus licheniformis, B. subtilis* and *Xanthomonas* sp. and gram negative bacteria including *Pseudomonas aeruginosa* and *Salmonella typhi*. Tris-tricine SDS-PAGE revealed major protein bands approximately 10.2, 15.7 and 21.5 kDa. Partial purification by Hitrap Q XL and SOURCE 15RPC column chromatography revealed that the active proteins might bear negative charges. A strong antibacterial activity suggests this plant species could be a good source for antibacterial agents. Further works on pure active compounds characterizations such as molecular structure and bacterial killing mechanism are still needed.

Key Words: Antibacterial; Eggplant; Peptide; Solanaceae; *Solanum stramonifolium*

INTRODUCTION

Antimicrobial peptides (AMPs) are conserved peptides found all domain organisms including vertebrates and invertebrate as well as plants (Thomma *et al*., 2002). Organisms produced antimicrobial peptides to protect themselves from pathogens infection. These molecules were called defensins, which were first isolated from barley and wheat grains. These types of molecules are believed to be found in every plant (Lay & Anderson, 2005) and in every tissue (Chehregani *et al*., 2007). However, most AMPs are found high amount in plant seeds and some of these peptides were isolated for molecular, biochemical as well as structural studies (Odintsova *et al*., 2007).

Members of the family Solanaceae are shown to have the growth inhibition ability toward bacteria, fungi and virus (Chah *et al*., 2000; Arthan *et al*., 2002; Wiart *et al*., 2004). The extract of *Solanum straminifolium* inhibits anti-Leishmaniasis (Estevez *et al*., 2007). Methanol extracts of *S. incanum* and *S. nigrum* exhibited antioxidant antimicrobial and cytotoxic activities (Al-Fatimi *et al*., 2007). Those bioactive molecules are organic compounds such as flavonoids, iso-flavonoids and phenolic acids.

Apart from non-protein bioactive compounds, proteins with antimicrobial activities in Solanaceae plants have also been reported. For examples, antibacterial peptides from seeds of *Capsicum annuum* L. (Ribeiro *et al*., 2007) and *S. tuberosum* (Feng *et al*., 2003) have been reported. Thailand situated in tropical zone that has been rich in biodiversity of plants. To our knowledge, there are few reports about antibacterial activity from Solanaceae plants found in Thailand. Recently, we have screened this plant genus for antibacterial activity and found that *S. stramonifolium* showed the strongest antibacterial activity amongst other tested species. In this study we reported antibacterial activity against certain pathogenic bacteria both gram positive and gram negative ones. We also proposed whether the bioactive compounds are peptides or small proteins by mean of its activity after subjected to proteolysis and purification trials by column chromatography techniques.

MATERIALS AND METHODS

Preparation of seed extract: The fruits of *S. stramonifolium* were purchased from the local market in Nong Khai province (northeastern, Thailand). Its seeds were removed out from its fruits by hands, cleaned with distilled water, powdered under liquid N_2 and extracted with 0.01 M HCl containing 0.15 M NaCl. The extraction procedure was done at a ratio of sample/extract solution of 1:3 (w/v). The residue was then removed by filtering through cheese cloth (if needed); the filtrate was then centrifuged at 8,100 x g, for 5 min. These seed extract was subjected to antibacterial activity experiments and protein determination.

Determination of protein content and protein pattern analysis: Protein content was determined as described by Bradford (1976) using a Bio-Rad protein assay reagent (Bio-Rad, USA) and using bovine serum albumin (BSA) as a protein standard. Absorbance was measured at 595 nm after stand the mixture for 5 min at room temperature.

To cite this paper: Sarnthima, R. and S. Khammuang, 2012. Antibacterial activities of *Solanum stramonifolium* seed extract. *Int. J. Agric. Biol.*, 14: 111–115

Protein profile analysis of seed extract was performed by a 15% separating Tris-tricine SDS-PAGE from the slightly modified method of Schagger and von Jagow (1987). To run the gel an initial voltage of 30 V for 15 min and then a constant voltage at 200 V were applied. The protein bands in the gel were visualized by silver staining.

Bacterial cultures: Bacteria were obtained as a kind gift from Associate Prof. Dr. Sompong Thammasirirak, Department of Biochemistry, Faculty of Science, Khon Kaen University and from Dr. Rungruedee Thiewthong, Department of Biology, Faculty of Science, Mahasarakham University. The bacteria used in this study consisted of eight strains of bacteria both gram positive and gram negative, including *Stapyllococcus aureus*, *B. licheniformis*, *B. subtilis*, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. They were grown and maintained on Luria-Bertani (LB) medium.

