Pak. J. Bot., 45(5): 1693-1700, 2013.

ANTIBACTERIAL STUDIES ON *IN VIVO* PLANT PARTS OF MEDICINALLY IMPORTANT *EURYCOMA LONGIFOLIA* (TONGKAT ALI)

MONICA DANIAL[1], GEETHAA SAGHAL[2], SAFIAH AHMAD MUBBARAKH[1], JEEVANDRAN SUNDARASEKAR[1] AND SREERAMANAN SUBRAMANIAM[1*]

[1]School of Biological Sciences, Universiti Sains Malaysia, 11800 Gelugor, Penang, Malaysia
[2]Institute For Research in Molecular Medicine, Universiti Sains Malaysia, 11800 Gelugor, Penang, Malaysia.
[*]Corresponding author's email: sreeramanan@usm.my/sreeramanan@gmail.com

Abstract

The various crude extracts of the *In vivo* plant parts of medicinally important *Eurycoma longifolia* were observed to demonstrate antibacterial activity to all the tested pathogenic bacteria. The most effective antibacterial agent was the extracted compound from the roots of *Eurycoma longifolia* on *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* (CDR), *Staphylococcus aureus* ATCC 25923 and *Shigella flexneri* ATCC 12022. In addition, there are also several other parts of this plants extract that produced good antibacterial activity against some tested pathogenic bacteria. Thus, the results obtained can be used to facilitate the development of antibacterial medicines for specific pathogenic bacteria to treat diseases caused by these bacteria.

Introduction

The tropical rain forest plants contains diverse resources of biologically and chemically important components as they synthesize various chemicals as defence agents against pests, diseases and predators. Hence, the tropical rain forest serves as an excellent reservoir of medicines and chemical leads with which researchers can design and synthesize new drugs (Ibrahim, 2004). However, fewer than 5% of tropical forest plant species have been examined for chemical compounds and medicinal values (Zakrzewski, 2002).

The use of plants directly or indirectly as remedies in treating ailments is because plants are rich in alkaloids and other phytochemical contents and many of them work effectively in curing a wide range of ailments. The alkaloids and the phytochemicals present in all organs of the plant including roots, stems, buds, leaves, flowers and fruits (Chitravadivu *et al*., 2009). There are numerous reports on the bioactive compounds which are mainly found in herbs and other plants (Tariq *et al*., 2011), which have been reported to posses possible health benefits with antioxidative, anticarcinogenic, antihypertensive, antimutagenic, and angiogenesis inhibitory activities (Cao & Cao, 1999; Geleijnse *et al*., 1999; Kahkonen *et al*., 1999; Yen *et al*., 2002). Medicinal plants contain large amounts of phenolic antioxidants in addition to compounds like vitamin C, vitamin E, and carotenoids. Phenolic antioxidants in medicinal plants are mainly composed of phenolic acids (Cao & Cao, 1999) and flavonoids (Madsen & Bertelsen, 1995).

The worldwide interest in medicinal plants reflects the recognition towards many traditional claims regarding the value of natural products in health care. Thus, this has lead to the investigation of the antimicrobial activity of medicinal plants (Chitravadivu *et al*., 2009). This is because bacterial resistance to currently used antibiotics is becoming less effective thus inflicts the concern on public health (Cock, 2008). The reason for this incident is due to the origin of super resistant bacterial strains that causes the failure of the currently used antibiotic agents failing to cure many bacterial infections (Cock, 2008). Therefore, there are many researches were conducted to uncover new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources (Bhavnani & Ballow, 2000). Specifically, the herbal based medications have gained the interest due to the belief that there is a lower incidence of adverse reactions and toxicity (Chariandy *et al*., 1999; Rodríguez *et al*., 2010) to plant preparations compared to synthetic pharmaceutical. In addition, the plant based natural therapeutics requires lesser production cost compared to synthetic remedies thus makes the search for natural therapeutics an attractive option (Cock, 2008). Thus, the objectives of the present study is to evaluate the antibacterial activity of crude extracts of *in vivo* plant parts of *Eurycoma longifolia* on American Type Culture Collection (ATCC) and a lab isolate strains of pathogenic bacteria.

Materials and Methods

Plant materials: *Eurycoma longifolia* plant with the age of 5 years old were purchased from supplier were used in this study. Different plant parts were obtained and dried separately in the incubator at 40ºC until a constant weight was obtained. The plant parts used are roots (RT), leaves (L), branches (BR), seeds (SE), Bark (BK) and stem core (SC). After obtaining a constant dried weight, the plant part were grind using dry blender (Panasonic) to obtain powdered form. The powdered form were then subjected to sonication treatment by the dissolving the powdered plant material using 99% methanol at the ratio of 5:1 (v/v) for 20 minutes for each session. The process was repeated for three times for each plant sample. Then, the sonicated solutions were separated using filtration method. After that, the filtrate was dried in the oven at 40ºC until all the methanol was evaporated out. Finally, the crude extracts of the different plant materials were used in the antibacterial assay. The working stock concentrations for the crude extracts of the plant materials were prepared at 50mg/mL by dissolving 100mg of the dried crude extracts into 2ml of 25% methanol. Chloramphenicol (+) at the concentration of 30µl/mg was used as positive control in this study. Chloramphenicol (Sigma-Aldrich) was prepared by dissolving 0.3mg of Chloramphenicol into 0.3mL 25% methanol. The negative control used was 25% methanol (-).

