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# Antimicrobial Activities of Three Different Seed Extracts of *Lansium* Varieties

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## ABSTRACT

*Lansium domesticum* Corr. is a fruit tree of the Meliaceae family, which is commonly found in South-East Asia with a wide range of varieties. This study investigated three varieties of *L. domesticum*; *Duku*, *Langsat* and *Dokong* for the phytochemical screening and antimicrobial activity. Seeds from the matured fruits were extracted using hexane, methanol and water. The crude extracts were screened for antimicrobial activities toward three bacteria, namely, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*. The findings showed that *Langsat* seed extracts contained more groups of compounds compared with the other two varieties, and its methanol extract demonstrated the highest inhibition zones against the three bacteria. The crude methanol extract of *Duku* seeds showed inhibition zones only towards *Bacillus subtilis* at a high concentration (1.0 $mgL^{-1}$), whilst the seed extracts of *Dokong* showed no inhibition zones towards any of the tested bacteria.

**Keywords**: *Lansium domesticum*, antimicrobial activities, inhibition zones

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## INTRODUCTION

*Lansium domesticum* Corr. or lanzones, a species of Meliaceae family, are evergreen tropical trees that grow upright, tall, slim, and can reach up to 30 metres in height. *L. domesticum* is a species that vary widely, and leads to a classification of several varieties as different species by some taxonomists. Difficulties in classifying the *L. domesticum* varieties have led to the usage of high-

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technology methods to determine the differences between them. Beng *et al*. (2000) classified the *L. domesticum* varieties of Peninsular Malaysia into four main groups (*Dokong*, *Duku*, *Langsat* and *Duku-langsat*) based on random amplified polymorphic DNA (RAPD) analysis, while the researchers from our neighbouring countries, Thailand and Indonesia, classified these varieties into three main groups (*Dokong*, *Duku* and *Langsat*), which are clearly differentiated through several techniques mainly RAPD, RAPD-based PCR and flow-cytometry (Te-chato *et al*., 2005; Sri Yulita, 2011). Our study focused on three varieties of *L. domesticum*, namely, *Duku*, *Langsat* and *Dokong*, due to their availability during the period of study.

The fruit from all the three varieties emerge from the flower inflorescences that grow and hang from large branches or trunk. The fruit can be elliptical, oval, or round, measuring 2 to 7 cm (0.79 to 2.8 in) by 1.5 to 5 cm (0.59 to 2.0 in) in size. The thickness of the fruit's skin varies. The skin of *Langsat* is thin and has latex, but there is little latex in the thicker skin of *Duku* and very little latex or none in the thick skin of *Dokong* (Yunus, 1997). The fruit usually contain 1 to 3 seeds, flat, and bitter tasting, whereas the seeds are covered with a thick, clear-white aril that has sour flavour and sweet taste (Sapii *et al*., 2000). The sweet juicy flesh contains monosaccharides such as sucrose, fructose and glucose. Among the three varieties, *Dokong* is sweeter, with very little seeds or no seed and relatively small size (Te-chato *et al*., 2005).

In addition to food, some parts of the tree are traditionally used as a remedy for treating parasites in the intestine and diarrhoea. The dried fruit with burnt skin are known to be used as mosquito repellent and treat malarias and scorpion stings. Meanwhile, the seed powder of *Langsat* is used to reduce fever (Morton, 1987). These traditional practices have initiated numerous research in identifying the chemical constituents of the *Lansium* seeds. A study on the *Lansium* plant has reported the presence of high amount of alkaloid content in barks (Lense, 2011) but only trace amount in their seeds (Morton, 1987). Saewann *et al.* (2006) successfully isolated five tetranortriterpenoids and 11 known triterpenoids.

A recent report shows that the methanol extract of the dried seeds and bark of *Lansium domesticum* cv. kokossan yielded two tetranortriterpenoids, kokosanolide A and C, and an onoceranoid-type triterpenoid, kokosanolide B, along with two onoceranoid-type triterpenoids; 8,14-*seco*gammacera-7,14-diene-3,21-dione, and a mixture of 8,14-*seco*gammacera-7,14(27)-diene-3,21-dione and 8,14-*seco*gammacera-7,14-diene-3,21-dione (1.5:0.5) (Mayanti *et al*., 2011). Several compounds isolated from *Lansium* seeds have been identified to exhibit anti-malarial activity against *Plasmodium falciparum* (Saewann *et al*., 2006), anti-feedant activity against the fourth instar larvae of *Epilachna vigintioctopunctata* (Mayanti *et al*., 2011), and anti-bacterial activity against Gram-positive bacterium (Dong *et al*., 2011).