Disc diffusion assay: Antibacterial activity was assayed by disc diffusion method according to Lo Cantore *et al*. (2009) with some modifications as previously described (Phansri *et al*., 2011) by using the crude extract of *S. stramonifolium*, 100 µg protein per each sterile disc. Kanamycin (2 mg) was used as a positive control. The experiments were carried out in triplicate. Inhibition zones are shown in mean ± S.D.

Treatment of crude extract by Pronase: In order to prove whether antibacterial activity is the responsibility of peptides (or proteins) in the crude extract, the crude extract was digested with proteolytic enzyme, Pronase. The Pronase was added into the 200 µL of the crude extract (protein conc. 3.55 mg/mL), in which the protein amount ratio of protein substrate and Pronase equaled to 1:8 (w/w). The treatment reaction was performed at 37°C for 20 h. After digested, the supernatant was obtained by centrifuged at 10,000 rpm for 5 min and used for antibacterial activity assay. Aliquots of pronase treated mixture were subjected to a 15% Tris-tricine SDS-PAGE and stained with silver.

Purification of active proteins from *S. stramonifolium* seed extracts: Seed extract of the plant was freeze dried and re-suspended, adjusted to pH 8.0 and conductivity <3.2 mS/cm with citrate-phosphate buffer (10x dilution). The sample was loaded onto anion exchange Hitrap Q XL pre-equilibrated with citrate-phosphate buffer pH 8.0 using a flow rate of 2 mL/min. Unbound proteins were washed out first with the same buffer whereas bound proteins were eluted out with a linear gradient of citrate-phosphate buffer pH 8.0-2.0 for 10 column volumes (CV), following with pH 2.0 washing step and further with 1.0 M NaCl in the same buffer. Fractions were collected and protein in each fraction was followed up using absorbance at 280, 254 and 215 nm. Each pool fraction was assayed for antibacterial activity against *P. aeruginosa*. Pool fraction that exhibited antibacterial activity will be further purified by SOURCE 15RPC ST 4.6/100 pre-equilibrated with solution A (0.065% TFA in water), flow rate 1.0 mL/min. the unbound proteins were washed out with solution A for 10 CV, whereas the bound proteins were eluted out with a linear gradient of 0-100% solution B (0.05% TFA in acetonitrile) for 20 CV. Fractions were detected for protein at A280, 254 and 215 nm.

RESULTS AND DISCUSSION

Antibacterial activity: Our group interest in peptides with antibacterial activity then, in this research, we used the aqueous solution to extract the bioactive molecules from *S. stramonifolium* seeds, which contained high amount of proteins. By the extraction as mention in the materials and methods, protein content of *S. stramonifolium* seed extract was approximately 0.45 mg/g fresh weight of seeds. The seed extracts showed a strong antibacterial activity according to disc diffusion method. This seed extract inhibited the growth of gram positive bacteria better than gram negative strains (Table I). However, no inhibition was observed against *E. coli* and *K. pneumonia*, which were gram negative bacterial strains. Proteins isolated from mature leaves of *S. villosum* were found to have antibacterial properties. This leaves protein extract could inhibit growth of four pathogenic bacteria, *S. aureus* MTCC 2940, *B. subtilis* MTCC 441 (Gram positive) and *E. coli* MTCC 739, and *P. aeruginosa* MTCC 2453 (Gram negative) (Chowdhury *et al*., 2008). This result seems not agree well with our result that *E. coli* could not be inhibited. However, this might be due to the difference between protein sources, seeds and leaves, which might contain different types of bioactive proteins. In another *Solanum* species, *S. nigrum*, even though it showed good antioxidant activity but water extract of leaves showed no inhibition toward the tested bacteria both gram negative and gram positive strains including *B. cereus*, *S. epidermidis*, *S. aureus*, *Micrococcus kristinae* , *Streptococcus pyrogens*, *E. coli*, *S. pooni*, *Serratia marcescens*, *P. aeruginosa*, *K. pneumonae* (Jimoh *et al*., 2010). This result showed that different species might contain different bioactive molecules with different activities.