Bacterial strains: Five American Type Culture Collection (ATCC) pathogenic strains and one lab isolate strain that was kindly provided by Center of Drug Research (CDR) USM were used in this study. The bacteria used for the antibacterial studies are *Escherichia coli* ATCC 25922,

Pseudomonas aeruginosa ATCC 27853, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923 and *Shigella flexneri* ATCC 12022 and generally regarded as safe (GRAS) bacteria Bacillus *subtilis* (CDR). Prior to be used in the antibacterial assay, the bacteria were grown on both Mueller Hinton (MH) broth and agar and placed in an incubator at 37ºC and observed for confluency of its growth.

Agar disc diffusion assay: The disk diffusion (Bauer *et al*., 1966) technique, based on the recommended standards of the National Committee for Clinical Laboratory Standards (NCCLS), was used for antimicrobial test. An overnight suspension culture of the six microbial strains was spread on MHA media by adding 100µl of the suspension culture in the middle of the plate and spreading it evenly on the plate using L-rod. Sterile discs were prepared (diameter = 6mm) and placed on the culture spread agar media. The discs were impregnated with the 20 µl of the working stock of each of the crude plant extracts (Fig. 1).

Chloramphenicol (+) was used as positive control to check the sensitivity of the strains. Twenty five percent (25%) of methanol (+) was used as negative control. The inoculated plates were incubated at 37ºC for 24 h (Karaman *et al*., 2003). The antimicrobial activity was evaluated by measuring diameter of the inhibition zone around the disc. The placement of the disc containing the crude plant extracts were done as shown below:

Statistical analysis: Each set of experiment was done in triplicates and repeated thrice. Means were analyzed using univariate analysis of variance (SPSS Inc/PASW Statistics 17.0) and differentiated with Duncan's test.

Results and Discussion

Five pathogenic strains namely *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923, *Shigella flexneri* ATCC 12022 and one generally regarded as safe (GRAS) bacteria *Bacillus subtilis* were used in this study.

The overall results indicated that the crude extract of the root of *Eurycoma longifolia* was the most effective antibacterial agent. The result was proven true for all the tested pathogenic bacteria except for a gram positive bacteria *Bacillus cereus*. This is because application of the crude extract of the root on the bacteria created the widest inhibition zone on *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* (CDR), *Staphylococcus aureus* ATCC 25923 and *Shigella flexneri* ATCC 12022. In addition, the significance value recorded for these bacteria were significantly higher than the crude extracts of other parts of this plant namely branches (BR), leaves (L), stem core (SC), bark (BK), roots (RT) and seeds (SE).

Shigella flexneri is Gram-negative, non-spore forming, facultative anaerobic bacilli closely related biochemically and antigenically to *Escherichia coli* (Alfredo, 2004). *Shigella* causes shigellosis or bacillary dysentery disease. *Shigella* causes an estimated 1 million deaths and 163 million cases of dysentery annually worldwide (Alfredo, 2004). The onset of the disease progression is marked by an infection of the large bowel followed by abdominal cramps, diarrhea, and fever. Initially, the diarrhea may be copious and the liquid stools often contain blood and mucus (Ryan & Falkow, 1994). Shigellosis is usually acquired by drinking water contaminated with human feces or by eating food washed with contaminated water. *Shigella* portrays extraordinary competence for acquiring plasmid encoded multi antibiotic resistance which was used in first-line therapy (Kotloff *et al*., 1999). Therefore, crude extract of *Eurycoma longifolia* is tested as potential antibacterial agent to combat the prevalence of *Shigella.*

The inhibition zones values recoded for *Shigella flexneri* ATCC 12022 indicates widest diameter of inhibition zone value from crude extracts of roots which is about 16.33±3.04 mm, followed by stem core, seeds and branches each with the inhibition diameter of 13.22±3.19 mm, 12.78±2.44 mm and 12.22±1.64 mm. On the contrary, bark and leaves has lower diameter of inhibition zone which is about 9.56±2.51 mm and 10.44±2.74 mm (Fig. 2). The crude root extract had the most significant inhibition effect on *Shigella flexneri* ATCC 12022 (Fig. 2). The second highest significance in terms of inhibitory effect on *Shigella flexneri* ATCC 12022 has been recorded for crude extracts of seeds and stem core. However, no significance in terms of inhibitory effects was recorded for crude extracts of bark, branches and leaves. In addition, for all the tested plant parts crude extracts the values obtained were significantly higher and different from the positive and negative control for the pathogenic *Shigella flexneri* ATCC 12022 (Fig. 2).

Figure 3 shows the inhibition zone when tested with for *Shigella flexneri* ATCC 12022. From the morphological observation, the widest inhibition zone can be observed for the crude extract obtained from the roots of *Eurycoma longifolia*. Moreover, clear inhibition zone were also observed for other plant parts crude extracts like seeds, stem core, bark and branches.