Generally, *Lansium* has been reported in very few studies focusing on its varieties. In this study, the potential of the seed crude extracts of the three closely related varieties of *Lansium* (*Duku*, *Langsat*, and *Dokong*) in inhibiting the growth of the three selected bacteria, *Pseudomonas aeruginosa*, *Staphyllococcus aureus* and *Bacillus subtilis,* was investigated. These bacteria are common in Malaysia and in most places in the world. For example, *Staphylococcus aureus* is a facultative anaerobic Gram-positive bacterium that is usually found

to coexist with human in normal skin flora and in nasal passages (Foster, 2004). However, it can cause a wide range of infections from minor ones such as pimples and impetigo to severe infections such as the methicillin-resistant *S. aureus* (MRSA) that are becoming a serious problem in many places in the world as these virus clones are resistant to many antibiotics (Foster, 2004; Grundmann *et al*., 2012). *S. aureus* produces enterotoxin, which is responsible for staphylococcal food poisoning that cause rapid onset, nausea, violent vomiting, and may cause chronic diarrhoea (Argudin *et al*., 2010). In fact, scientists are still working on searching for the right vaccine or special drug to prevent or to cure this problem. *Pseudomonas aeroginosa*, a Gram-negative bacterium, is capable of living in both aerobic and anaerobic conditions. It is an important pathogen of plants and animals, which can infect damaged tissues or those with reduced immunity, and may become fatal if colonized in vital organs such as kidney, liver and urinary tracts (Stojek *et al*., 2008). As this bacterium has high prevalence of antibiotic resistant strains, a new therapeutic agent is required to overcome this problem. Another bacterium chosen in this study, *Bacillus subtilis,* is a well-known Gram-positive model bacterium for laboratory studies. Many *B. subtilis* strains have been genetically manipulated, selected, improved, and well studied (Buxton & Ward, 1980; Tam *et al*., 2006). On this basis, the researchers hoped that the results from the current work would complement excellent findings previously reported for the *Lansium* species.

## MATERIALS AND METHODS

### *Preparation of the Seed Extracts*

The matured seeds of *Duku*, *Langsat*, and *Dokong* were collected from several areas in Kelantan, Malaysia, from July to October 2010. The plant samples were identified by the plant systematic experts. The seeds were separated from the pulps and dried in an oven at 40-45°C for 5 days. From 1 kg of fruits, approximately 30 g, 25 g, and 20 g of the dried seeds of *Langsat*, *Duku* and *Dokong* were produced, respectively. The dried seeds were ground to coarse powder before extracted with hexane, methanol and water in cold condition. The various extracts were filtered and evaporated using rotavap to give crude extracts. The crude extracts produced from hexane, methanol and water of *Langsat's* dried seeds were 1.44 g, 2.75 g, and 1.00 g, respectively, *Duku* (1.20 g, 1.69 g and 1.00 g) and *Dokong* (1.12 g, 11.27 g and 1.00 g). All the crude extracts were stored in -4 $^0$C until to be used for antimicrobial activity.

### *Preparation of the Stock Solutions of Plant Extracts*

Stock solutions of the plant extracts were prepared by dissolving 200 mg of each plant extract in 1 mL of sterilized distilled water. The mixture was vortexed to ensure that the extracts were homogeneous. The working stock solutions were protected from light by covering the bottle with aluminium foil. The seed extracts of stock solutions were used in disc diffusion test for the antimicrobial activity and for the phytochemical screening.

### *Phytochemical Screening of Seed Extracts*

Seed extracts were subjected to phytochemical screening to identify the chemical constituents such as alkaloids, flavonoids, saponins and tannins using standard procedures described below.

### *Alkaloids*

The test for alkaloids was carried out by basifying 20 g of crushed seeds in 10 % of ammonia solution and soaking with dichloromethane, heated and filtered. The dichloromethane extract was re-extracted with 5 % HCl and the aqueous layer was tested with the Mayer's reagent (Bruneton, 1999). A positive test for alkaloids was indicated by the production of a turbid solution or a yellowish creamy precipitate colour.

### *Flavonoids*

The presence of flavonoids was determined by adding a few drops of sodium hydroxide into the extracts. An intense colour was produced and it later became colourless with an addition of a few drops of dilute acid (Kumar *et al*., 2009).

### *Saponins*

The extract was subjected to Froth test to identify saponin (Onwukaeme *et al*., 2007). A small quantity of the seed extract was boiled in 20 ml of distilled water in a water bath and filtered. The mixture was then filtered and 10 ml of the filtrate was added into 5 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and the mixture was then observed in the formation of emulsion.