Tris-tricine SDS-PAGE: Tris-tricine SDS-PAGE revealed the major protein bands at 10.2 kDa and 15.7 kDa whereas a minor band around 21.5 kDa as shown in Fig. 1. Previous antimicrobial peptides reports showed that pepper (*Piper nigrum*) extract contained protein bands at MW approximately 6-10 kDa (Diz *et al*., 2006) and 6-16 kDa (Ribeiro *et al*., 2007). The peptide snakin-2 (StSN2) from potato (*S. tuberosum* cv Jaerla) tubers has MW about 7 kDa (Berrocal-Lobo *et al.*, 2002). Therefore, we proposed from this SDS-PAGE result those protein or some of them probably are antimicrobial peptides.

Antibacterial activity and protein pattern of Pronase treated seed extract: *S. stramonifolium* seed aqueous extracts after treated with pronase, antibacterial activity was only slightly decreased as shown in Table II. Antibacterial activity was related to the results of Tris-tricine SDS-PAGE, which no difference could be observed between treated and

Table I: Antibacterial activity of *S. stramonifolium* seed extracts against certain gram positive and gram negative bacteria by disc diffusion method

Character	Bacteria	Inhibition zone[a] (mm)
	S. aureus	2.00 ± 0.00
Gram positive	*B. subtilis*	8.33 ± 1.15
	B. licheniformis	8.00 ± 0.00
	Xanthomonas sp.	6.00 ± 0.00
	P. aeruginosa	9.50 ± 0.50
Gram negative	*E. coli*	0.00 ± 0.00
	S. typhi	6.67 ± 0.29
	K. pneumonia	0.00 ± 0.00

[a]data showed the mean inhibition zone from a triplicate subtracted from negative controls

Table II: Anti-*B. subtilis* and *anti-P. aeruginosa of S. stramonifolium* seed extract treated with pronase

Sample	Protein content (µg/µL)	Loaded protein (µg)	Inhibition zone (mm)			
			B. subtilis		*P. aeruginosa*	
			C^+	*S. Stramonifolium*	C^+	*S. stramonifolium*
Crude extract	6.8	50.0	18.0	9.50 ± 0.71	17.0	11.00 ± 0.00
Untreated	2.4	50.0	18.0	10.00 ± 0.00	17.0	10.75 ± 1.77
Pronase treated	2.4	50.0	18.0	9.75 ± 0.35	17.0	10.00 ± 0.00

Table III: Purification table of *S. stramonifolium* seed extracts

Purification steps	Volume (mL)	Protein (µg/mL)	Total protein (mg)
Crude extract 1	30	153.0	4590
HiTrapQ 1	21	153.0	3.21
Unbound	620	2.8	1.76
Q1	12	23.0	0.28
Q2	10	39.0	0.39
Q3	10	40.7	0.41
Q4	16	24.0	0.38
SOURCE 15RPC			
S1	6	15.7	0.094
S2	2	18.0	0.036
S3	7	13.4	0.094

untreated seed extracts (Fig. 2). This indicated that protein in this solanaceae seed aqueous extract could not be degraded by this proteolytic enzyme in the experimented condition. It will also be possible that there might be some peptides (or protein) in the seed aqueous extract that act as protease inhibitors. Proteinase inhibitor II (PIN2) proteins from the Solanaceae family have been reported (Chye et al., 2006). Six diverse representative *Capsicum annuum* (common name: hot pepper; Solanaceae) protease inhibitor genes were found (Mishra *et al*., 2010). These indicate that there might be also protease inhibitor genes or protein inhibitors of proteases in this *S. stramonifolium* seed extract. However, further study in more detail is still needed.

Purification of active protein: The crude extract of *S. stramonifolium* after purified by Hitrap Q XL column, one fraction of unbound protein and 10 bound protein fractions were collected. Protein concentration and total protein contents of each fraction are as shown in Table III. Purification profile is as shown in Fig. 3. The results

Fig. 1: A 12% tris-tricine SDS-PAGE gel stained with silver of seed extracts. Lane 1: protein markers; 2: *S. stramonifolium* (3 µg protein). The arrows indicate proteins bands expressed in various treatments