Escherichia coli are Gram negative rod shaped bacteria. Most of the *Escherichia* strains have flagella and the bacteria are motile. *Escherichia coli* grow well on a variety of media at 37°C. The bacteria are usually found in the intestinal tract of the warm blooded animals including humans. Upon infection to humans, it causes diarrheal disease. Diarrheal illness is a major public health problem worldwide, with over 2 million deaths occurring each year (Danilo *et al*., 2009). The antibacterial assay on *Escherichia coli* facilitated the identification of potential vaccine candidates based on the plant extracts of *Eurycoma longifolia*.

Highest inhibition zone value was recorded for *Escherichia coli* ATCC 25922 for the crude extracts from roots which is about 19.67±2.45 mm, followed by crude extract of the stem core and branches (Fig. 4). The inhibition zone recorded for both the stem core and branches were 15.00±3.74 mm and 13.22±2.68 mm respectively. The inhibition zone value recorded for seeds, bark and leaves were not very much different from each other which are 11.11±2.03 mm, 10.11±2.37 mm and 10.89±2.76 mm, respectively. However, all the inhibition zone value recorded were lower than the positive control value in plate 1 (Fig. 4).

The root extract of *Eurycoma longifolia* inhibited the pathogenic *Escherichia coli* ATCC 25922 most significantly compared to other plant parts. On the other hand, the significance value obtained for all other plants parts crude extract like bark, stem core, seeds and leaves were not significantly different from each other. Furthermore, the significance values of crude extracts of these plant parts were significantly lower or equal compared to the positive control despite recorded higher significant value compared to the negative control.

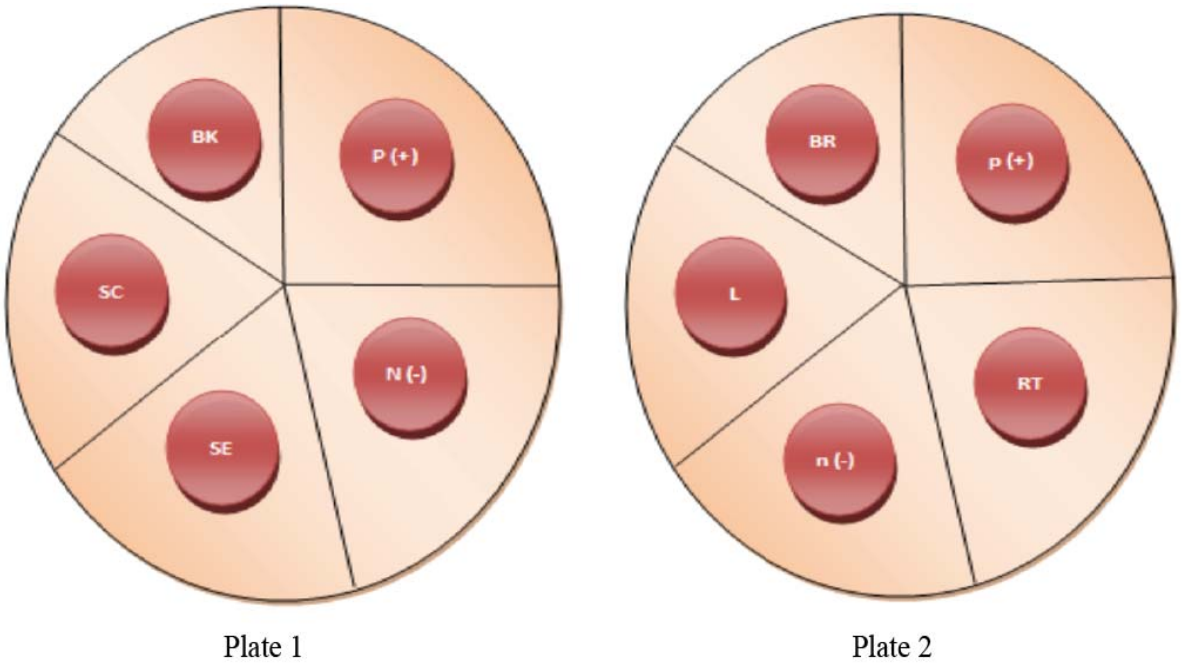

Fig. 1. The placement of the disc containing the crude plant extracts; bark (BK), stem core (SC), seeds (SE), branches (BR), leaves (L), roots (RT). Positive control used is Chloramphenicol (30µl/mg) is denoted as P (Plate 1) or p (Plate 2) (+) and the negative control used is 25% methanol and is denoted as N or n (-).

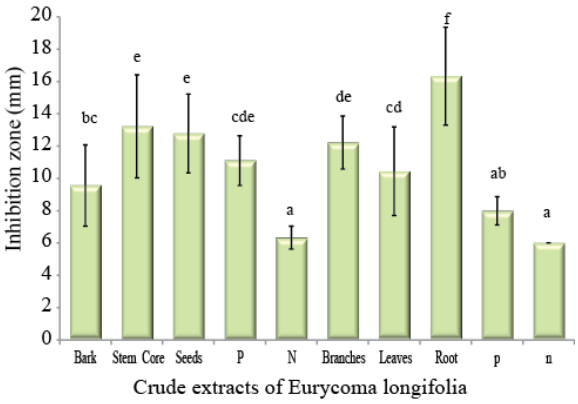

Fig. 2. Inhibition zone (mm) for *Shigella flexneri* when tested with extract from various parts of *in vivo Eurycoma longifolia.* Means underscored by the same letter are not significantly different at p≤0.05.