### *Tannins*

The tests for tannins were carried out by subjecting the seed extracts in 1 ml of 10% of potassium hydroxide and the formation of dirt precipitation showed the existence of tannin (Kumar *et al*., 2009).

### *Culture Media*

The bacterial cultures used in this study were *Staphylococcus aureus* (ATCC 1026), *Bacillus subtilis (B. spizizenii*) (ATCC 6633) and *Pseudomonas aeruginosa* (ATCC 10145). The bacteria were purchased from Choice Care Sdn. Bhd. The test organisms were purified and maintained on slant agar kept at 4°C until further use.

### *Preparation of the Media*

Nutrient agar was used as the media for bacterial enumeration. Nutrient broth was also used for the generation of exponential culture of each organism.

*Nutrient Agar*

15 g nutrient agar was suspended with 1 litre of distilled water in a Duran bottle. The media was dissolved by fast cooking in the microwave and autoclaved for about 15 min at 121°C. The sterilized melted agar was poured into sterile Petri dishes in the laminar flow for the agar plate preparation.

*Nutrient Broth*

13 g nutrient broth was suspended with 1 litre of distilled water. The media dissolved by fast cooking in the microwave. 5 ml of melted broth were pipetted into test tube and sterilized by autoclaving for about 15 min at 121°C.

*Screening for Antibacterial Activity*

The antibacterial activity of the extracts on the microorganisms was determined by using the disc diffusion methods.

*Disk Diffusion Method*

The disk diffusion method was used to measure the rate of inhibition in growth of bacteria by different concentrations of the plant extract on paper disc. The volumes of the extract used were 20 µl, 30 µl, 40 µl, and 50 µl from the 200 mg/ml of each stock solution. The discs were allowed to dry in the laminar flow before they were placed on top of the agar. The 24-hour broth culture of each test bacterium species was aseptically introduced and spread on the surface of sterile nutrient agar using sterile cotton swab. The sterile paper discs (6 mm) impregnated with extract were placed on the cultured plates and sterile forceps were used to gently press down each disc to ensure complete contact with agar surface. A disc with distilled water alone served as negative control. The plates were incubated at $37^0$C for 48 hours. All the experiments were performed in four replicates and each experiment was reproduced a minimum of three times and all these procedures were carried out aseptically.

*Determination of Antibacterial Properties*

Reading of the inhibition zones was done at 48 hour-intervals. The antibacterial activity was interpreted from the size of the diameter of zone inhibition measured to the nearest mm as it was observed from the clear zone surrounding the disc. The zone of inhibition is measured from the edge of the disc to the edge of the growth. It is measured on the undersurface of the plate without opening the lid.

## RESULTS AND DISCUSSION

Firstly, the mature seeds of *Duku*, *Langsat* and *Dokong* were screened to determine their phytochemical components. In some previous studies, phytochemical compounds such as tannins, saponins, flavonoids, steroids, and glucose-lowering were found in many plant seeds (Anago *et al*., 2011). The phytochemical screening in this study revealed the presence of

tannins and flavonoids in the seeds from all the *Lansium* varieties (see Table 1). Alkaloid, a substance that is always related to bitterness taste, was only found in the *Langsat* seed extract, but not in the other two varieties. Several findings have shown a high amount of alkaloid in the bark extract (Lense, 2011) and fruit peels (Solidum, 2012) of *Lansium domesticum*, which exhibit its medicinal potential. *Langsat* is also the only variety studied that contains saponin, another bitter taste secondary metabolite. The presence of the compounds screened in the *Langsat* seed extracts shows its potential in anti-microbial activity as these compounds have been identified to exhibit this particular effect (Cushnie & Lamb, 2005).

TABLE 1
Phytochemical screening of the seed extracts from *Lansium domesticum* varieties

| *Lansium domesticum* variety | Constituents | | | |
|---|---|---|---|---|
| | Alkaloid | Saponin | Tannin | Flavonoid |
| *Langsat* | + | + | ++ | + |
| *Duku* | - | - | + | + |
| *Dokong* | - | - | + | + |

++ = present in high amount; + = present; - = absent

In order to determine the potential of the *Duku*, *Langsat* and *Dokong* seed extracts as antimicrobial agents, seed extraction was done using hexane, methanol and water. All the crude extracts were tested against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*, and observed after 48 hours. The results are shown in Tables 2, 3 and 4, respectively.