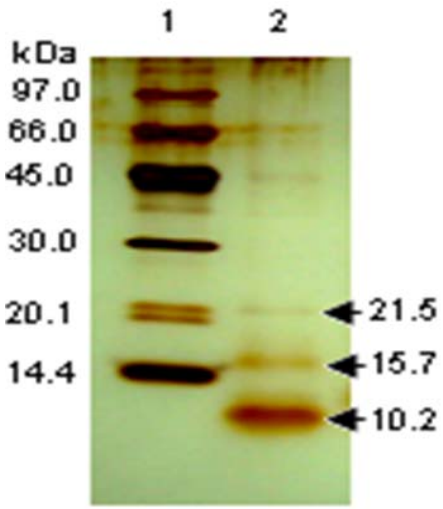
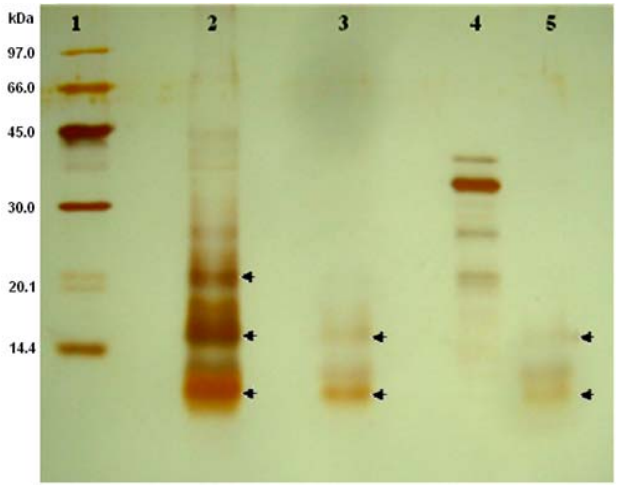


Fig. 2: A 12% tris-tricine SDS-PAGE stained with silver of pronase treated seed extract. Lane 1: protein markers; 2: crude extract of *S. stramonifolium* seed; 3: control; 4: Pronase; 5: crude extract of *S. stramonifolium* seed treated with pronase. The arrows indicate proteins bands expressed in various treatments



showed that Q4 exhibited antibacterial activity against *P. aeruginosa*. Active fraction bound to Hitrap Q XL at pH 8.0, which indicated that at this pH they bare net negative charges on their molecular structures and their p*I* is supposed to be less than 8.0. Unlike most reports antimicrobial peptides, which normally are highly basic (p*I*>10) such as plant AMPs from the seed of *Mirabilis jalapa* L. (Cammue *et al*., 1992; Broekaert *et al.*, 1992). Some other plant AMPs have p*I* > 8.0 such as reported by Canales *et al*. (2011). Snakin-2 (StSN2) has been isolated

Fig. 3: Hitrap Q XL column profile of *S. stramonifolium* seed extract. Bound proteins eluted with a linear gradient of citrate-phosphate buffer, pH 8.0-2.0

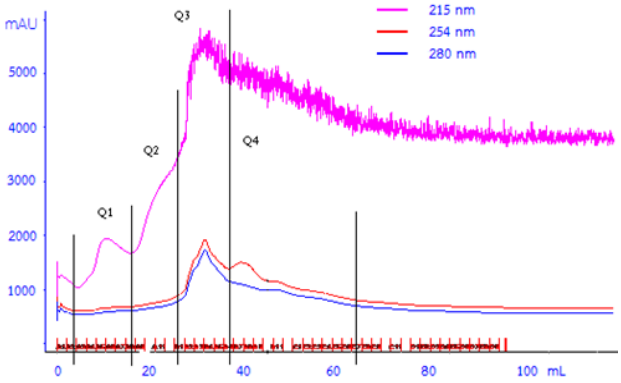
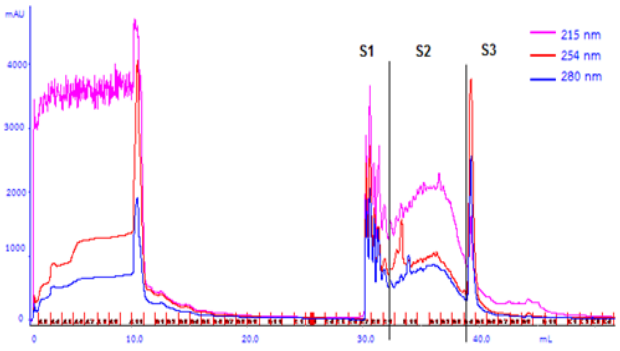


Fig. 4: SOURCE 15RPC ST 4.6/100 column profile of *S. stramonifolium* (Q4 fraction). Unbound proteins were washed with solution A (0.065% TFA in water) and bound proteins were eluted with a linear gradient of 0-100% solution B (0.05% TFA in acetonitrile)



from potato (*S. tuberosum* cv Jaerla) tubers also has p*I* = 9.16 (Berrocal-Lobo *et al*., 2002). Those antimicrobial peptides contain positive net charges. In our work, we suggest that peptide fractions contain antibacterial activity might have negative net charges. According to report of Canales *et al*. (2011), there are some plant AMPs bearing negative net charges and had their p*I* values less than 7.0 such as from *Sorghum bicolor*, *Zea mays*, *Picea sitchensis*, *Pinus taeda* and *P. pinaster*.