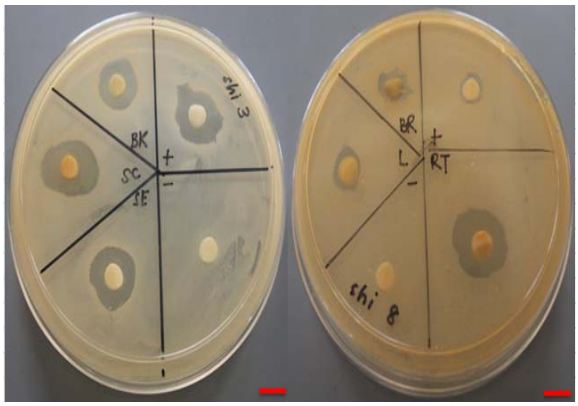

Fig. 3. Inhibition zone (mm) for *Shigella flexneri* when tested with extract from various parts of *in vivo Eurycoma longifolia* (scale bar in red indicates 1 unit = 0.5cm).

Pseudomonas *aeruginosa* is a gram-negative rod that belongs to the family Pseudomonadaceae. Pseudomonas *aeruginosa* can be found commonly in nature, inhabiting soil, water, plants, animals and humans. *Pseudomonas aeruginosa* is the most common pathogen responsible for both acute respiratory infections in ventilated or immunocompromised patients and chronic respiratory infections in cystic fibrosis patients (Chastre & Trouillet, 2000; Chastre & Fagon, 2002). Furthermore, due to problems of high incidence and infection severity, the resistance of Pseudomonas *aeruginosa* to conventional antimicrobial treatment has increased over the past decade highlighting the need for new therapeutic options (Kipnis *et al*., 2006).

The widest inhibition zone recorded for *Pseudomonas aeruginosa* ATCC 27853 was by the root crude extracts of *Eurycoma longifolia.* The diameter recorded was 18.56±2.19 mm. The second widest inhibition zone was recorded for crude extract from stem core which is about 11.56±2.40 mm (Fig. 5). Almost similar inhibition zone values were recorded for crude extracts from seeds, leaves, bark and branches. The values of the inhibition zone are 10.44±2.51 mm, 10.11±2.03 mm, 9.56±2.01 mm and 9.00 ±2.60 mm respectively. The positive control value from plate 1 (Fig. 5) were higher than all the tested plant crude extracts except for roots. Despite that, the all the crude extracts recorded higher values of inhibition zone when compared with the negative control.

The growth of the pathogenic bacteria *Pseudomonas aeruginosa* ATCC 27853 is significantly inhibited by the root extract of *Eurycoma longifolia.* However, the value obtained for all other parts of the plant were recorded significantly lower or equal to the positive control (positive control plates 1 and 2) (Fig. 5). The inhibition zone for bark, stem core and branches were significantly different from each other but inhibition of plant parts like seeds and leaves were not significant from each other as shown in Fig. 5. In addition, all the values obtained were significantly higher than the negative control.

Staphylococcus aureus is a facultative anaerobic gram-positive bacteria. *Staphylococcus aureus* is non-motile and non-spore forming bacteria. *Staphylococcus aureus* is a versatile pathogen capable of causing a wide range of human diseases. However, the role of different virulence factors in the development of staphylococcal infections remains incompletely understood (Rachel & Franklin, 2008). The antibacterial assay on *Staphylococcus aureus* is hoped to facilitate the identification of potential vaccine candidates based on the plant extracts of *Eurycoma longifolia*.

Higher values for inhibition zone were recorded for pathogenic *Staphylococcus aureus* ATCC 25923 compared to the 5 other pathogenic bacteria used in this study. This indicates that the extract of *Eurycoma longifolia* works well in inhibiting *Staphylococcus aureus* ATCC 25923. Wide inhibition zone for the pathogenic *Staphylococcus aureus* was obtained from the crude extracts of roots (17.22±2.44 mm) and stem core (13.56±2.07 mm) (Fig. 6). In addition, crude extracts of plant part like branches, seeds, leaves and bark also inhibited the pathogenic *Staphylococcus aureus* ATCC 25923 by creating the inhibition zones at the diameter of 11.89±2.09 mm, 11.89±1.76 mm, 11.56±2.70 mm and 10.67±2.18 mm (Fig. 6).

