Journal of King Saud University – Science (2014) **26**, 79–82

King Saud University
**Journal of King Saud University – Science**
www.ksu.edu.sa
www.sciencedirect.com

SHORT COMMUNICATION

# Antimicrobial activity of *Litchi chinensis* and *Nephelium lappaceum* aqueous seed extracts against some pathogenic bacterial strains

**Ramesa Shafi Bhat *, Sooad Al-daihan**

*Biochemistry Department, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia*

Received 3 March 2013; accepted 27 May 2013
Available online 6 June 2013

**KEYWORDS**
*Litchi chinensis*;
*Nephelium lappaceum*;
Peptides;
Seeds;
Antibacterial

**Abstract** Seeds aqueous extracts from *Litchi chinensis* and *Nephelium lappaceum* were investigated for antibacterial activity by disc diffusion method and protein profile. Both seed aqueous extracts show moderate inhibition against pathogenic bacteria, both gram positive including *Staphylococcus aureus, Streptococcus pyogenes, and Bacilllus subtillis* and gram negative bacteria including *Escherichia coli and Pseudomonas aeruginosa*. Overall analysis of the antibacterial activity of tested samples revealed that the highest inhibitory activity was produced by *Litchi chinensis* (15 ± 0.55 mm) against *S. pyogenes*. Tris glycine SDS PAGE revealed major protein band approximately 15.5 kDa and 22-kDa. Protein contents of Seeds of *Litchi chinensis* and *Nephelium lappaceum* were approximately 7.5 and 13.5 mg/g, respectively.



## 1. Introduction

Plants defend themselves against microbial pathogens by various defence responses like production of antimicrobial peptides, secondary metabolites, lytic enzymes, membrane-interacting proteins and reinforcement of cell walls (Scheel, 1998; Karin et al., 2000; Freeman and Beattie, 2008; Walter, 2012). Most antimicrobial proteins that have been identified belong to a group of small molecular mass antimicrobial peptides (Garćıa-Olmedo et al., 1998, 2001). Antimicrobial proteins and peptides in plants have most commonly been discovered in seeds, where they accumulate to high levels and may also function as storage proteins (Candido et al., 2011). Homologues of the seed proteins have often been found subsequently at much lower concentrations in vegetative and floral tissues (Terras et al., 1995; Candido et al., 2011). These antimicrobial agents are very similar to those of human antimicrobial peptides in structure and function and are produced as a part of their defence systems (Thevissen et al., 2007). Most of these are small Cystein-rich basic peptides which have been also classified as Cationic Antimicrobial Peptides (CAMPs) in all living organisms (Peschel and Sahl, 2006). These antimicrobial peptides are now known as good antifungal substances and occasionally have antibacterial activities *in vitro* (Portieles et al., 2006; Thevissen et al., 2007).

* Corresponding author. Tel.: +966 14785968x1204.
E-mail addresses: ramesa.aftab@gmail.com, rbhat@ksu.edu.sa (R.S. Bhat).

Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

1018-3647 © 2013 Production and hosting by Elsevier B.V. on behalf of King Saud University.
http://dx.doi.org/10.1016/j.jksus.2013.05.007

The plants chosen for the present study are *L. chinensis* (litchi) and *N. lappaceum* (Rambutan), subtropical and tropical fruit of the Sapindaceae family (Marisa, 2006). *L. chinensis* is a subtropical species originating from South China, while *N. lappaceum* is a tropical species common to Southeast Asia and but nowadays cultivation has been extended to China, India, Thailand, Taiwan, Malaysia, and Australia (Davidson et al., 2006; Jalikop, 2012). Traditionally the *L. chinensis* is used to relieve coughing, cure stomach ulcers diabetes, obesity and also has analgesic action (Obrosova et al., 2010; Morton, 1987). It is also used to kill intestinal worms. Recent medical reports have shown that *L. chinensis* fruit and seeds impede the growth of cancerous cells (Xiao et al., 2004). Its seeds also show antihyperglycemic, antihyperlipidemic, antiplatelet, and antiviral activities (Chen et al., 2007). The fruit is a rich source of flavonoids which are very effective against breast cancer (Xu et al., 2011). Rambutan has been used as traditional medicine from centuries especially as a remedy for diabetes, high blood pressure (Kaushik et al., 2010; Peter, 2010). Although antibacterial activity of various extracts of *L. chinensis* and *N. lappaceum* have been reported (Thitilertdecha et al., 2008, 2010; Wisitsak et al., 2012) but antibacterial activity of their aqueous seed extract (which has high concentration of protein) has been found lacking in literature. Therefore the present study was undertaken to compare the protein profile and antibacterial activity of aqueous seed extract of *L. chinensis* and *N. lappaceum* against human pathogens which included *S. aureus, S. pyogenes, B. subtillis*, *E. coli* and *P. aeruginosa*.