Inhibition zones could be clearly seen in the hexane and methanol extracts of *Langsat*, but not in the water extract (Table 2). The hexane extract of *Langsat* seeds inhibited the growth of two of the tested bacteria (*S. aureus* and *B. subtilis*), while the methanol extract of Langsat was shown to inhibit all the tested bacteria. The minimal inhibition concentration (MIC) of the hexane and methanol extracts towards *S. aureus* and *B. subtilis* was at 0.25 g/ml and the MIC for methanol extract towards *P. aeroginosa* was at 0.50 g/ml. The increase in the inhibition zones was observed with the increasing concentration of the hexane and methanol seed extracts. The biggest inhibition zones occurred in the hexane extract against *S. aureus* and in the methanol extract against *B. subtilis*. In both solvents, increase in the inhibition zones was observed when the concentration of the extracts increased with the highest inhibition zones shown against *S. aureus* for the hexane extract and *B. subtilis* for the methanol extract.

TABLE 2
The inhibition zones of the *Langsat* seed extracts after 48 hours of incubation

| Solvent | Bacteria | Concentration of plant extracts (g/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 0.0625 | 0.125 | 0.25 | 0.5 | 1.0 |
| | | Inhibition zones (cm) | | | | |
| Hexane | PA | - | - | - | - | - |
| | SA | - | - | 0.67 ± 0.12 | 0.77 ± 0.09 | 1.07 ± 0.09 |
| | BS | - | - | 0.27 ± 0.15 | 0.23 ± 0.12 | 0.70 ± 0.06 |
| Methanol | PA | - | - | - | 0.33 ± 0.09 | 0.73 ± 0.09 |
| | SA | - | - | 0.23 ± 0.15 | 0.80 ± 0.10 | 0.97 ± 0.09 |
| | BS | - | - | 0.83 ± 0.07 | 0.90 ± 0.06 | 1.13 ± 0.12 |
| Water | PA | - | - | - | - | - |
| | SA | - | - | - | - | - |
| | BS | - | - | - | - | - |

PA = *Pseudomonas aeruginosa* SA = *Staphylococcuss aureus* BS = *Bacillus subtilis*

An inhibition zone was observed in the methanol seed extract of *Duku* towards *B. subtilis* at the highest concentration (1.0 g/ml). Nonetheless, no inhibition against other bacteria was observed from either the methanol extracts or other extracts (Table 3).

TABLE 3
The inhibition zones of the *Duku* seed extracts after 48 hours of incubation

| Solvent | Bacteria | Concentration of plant extracts (g/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 0.0625 | 0.125 | 0.25 | 0.5 | 1.0 |
| | | Inhibition zones (cm) | | | | |
| Hexane | PA | - | - | - | - | - |
| | SA | - | - | - | - | - |
| | BS | - | - | - | - | - |
| Methanol | PA | - | - | - | - | - |
| | SA | - | - | - | - | - |
| | BS | - | - | - | - | 0.67 ± 0.03 |
| Water | PA | - | - | - | - | - |
| | SA | - | - | - | - | - |
| | BS | - | - | - | - | - |

PA = *Pseudomonas aeruginosa* SA = *Staphylococcuss aureus* BS = *Bacillus subtilis*

On the other hand, none of the *Dokong* seed extracts showed any inhibition zones against any bacterium (Table 4). The finding revealed that using any of the solvent in this study, the *Dokong* seed extract is an ineffective inhibitor of all the three bacteria.

TABLE 4
The inhibition zones of the *Dokong* seed extracts after 48 hours of incubation

| Solvent | Bacteria | Concentration of plant extracts (g.mL-1) | | | | |
|---|---|---|---|---|---|---|
| | | 0.0625 | 0.125 | 0.25 | 0.5 | 1.0 |
| | | Inhibition zones (cm) | | | | |
| Hexane | PA | - | - | - | - | - |
| | SA | - | - | - | - | - |
| | BS | - | - | - | - | - |
| Methanol | PA | - | - | - | - | - |
| | SA | - | - | - | - | - |
| | BS | - | - | - | - | - |
| Water | PA | - | - | - | - | - |
| | SA | - | - | - | - | - |
| | BS | - | - | - | - | - |

PA = *Pseudomonas aeruginosa* SA = *Staphylococcuss aureus* BS = *Bacillus subtilis*

This study employed the disc diffusion method to determine the antimicrobial activity of the *Lansium* seed extracts against the three selected bacteria. In some previous reports of antimicrobial activity studies, the disc diffusion methods have been shown to yield similar results with an excellent agreement using broth dilution or microdilution methods and other tests (Serrano *et al.*, 2004; Milici *et al*., 2007; Kumar *et al.*, 2010). Therefore, the disc diffusion method was applied in this study as it is cheaper, reliable and easy to perform (Wiegand & Hilpert, 2008).