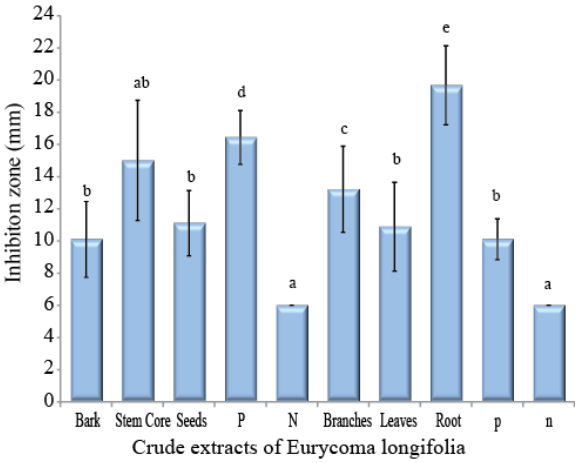

Fig. 4. Inhibition zone (mm) for *Escherichia coli* when tested with extract from various parts of *in vivo Eurycoma longifolia.* Means underscored by the same letter are not significantly different at p≤0.05.

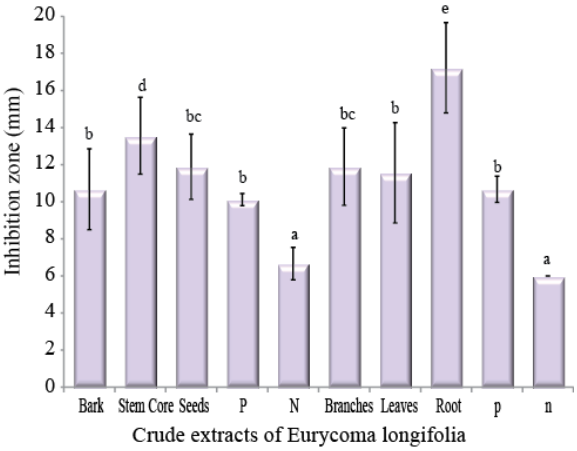

Fig. 6. Inhibition zone (mm) for *Staphylococcus aureus* when tested with extract from various parts of *in vivo Eurycoma longifolia.* Means underscored by the same letter are not significantly different at p≤0.05.

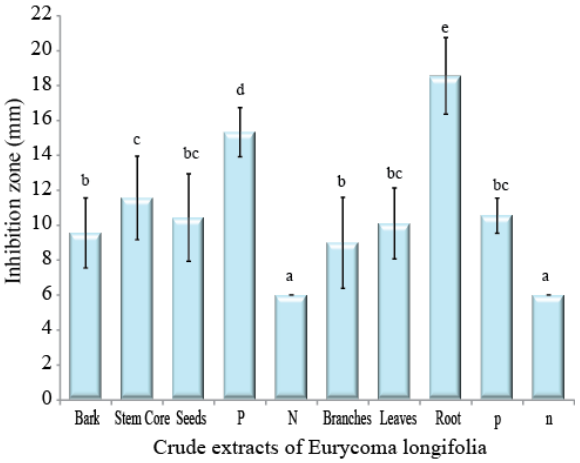

Fig. 5. Inhibition zone (mm) for *Pseudomonas aeruginosa* when tested with extract from various parts of *In vivo Eurycoma longifolia.* Means underscored by the same letter are not significantly different at p≤0.05.

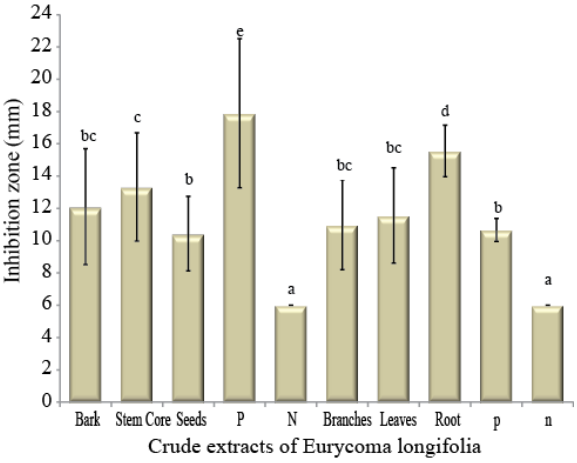

Fig. 7. Inhibition zone (mm) for *Bacillus cereus* when tested with extract from various parts of *In vivo Eurycoma longifolia.* Means underscored by the same letter are not significantly different at p≤0.05.

The inhibition zone obtained for the root crude extract revealed the most significant value compared to other parts of the plant. The clearance zone can be observed in Fig. 6. In addition, the crude extract of stem core of *Eurycoma longifolia* also works well in inhibiting *Staphylococcus aureus* ATCC 25923. This is supported by the significant value obtained for stem core compared other plant parts (Fig. 6). On the contrary, the significance values obtained for all other plant parts namely bark, seeds, branches and a leaf were not significantly different from each other and also from the positive control which is chloramphenicol but they are significantly higher than the negative control (Fig. 6).

Bacillus cereus is a gram-positive and spore-forming bacterium that produces multiple toxins which cause food poisoning in human (In-Cheol *et al*., 2011). *Bacillus cereus* produces emetic and diarrheal toxins as an opportunistic human pathogen (Bhunia, 2008). The diarrheal is caused by enterotoxins in the human small intestine (Granum & Lund, 1997). Furthermore, *Bacillus cereus* has been associated with more severe infection such as endophthalmitis and pneumonia (Miller *et al*., 1997). The endospores of *Bacillus cereus* are able to survive in harsh environments because of their thermal stability and ability to be easily transmitted to food through other environments such as soil or water (Kortiranta *et al*., 2000). Therefore, *Bacillus cereus* has been emerging as a food-borne pathogen with a distribution similar to Salmonella spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* in food industry (In-Cheol *et al*., 2011).