## 2. Materials and methods

### 2.1. Medicinal plants

Commercially available fresh fruit samples of *L. chinensis* and *N. lappaceum* were collected from Riyadh. The seeds were separated out from the fruits by hands, cleaned with distilled water and shade dried for 2 weeks.

### 2.2. Microorganisms for antibacterial assay

Reference bacterial strains which included *S. aureus, S. pyogenes, B. subtillis*, *E. coli* and *P. aeruginosa* (clinical isolates) were provided by the Department of Botany and microbiology, King Saud University. The strains were maintained on agar slant at 4 °C and sub cultured at 37 °C for 24 h on nutrient agar (Sigma–Aldrich, Germany) prior to any screening.

### 2.3. Preparation of seed extract

One gram of dry powder of samples was dissolved in 3 ml of 0.01 M HCl containing 0.15 M NaCl. (Ratio of sample/extract solution of 1:3 w/v). The residue was then removed by filtering through cheese cloth; the filtrate was then centrifuged at 8,100*g*, for 5 min. This seed extract was subjected to antibacterial activity experiments and protein determination on the same day (Franco et al., 2006).

### 2.4. 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 the mixture was allowed to stand for 5 min at room temperature. Protein profile analysis of seed and fruit extract was performed by a 12% separating Tris–tricine sodium dodecyl sulphate polyacrylamide gel electrophoresis (Tris–glycine SDS–PAGE) and stained with coomassie brilliant blue R250.

### 2.5. Media preparation and antibacterial activity

The antibacterial assay was performed using the paper disc diffusion method (Bhat et al., 2012). Using a sterile cotton swab, the nutrient broth (Sigma–Aldrich, Germany) cultures were swabbed on the surface of sterile nutrient agar (Sigma–Aldrich, Germany) plates and allowed to dry for 5 min. Sterile filter paper discs (6 mm in diameter) impregnated with different test extracts (100 µl/disc) were then placed on the surface of inoculated agar plates. Kanamycin (30 µg/disc) was used as positive control. The plates were then incubated at 37 °C for 24 h after which microbial growth was determined by measuring the diameter of the inhibition zone (mm) using a transparent scale. Each extract was analysed in triplicate, the mean values are presented.

### 2.6. Minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) was determined by using the serial dilution technique (Anu Kiruthika et al., 2011). In this technique copious of test tubes were used and each test tube was filled with 1 ml of sterile nutrient broth media and graded doses of sample solution were added. Then these test tubes were inoculated with the selected organisms (inoculums contains $1 \times 106$ cells/ml) followed by incubation at 37 °C for 24 h to allow the growth of the bacteria. The test tubes which showed minimum concentration as well as clear content were selected. This lowest or minimum concentration was considered as the Minimum Inhibitory Concentration (MIC).

### 2.7. Statistical analysis

The results were analysed by using standard deviation (SD) statistical methods.

## 3. Results and discussion

### 3.1. Protein content of crude extract from ***L. chinensis*** and ***N. lappaceum*** seed

Ten grams of dry Seeds of *L. chinensis* and *N. lappaceum* were extracted with 30 ml of extraction solution. Protein contents of Seeds of *L. chinensis* and *N. lappaceum* were approximately 7.5 and 13.5 mg/g, respectively.

**Table 1** Antibacterial activity of *Litchi chinensis L.* and *Nephelium lappaceum L.* seed extracts against certain gram positive and gram negative bacteria by disc diffusion method.

| Bacteria | Inhibition zone [a]mm | | |
|---|---|---|---|
| | *L. chinensis* | *N. lappaceum* | *Kanamycin* |
| *S. pyogenes* | 15 ± 0.55 | 12 ± 0.10 | 28 |
| B. subtilis | 11 ± 0.00 | 12 ± 0.40 | 21 |
| *S. aureus* | 10 ± 0.25 | 13 ± 0.80 | 26.5 |
| *E. Coli* | 7.5 ± 0.30 | 6.5 ± 0.66 | 20 |
| *P. aeruginosa* | 9 ± 0.45 | 10 ± 0.55 | 25 |

[a] Data showed the mean inhibition zone from a triplicate.