When the effectiveness of the solvents was compared, methanol was shown to have exhibited a better performance in extracting the antimicrobial compounds from the *Lansium* seed extracts compared to hexane, as the methanol extract showed better inhibition zones and inhibited more bacteria. Meanwhile, alcoholic solvents such as ethanol and methanol showed better performance in extracting flavonoids and phenolic compounds in many medicinal plants (Rispail *et al*., 2005; Sultana *et al.,* 2009; Tomsone *et al*., 2012). Methanol is an efficient solvent to degrade the cell walls and penetrate the cellular membranes, causing the intercellular compounds to be released from the cells (Tiwari *et al*., 2011). On the other hand, hexane is efficient in extracting oils and other non-polar compounds from seeds and plant parts (Gidwani *et al.,* 2010; Singh *et al*., 2012). As oil is difficult to dissolve in water or agar medium, this might have affected the results of the antibacterial activity using disc diffusion method, which was employed in the current study. However, several studies have shown that the hexane extracts of the seeds and other plant parts are capable of inhibiting the growth of *B. subtilis* and *S. aureus* using either disc diffusion or agar dilution methods (Gidwani *et al*., 2010; Ahmed-Hassan *et al*., 2011; Singh *et al*., 2012). In the present study using hexane as a solvent, the *Langsat* seed extracts demonstrated the ability to inhibit two of the bacteria studied. Nevertheless, no positive results were found from any water extracts of the *Lansium* varieties. In traditional medicine, water is always used to extract different

parts of plant (Abdalah, 2011; Borhade, 2012). However, higher water content in an extract increases the concomitant extractions of other compounds and reduces the selectivity of the extracted compounds (Tomsone *et al*., 2012). This reason may result in lower extraction rate of antimicrobial compounds in the water extracts of *Lansium* seeds.

The antimicrobial activity results show that the *Langsat* seed extract is the most effective inhibitor against the bacteria tested compared with *Duku* and *Dokong*. Some previous studies have reported the effectiveness of *Langsat* seed and organ extracts as antimicrobial agents. For examples, the fruit skin, leaf and seed methanol extracts of *L. domesticum* were found to be effective in inhibiting the growth of *Plasmodium falciparum,* demonstrating the potential as antimalarial agent (Yap & Yap, 2003; Saewann *et al*., 2006). Mayanti *et al.* (2011) reported that the methanol extract of *L. domesticum* showed a strong antifeedant activity against the fourth instar larvae of *Epilachna vigintioctopunctata.* The potential of the *Lansium* ethanol extract as antimicrobe had also been studied by Dong *et al.* (2011). The researchers reported a class of onoceranoid-type triterpenoids, found in the plant twigs (Dong *et al*., 2011), exhibited a moderate antibacterial activity against Gram-positive bacteria. The finding is in agreement with the results of the present study which showed that the *Langsat* and *Duku* seed extracts exhibited antibacterial activities towards Gram-positive bacteria, namely, *S. aureus* and *B. subtilis*. Nevertheless, has been reported on the potential of the *Lansium* seed methanol extract against the Gram-negative bacteria. The findings of this study showed that the *Langsat* seed extract showed a great potential in inhibiting the growth of harmful bacteria such as *P. aeruginosa*.

Mohamed *et al.* (1994) studied *Duku* fruit skin extracts and found their activities against *Candida lypolytica* but none was reported on the seed extracts. In the present study, the methanol extract of *Duku* seeds was found to exhibit the antibacterial activity towards *B. subtilis*.

## CONCLUSION

Three varieties of *Lansium domesticum* (*Langsat*, *Duku* and *Dokong*) have been shown to have interesting patterns in their antimicrobial activities towards *Staphylococcuss aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Even though these varieties shared similar morphological characteristics, the findings revealed that *the Langsat* seed extract exhibits a good potential in inhibiting the growth of two Gram-positive bacteria (*S. aureus* and *B. subtilis*) whilst moderately inhibiting the growth of Gram-negative bacterium, *P. aeruginosa.* Meanwhile, the methanol seed extract of *Duku* showed a moderate inhibition only towards *B. subtilis*. On the other hand, the seed extracts of *Dokong* did not show any potential antimicrobial inhibition. Therefore, the results of this study provide a basis for further phytochemical and antimicrobial studies on the seed extracts of the *Lansium* varieties.

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