The values for the inhibition zone recorded for *Bacillus cereus* ATCC 10876 shows that the tested plant extracts were not effective in inhibiting these pathogenic bacteria. This is because the values obtained from the inhibition zone of the positive control plate 1 (Fig. 7) were higher compared to the plant parts (Fig. 7). The diameter of the zone of inhibition recorded for the positive control plate 1 (Fig. 7) is 17.89±4.62 mm. Despite it, the inhibition value recorded for the root crude extract was significantly higher than the rest of the explants which is about 15.56±1.59 mm. In addition, the crude extracts of the stem core and bark also recorded clear inhibition zones at the diameter of 13.33±3.35 mm and 12.11±3.59 mm. Moreover, almost similar values of the inhibition zone were recorded for other *Eurycoma longifolia* plant crude extracts like seeds, branches and leaves as shown in Fig. 7. The inhibition zone recorded for these crude extracts are 10.44±2.30 mm, 11.00±2.78 mm and 11.56±2.96 mm. However, the inhibition zone values for all tested crude extracts were higher than the negative control (Fig. 7).

Bacillus subtilis is a naturally occurring gram-positive saprophytic bacterium that is commonly found in soil, water, air and decomposing plant material (Backman *et al*., 1997; Perez *et al*., 2000). However, in most of the conditions, *Bacillus subtilis* is not biologically active and remain in the spore form. Different strains of *Bacillus subtilis* are being used as biological control agents under different situations (Backman *et al*., 1997). Several *Bacillus* species produces antibiotics with broad spectrums and various structures such as bacteriocin-like substances and antimicrobial lipopeptides (Stein, 2005). *Bacillus subtilis* is widely used to inhibit food-borne pathogens and has importance in fermented foods production because its genetic and biochemical properties have been well-studied, and antimicrobial substances produced by *Bacillus subtilis* have low toxicity, high biodegradability, and are environmentally-friendly (Raaijmakers *et al*., 2010). *Bacillus subtilis* has also been granted the Generally Recognized as Safe (GRAS) status (Cladera, *et al*., 2004).

The inhibition zone for *Bacillus subtilis* (CDR) can be observed in Fig. 8. Highest inhibition zone value was recorded from the crude extracts of roots (12.89±3.18 mm). This is followed by the inhibition zone value of the positive control in plate and branches (Fig. 8). The inhibition zone values for both the positive control in plate and branches are 11.78±2.64 mm and 10.44 ±3.28 mm respectively. Not much difference in diameter can be observed for bark, leaves and stem core each with the inhibition zone value of 8.67±2.24 mm, 8.56±1.88 mm and 7.44±2.65 mm (Fig. 8).

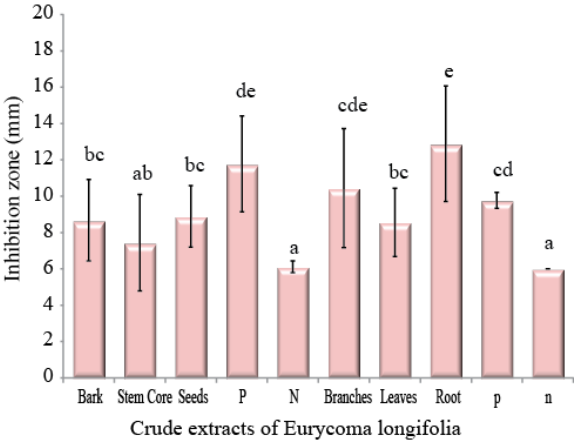

Fig. 8. Inhibition zone (mm) for *Bacillus subtilis* when tested with extract from various parts of *in vivo Eurycoma longifolia.* Means underscored by the same letter are not significantly different at p≤0.05.

The significance value for all plant crude extracts in inhibiting *Bacillus subtilis* (CDR) can be divided into three major groups with similar significance that are positive control in plate 1, branches and roots (Fig. 8). The second group with similar significance consist of bark, stem core, seeds and leaves and finally the third group that consist of negative controls, that have lower significance value compared to all other tested extracts (Fig. 8).

Almost all the tested pathogenic bacteria are inhibited by the root extracts of *Eurycoma longifolia* as reported for the root extracts of *Leptadenia pyrotechnica* (Mehmooda *et al.,* 2012). High antibacterial activity from the root extract may due to the reason that plant roots continuously produce and secrete compounds into the rhizosphere (Gleba *et al*., 1999; Bais *et al*., 2001). Root exudation consist of compounds like of ions, free oxygen

and water, enzymes, mucilage, and a diverse array of carbon-containing primary and secondary metabolites (Uren, 2000; Bertin *et al*., 2003). In addition, root exudation can be broadly divided into two active processes (Bais *et al*., 2006). The first active process of root excretion involves gradient dependent output of waste materials with unknown functions, whereas the second, secretion, involves exudation of compounds with known functions, such as lubrication and defense (Bertin *et al*., 2003; Bais *et al*., 2004).