**Table 2** The MIC of *Litchi chinensis L.* and *Nephelium lappaceum L.* seed extracts against *S. pyogenes*.

| Extracts | *S. pyogenes* | | | | |
|---|---|---|---|---|---|
| Con. (mg/ml) | 0 | 5 | 10 | 15 | 20 |
| *Litchi chinensis L.* | – | – | – | – | + |
| *Nephelium lappaceum L.* | – | – | – | + | + |

### 3.2. Antibacterial activity

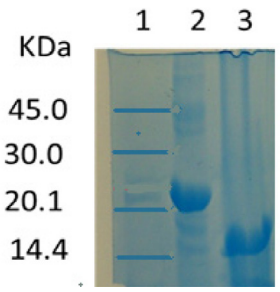
In our study we used aqueous solution for extraction of seed material which contain higher amount of protein. Four of the five selected strains were found sensitive to the protein extracts of *L. chinensis* and *N. lappaceum* seeds. The seed extracts inhibited the growth of gram positive bacteria better than gram negative strains (Table 1). Among gram (+) bacteria, the strongest activity of *L. chinensis* was observed against *S. pyogenes* (15 ± 0.55) followed by *B. subtilis* and *S. aureus* and *N. lappaceum* seed extract shows highest activity against *S. aureus*. However, *P. aeruginosa* and *E. coli* exhibited moderate to weak activities, respectively for both the test extracts. Gram (−) bacteria are surrounded with the cell wall which restricts diffusion of hydrophobic compounds through its lipo polysaccharide covering. The absence of this barrier in gram (+) bacteria allows the direct contact of the essential oil's hydrophobic constituents with the phospholipid bilayer of the cell membrane, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems (Zhao et al., 2001).

### 3.3. Minimum inhibitory concentration

*S. pyogenes* was found most sensitive among all the strains. Therefore MIC was determined only against *S. pyogenes*. To determine MIC different concentrations of sample extracts were used against selected bacteria. Results are presented in Table 2.

### 3.4. Tris–glycine SDS–PAGE

Tris–glycine SDS–PAGE revealed the major protein bands at 15.5 kDa for *L. chinensis* and 22-kDa for *N. lappaceum* as shown in Fig. 1. Earlier antimicrobial peptide studies have revealed that extracts contain protein bands in the range from 6 to 20 kDa (Diz et al., 2006; Ribeiro et al., 2007; Berrocal-Lobo et al., 2002). Therefore, we deem from this SDS–PAGE result that those proteins or some of them probably are antimicrobial peptides.




Lane I *marker,* lane 2 *Nephelium lappaceum L,* lane 3 *Litchi chinensis*

**Figure 1** SDS–Polyacrylamide Gel Electrophoresis pattern of *Nephelium lappaceum L.,* and *Litchi chinensis* crude extracts. About 55 μg of sample was electrophorised on 12% polyacrylamide gel in presence of 0.2% SDS. Electrophoresis buffer used was 0.01 M Tris–glycine containing 0.1% SDS, pH 8.4. Electrophoresis was performed at the current of 25 mA in the stacking gel region and at 30 mA as the sample entered separating gel.

## 4. Conclusion

The overall result of the present study concluded that aqueous seed extracts of *L. chinensis* L and *N. lappaceum L.* exhibit moderate antibacterial activity. The protein profile of the studied aqueous seed extracts showed *N. Lappaceum* is a rich source of protein as compared to *L. chinensis*. Furthermore, evaluation of active compounds responsible for the activity warns extensive investigation.

## Acknowledgement

We extend our appreciation to Deanship of Scientific Research, King Saud University for funding the work through the research group project no RGP-VPP-063.

## References

Anu Kiruthika, K., Amutha Jaisheeba, A., Sornaraj, R., 2011. Evaluation of the antimicrobial property of selected flower extracts when exposed in a hospital environment. Int. J. PharmTech Res. 2, 769–774.

Berrocal-Lobo, M., Segura, A., Moreno, M., López, G., García-Olmedo, F., Molina, A., 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.

Bhat, R.S., Al-Daihan, S., 2012. Antibacterial properties of different cultivars of Phoenix dactylifera L and their corresponding protein content. Ann. Biol. Res. 3 (10), 4751–4757.

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.

Candido, E.S., Pinto, M.F., Pelegrini, P.B., Lima, T.B., Silva, O.N., Pogue, R., Grossi-de-Sá, M.F., Franco, O.L., 2011. Plant storage

proteins with antimicrobial activity: novel insights into plant defense mechanisms. FASEB J. 25, 3290–3305.

Chen, Y.B., Wu, K.S., Gu, Y., Chen, J.Z., 2007. Research progress in the chemical constituents and pharmacological effects of lychee seeds. Chin. J. Inf. TCM 14 (5), 97–98.

Davidson, J.L., Davidson, A., Saberi, H., Jaine, T., 2006. The Oxford Companion to Food Oxford [Oxfordshire]. Oxford University Press.

Diz, M.S.S., Carvalho, A.O., Rodrigues, R., Neves-Ferreira, A.G.C., Da Cunha, M., Alves, E.W., Okorokova-Façanha, A.L., Oliveira, M.A., Perales, J., Machado, O.L.T., Gomes, V.M., 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.

Franco, O.L., Murad, A.M., Leite, J.R., Mendes, P.A.M., Prates, M.V., Bloch Jr., C., 2006. Identification of a cowpea g-thionin with bacteria activity. FEBS J. 273, 3489–3497.