Most AMPs have been reported baring positive charges, which be hypothesized to interact with negative charges of phospholipid on bacterial membrane. This interaction cause alteration of membrane or disruption and cause the death of such bacteria (Zaloff, 2002). The SOURCE 15RPC ST 4.6/100 chromatogram of Q4 fraction showed several peaks came out both unbound and bound fractions as shown in Fig. 4. This indicated that there are several compounds differently in size or polarity. Unfortunately, these small fractions of proteins are not enough for either antibacterial activity assay or protein pattern checking by SDS-PAGE. Improvement of purification is still needed in order to achieve the pure active compounds enough for characterizations.

CONCLUSION

Seed aqueous extracts of *S. stramonifolium* exhibited very good antibacterial activity against tested bacterial strains except for *E. coli* and *K. pneumonia* at protein amount of 100 µg/ disc. The small proteins or peptides with MW less than 14.4 kDa might involve in antibacterial activity of this specie. To confirm the peptides a corresponding molecule, further successful purification is still needed. Apart from a common vegetable food, seed proteins from this plant species might be a good source of antibacterial agents.

Acknowledgement: This work was financially supported by the National Research Council of Thailand (NRCT) under Mahasarakham University (Fiscal year 2010) and partial supports from the Faculty of Science (Fiscal year 2010). This work also supported in part by The Center of Excellence for Innovation in Chemistry (PERCH-CIC), Department of Chemistry, Faculty of Science, Mahasarakham University. The authors would like to thank S. Waengthisarn for laboratory assistants and Assoc. Prof. Dr. S. Thammasirirak and Dr. R. Thiewthong, for bacterial strains.

REFERENCES

Al-Fatimi, M., M. Wurster, G. Schroder and U. Lindequist, 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. *J. Ethnopharmacol.*, 111: 657–666

Arthan, D., J. Svasti, P. Kittakoop, D. Pittayakhachonwut, M. Tanticharoen and Y. Thebtaranonth, 2002. Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*. *Phytochemistry*, 59: 459–463

Berrocal-Lobo M., A. Segura, M. Moreno, G. López, F. García-Olmedo and A. Molina, 2002. Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to pathogen infection. *Plant Physiol.*, 128: 951–961

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248–254

Broekaert, W.F., W. Marien, F.R.G. Terras, M.F.C. De Bolle, P. Proost, J. Van Damme, L. Dillen, M. Claeys and S.B. Rees, 1992. Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine/glycine-rich domain of chitin-binding proteins. *Biochemistry*, 31: 4308–4314

Cammue, B.P.A., M.F.C. De Bolle, F.R.G. Terras, P. Proost, J. Van Damme, S.B. Rees, J. Vanderleyden and W.F. Broekaert, 1992. Isolation and characterization of a novel class of plant antimicrobial peptides form mirabilis jalapa L. seeds. *J. Biol. Chem.*, 267: 2228–2233

Canales, J., C. Avila and F. M. Ca´novas, 2011. A maritime pine antimicrobial peptide involved in ammonium nutrition. *Plant Cell Environ.*, 34: 1443–1453

Careaga, M., E. Fernandez, L. Dorantes, L. Mota, M.E. Jaramillo and H. Hernandez-Sanchez, 2003. Antibacterial activity of Capsicum extract against *Salmonella typhimurium* and *Pseudomonas aeruginosa* inoculated in raw beef meat. *Int. J. Food Microbiol.*, 83: 331–335

Chah, K.F., K.N. Muko and S.I. Oboegbulem, 2000. Antimicrobial activity of methanolic extract of *Solanum torvum* fruit. *Fitoterapia*, 71: 187–189

Chehregani, A., F. Azimishad and H.H. Alizade, 2007. Study on Antibacterial Effect of Some *Allium* Species from Hamedan-Iran. *Int. J. Agric. Biol.*, 9: 873–876