In addition, the data in this research revealed that the gram-positive bacteria namely *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus* were more resistant to the antibacterial assay compared to the pathogenic gram negative bacteria namely *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella flexneri*. It may due to the reason that the gram-positive bacteria are more sensitive than the gram-negative bacteria (Cynthia *et al.,* 2011). The differences between gram-positive and gram-negative bacteria are due to cell wall structural differences between these classes of bacteria (Cock, 2008; Lebleau *et al*., 2009). The bacterial cell-wall is composed of a specific layer of a cross-linked polymer which is known as peptidoglycan. As reviewed by Lebleau *et al*., (2009) in gram-negative bacteria the peptidoglycan layer thickness is around 2 to 6 nm, whereas the gram-positive bacteria has a thicker peptidoglycan layer which is about 20 to 80 nm. The differences in the thickness of the peptidoglycan layer causes different amount of resistance between the gram-negative and gram-positive bacteria (Sadr *et al*., 1999). This is because the peptidoglycan layer is responsible for the cell-wall mechanical strength (Yao *et al*., 1999). Therefore, since the gram-negative thickness of the peptidoglycan layer is much thinner thus higher likelihood for antibacterial compounds to penetrate or pass through the smaller pores. On the other hand, the large peptidoglycan layer thickness of the gram-positive bacteria limits the entry of the antibacterial compound (Yao *et al*., 1999).

As reviewed by Young *et al*., (2009), lipopolysaccharide (LPS) is the major etiologic component of pathogenic gram-negative bacteria (Morrison & Ryan, 1987). The major function of lipopolysaccharide in pathogenic gram-positive bacteria is to stimulate the host cells and leads it to the production of inflammatory mediators like cytokines, chemokines and lipid metabolism (Young *et al*., 2009). The lipopolysaccharide is only found in gram-negative bacteria but the gram-positive bacteria lack lipopolysaccharide and instead it contains lipoteichoic acid (LTA) on their cell wall (Zdenek *et al*., 2010).

The lipoteichoic acid plays a role as an important pathogen-associated molecular pattern (PAMP) in the gram-positive bacteria. The lipoteichoic acid is able to stimulate the innate immunity of the pathogenic gram-positive bacteria (Han *et al*., 2006). Therefore, the lipoteichoic acid plays the same role as lipopolysaccharide in gram-positive bacteria although there are some difference in the structural details and the signaling transduction in both lipoteichoic acid and lipopolysaccharide (Young *et al*., 2009). It was reported that the expression of the lipoteichoic acid were not only found in the pathogenic gram-positive bacteria but the expression was also found in non-pathogenic or beneficial gram-positive bacteria (Veckman *et al*., 2004). In gram-positive bacteria, the infection at the lipoteichoic acid region is first recognized by Toll-like receptor 2 (TLR2) (Han *et al*., 2003). Consequently, the Toll-like receptor 2 activation turns on intracellular messengers such as MyD88, TRAF6 and MAP kinases which in turn will lead to the activation of transcription factors like NF-κB and AP-1, which are required for the expression of inflammatory cytokines (Buckley *et al*., 2006).

Similar research on the antibacterial assay on *Eurycoma longifolia* plant parts were conducted by Farouk & Benarfi (2007). However, Farouk & Benarfi (2007) concluded that the extracts from leaves and stems of *Eurycoma longifolia* contained potent antibacterial agent. In addition, it was reported that the root extract did not have any antibacterial agent. Another recent report by Tzar *et al*., (2011) indicated that the root extract of *Eurycoma longifolia* did not show any antibacterial or antifungal effect at concentrations of equal to or less than 50 mg/ml and 10 mg/ml. Later it was reviewed and concerns were raised as the root extracts did not show any antibacterial activity (Rajeev & Karim, 2010). Differences in these studies may be contributed by factors like age of the plant parts, used in this study, the mode of processing the samples to obtain the crude extract and the external factors like samples handling before and during the test was conducted.

Conclusion

There are a lot of factors that influences the antibacterial activity. The factors may include the age used for the preparation of the plant extract, type of the solvent used for extraction of the plant materials and the organism tested. Therefore, the result indicated that the most effective antibacterial agent is the extracted compound from the roots of *Eurycoma longifolia* on *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* (CDR), *Staphylococcus aureus* ATCC 25923 and *Shigella flexneri* ATCC 12022. In addition, there are also several other parts of this plants extract that showed good antibacterial activity against some tested pathogenic bacteria like crude extracts of stem core.

References

Alfredo, G.T. 2004. Current aspects of *Shigella* pathogenesis. In: *Revista Latinoamericana de Microbiología*, 46(3-4): 89-97.

Backman, P.A., M. Wilson and J.F. Murphy. 1997. Bacteria for biological control of plant diseases. In: *Environmentally Safe Approaches to Crop Disease Control*, un Rechigl and Rechigl, editions, CRC Press, Washington, USA, 95-109.

Bais, H.P., L.W. Tiffany, G.P. Laura, G. Simon and M.V. Jorge. 2006. The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. *Annu Rev Plant Biol*., 57: 233-266.