Freeman, B.C., Beattie, G.A., 2008. An overview of plant defenses against pathogens and herbivores. Plant Health Instructor. http://dx.doi.org/10.1094/PHI-I-2008-0226-01.

Garćıa-Olmedo, F., Rodriguez-Palenzuela, P., Molina, A., Alamillo, J.M., Lopez-Solanilla, E., Berrocal-Lobo, M., Poza-Carrion, C., 2001. Antibiotic activities of peptides, hydrogen peroxide and peroxynitrite in plant defense. FEBS Lett. 498, 219–222.

Garćıa-Olmedo, F., Molina, A., Alamillo, J.M., Rodŕıguez-Palenzuela, P., 1998. Plant defense peptides. Biopolymers 47, 479–491.

Jalikop, S.H., 2012. Rambutan. <http://www.fruitipedia.com/>.

Karin, T., Rupert, W.O., David, P.A., Willem, F.B., 2000. Specific binding sites for an antifungal plant defensin from Dahlia (Dahlia merckii) on fungal cells are required for antifungal activity. Mol. Plant-Microbe Interact. 13, 54–61.

Kaushik, G., Satya, S., Khandelwal, R.K., Naik, S.N., 2010. Commonly consumed Indian plant food materials in the management of diabetes mellitus. Diabetes and metabolic syndrome. Clin. Res. Rev. 4 (1), 21–40.

Marisa, M.W., 2006. Ascorbic acid and mineral composition of longan (Dimocarpus longan), lychee (*Litchi chinensis*) and rambutan (*Nephelium lappaceum*) cultivars grown in Hawaii. J. Food Compos. Anal. 85 (2), 231–237.

Morton, J., 1987. Lychee. In: Fruits of Warm Climates. Julia F. Morton, Miami, FL, pp. 249–259.

Obrosova, I.G., Chung, S.S., Kador, P.F., 2010. Diabetic cataracts: mechanisms and management. Diabetes Metab. Res. Rev. 26, 172–180.

Peschel, A., Sahl, H.G., 2006. The co-evolution of host cationic 5. Antimicrobial peptides and microbial resistance. Nat. Rev. Microbiol. 4, 529–536.

Peter, B., 2010. The Tropical fruit rambutan: its nutrition and culinary uses. Food Nutr.

Portieles, R., Ayra, C., Borras, O., 2006. Basic insight on plant 3. Defensins. Biotecnol. Apl. 23, 75–78.

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

Scheel, D., 1998. Resistance response physiology and signal transduction. Curr. Opin. Plant Biol. 1, 305–310.

Terras, F.R.G., Eggermont, K., Kovaleva, V., Raikhel, N.V., Osborn, R.W., Kester, A., Rees, S.B., Torrekens, S., Van-Leuven, F., Vanderleyen, J., Cammue, B.P.A., Broekaert, W.F., 1995. Small cysteine-rich antifungal proteins from radish: their role in host defence. Plant Cell 7, 573–588.

Thevissen, K., Kristensen, H., Thomma, B.P.H.J., Cammue, B.P.A., Francois, I.E.J.A., 2007. Therapeutic potential of antifungal plants and insect defensins. Drug Discov. Today 12, 966–972.

Thitilertdecha, N., Teerawutgulrag, A., Rakariyatham, N., 2008. Antioxidant and antibacterial activities of *Nephelium lappaceum L.* extracts. Food Sci. Technol. 41, 2029–2035.

Thitilertdecha, N., Teerawutgulrag, A., Kilburn, J.D., Rakariyatham, N., 2010. Identification of major phenolic compounds from *Nephelium lappaceum L.* and their antioxidant activities. Molecules 15 (3), 1453–1465.

Walter, A., 2012. Plant defense mechanisms are activated during biotrophic and necrotrophic development of colletotricum graminicola in maize. Plant Physiol. 158 (3), 1342–1358.

Wisitsak, P., Nimkamnerd, J., Thitipramote, N., Saewan, N., Chaiwut, P., Pintathong, P., 2012. Comparison the bioactive compounds and their activities between longan and litchi seeds extracts. 1st Mae Fah Luang University International Conference.

Xiao, L.Y., Zhang, D., Feng, Z.M., Chen, Y.W., Zhang, H., Lin, P.Y., 2004. Studies on the antitumor effect of lychee seeds in mice. J. Chin. Med. Mater. 27 (7), 517–518.

Xu, X., Xie, H., Hao, J., Jiang, Y., Wei, X., 2011. Flavonoid glycosides from the seeds of *Litchi chinensis*. J. Agric. Food Chem. 59 (4), 1205–1209.

Zhao, W.H., Hu, Z.O., Okubo, S., Hara, Y., Shimamura, T., 2001. Mechanism of synergy between epigallocatechin gallate and β-lactams against methicillinresistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. 45, 1737–1742.