Chowdhury, N., S. Laskar and G. Chandra, 2008. Mosquito larvicidal and antimicrobial activity of protein of *Solanum villosum* leaves. *BMC Complement Altern Med.*, 8: 62

Chye, M.L., S.F. Sin, Z.F. Xu and E.C. Yeung, 2006. Serine proteinase inhibitor proteins: exogenous and endogenous functions. *In Vitro Cell Dev. Biol. Plant*, 42: 100–108

Cichewicz, R.H. and P.A. Thorpe, 1996. The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine. *J. Ethnopharmacol.*, 52: 61–70

Diz, M.S.S., A.O. Carvalho, R. Rodrigues, A.G.C. Neves-Ferreira, M. Da Cunha and E.W. Alves, A.L. Okorokova-Façanha, M.A. Oliveira, J. Perales, O.L.T. Machado and V.M. Gomes, 2006. Antimicrobial peptides from chilli pepper seeds causes yeast plasma membrane permeabilization and inhibits the acidification of the medium by yeast cells. *Biochim. Biophys. Acta*, 1760: 1323–1332

Estevez, Y., D. Castillo, M. Tangoa Pisango, J. Arevalo, R. Rojas, J. Alban, E. Deharo, G. Bourdy and M. Sauvain, 2007. Evaluation of the leishmanicidal activity of plants used by Peruvian Chayahuita ethnic group. *J. Ethnopharmacol.*, 114: 254–259

Feng, J., F. Yuan, Y. Gao, C. Liang, J. Xu, C. Zhang and L. He, 2003. A novel antimicrobial protein isolated from potato (*Solanum tuberosum*) shares homology with an acid phosphatase. *Biochem. J.*, 376: 481–487

Jimoh, F.O., A.A. Adedapo and A.J. Afolayan, 2010. Comparison of the nutritional value and biological activities of the acetone, methanol and water extracts of the leaves of *Solanum nigrum* and *Leonotis leonorus*. *Food Chem. Toxicol*., 48: 964–971

Lay, F.T. and M.A. Anderson, 2005. Defensins-components of the innate immune system in plants. *Curr. Protein Pept. Sci.*, 6: 85–101

Lo Cantore, P., V. Shanmugaiah and N.S. Iacobellis, 2009. Antibacterial activity of essential oil components and their potential use in seed disinfection. *J. Agric. Food Chem.*, 57: 9454–9461

Mishra, M., V.A. Tamhane, N. Khandelwal, M.J. Kulkarni, V.S. Gupta and A.P. Giri, 2010. Interaction of recombinant CanPIs with *Helicoverpa armigera* gut proteases reveals their processing patterns, stability and efficiency. *Proteomics*, 10: 2845–2857

Odintsova, T.I., T.A. Egorov, A.K.H. Musolyamov, M.S. Odintsova, V.A. Pukhalsky and E.V. Grishin, 2007. Seed defensins from *T. kiharae* and related species: Genome localization of defensin-encoding genes. *Biochimie*, 89: 605–612

Phansri, K., R. Sarnthima, S. Thammasirirak, P. Boonchalee and S. Khammuang, 2011. Antibacterial activity of *Bauhinia acuminata* L. seed protein extract with low hemolytic activity against human erythrocytes. *Chiang Mai J. Sci.*, 38: 242–251

Ribeiro, S.F.F., A.O. Carvalho, M.D. Cunha, R. Rodrigues, L.P. Cruz, V.M.M. Melo, André O. Carvalho, M.D. Cunha, R. Rodrigues, L.P. Cruz, V.M.M. Melo, I.M. Vasconcelos, E.J.T. Melo and V.M. Gomes, 2007. Isolation and characterization of novel peptides from chilli pepper seeds: Antimicrobial activities against pathogenic yeasts. *Toxicon*, 50: 600–611

Schagger, H. and G. Von Jagow, 1987. Tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis for the separation of proteins in the range from 1-100 kDalton. *Anal. Biochem.*, 166: 368–379

Thomma, B.P., B.P. Cammue and K. Thevissen, 2002. Plant defensins. *Planta*, 216: 193–202

Wiart, C., S. Mogana, S. Khalifah, M. Mahan, S. Ismail, M. Buckle, A.K. Narayana and M. Sulaiman, 2004. Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. *Fitoterapia*, 75: 68–73

Zaloff, M., 2002. Antimicrobial peptides of multicellular organisms, *Nature*, 415: 389–395

(Received 13 June 2011; Accepted 19 October 2011)