Bais, H.P., S.W. Park, T.L. Weir, R.M. Callaway and J.M. Vivanco. 2004. How plants communicate using the

underground information superhighway. *Trends Plant Sci.*, 9(1): 26-32.

Bais, H.P., V.V.M. Loyola, H.E. Flores and J.M. Vivanco. 2001. Root-specific metabolism: the biology and biochemistry of underground organs. *In vitro-Pl.*, 37(6): 730-741.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clinic Pathol.*, 36: 493-496.

Bertin, C., X.H. Yang and L.A. Weston. 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil*, 256: 67-83.

Bhavani, S.M. and C.H. Ballow. 2000. New agents for Gram-positive bacteria. *Curr. Opin. Microbiol*, 3: 528-534.

Bhunia, A.K. 2008. *Foodborne microbial pathogens*. In: *Springer*, New York. 135-147.

Buckley, J.M., J.H. Wang and H.P. Redmond. 2006. Cellular reprogramming by gram-positive bacteria components: a review. *J. Leukocyte Biol.*, 80(4): 731-741.

Cao, Y.H. and R.H. Cao. 1999 Angiogenesis inhibited by drinking tea. *Nature*, 6726: 381-398.

Chariandy, C.M., C.E. Seaforth, R.H. Phelps, G.V. Pollard and B.P. Khambay. 1999. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J. Ethnopharmacol.*, 64: 265-270.

Chastre, J. and J.L. Trouillet. 2000. Problem pathogens (*Pseudomonas aeruginosa* and *acinetobacter*). *Semin Respir Infec*, 15: 287-298.

Chastre, J. and J.Y. Fagon. 2002. Ventilator-associated pneumonia. *Am. J. Resp. Crit. Care Med.*, 165: 867-903.

Chitravadivu, C., S. Manian and K. Kalaichelvi. 2009. Antimicrobial Studies on Selected Medicinal Plants, Erode Region, Tamilnadu, India. *Middle-East J. Scient. Res.*, 4(3): 147-152.

Cladera, O.F., G.R. Caron and A. Brandelli. 2004. Bacteriocin-like substance production by *Bacillus licheniformis* strain P40. *Lett. Appl. Microbiol.*, 38(4): 251-256.

Cock, I.E. 2008. Antibacterial activity of selected Australian native plant extracts. *Internet J. Microbiol.*, 4(2): 1-8.

Cynthia, W., Z.K. Shinwari, I. Afzal, R.N. Malik. 2011. Antibacterial activity in herbal products used in Pakistan. *Pak. J. Bot.*, 43(1): 155-162.

Danilo, G.M., B. Isabella, S. Angela, M. Sara, R. Robert, N. Barbara, P. Ilaria, A. Vanja, C. Mariani, T. Giulia, C. Elena, S. Silvana, S. Maria, D. Ulrich, H. Jörg, T. Hervé, J.T. Luke, S. Steven, H. Lothar, E. Wielere Christa, P. Derek, D. Gordon, R.F. Maria, R. Rino, P. Mariagrazia and S. Laura. 2009. Identification of protective and broadly conserved vaccine antigens from the genome of extra-intestinal pathogenic *Escherichia coli*. *P. Natl. Acad. Sci.*, USAm 1-6.

Farouk, A.E. and A. Benafri. 2007. Antibacterial activity of *Eurycoma longifolia* Jack. A Malaysian medicinal plant. *Saudi Med. J.*, 28(9): 1422-1424.

Geleijnse, J.M., L.J. Launer, A. Hofman, H.A.P. Pols and J.C.M. Witteman. 1999. Tea flavonoids may protect against atherosclerosis – The Rotterdam study. *Arch. Intern. Med.*, 159: 2170-2174.

Gleba, D., N.V. Borisjuk, L.G. Borisjuk, R. Kneer, A. Poulev, M. Skarzhinskaya, D. Slavik, L. Sithes, Y.G. Yuri and R. Ilya. 1999. Use of plant roots for phytoremediation and molecular farming. *Proc. Natl. Acad. Sci.*, USA, 25: 5973-5977.

Granum, P.E. and T. Lund. 1997. *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiol. Lett.*, 157(2): 223-228.

Han, S.H., J.H. Kim, H.S. Seo, M.H. Martin, G.H. Chung, S.M. Michalek and H.N. Moon. 2006. Lipoteichoic acid-induced nitric oxide production depends on the activation of platelet activating factor receptor and Jak2. *J. Immunol*, 176(1): 573-579.

Han, S.H., J.H. Kim, M. Martin, S.M. Michalek and M.H. Nahm. 2003. Pneumococcal lipoteichoic acid (LTA) is not as potent as staphylococcal LTA in stimulating Toll-like receptor 2. *Infect Immunol*, 71(10): 5541-5548.

Ibrahim, J. 2004. *Medicinal Plant Research in Malaysia: Scientific Interests and Advances*. *J. Sains Kesihatan Malaysia*, 2(2): 27-46.

In-Cheol, Y., K.L. Nam, C. Chang-Jun and T.H. Young. 2011. Narrow antagonistic activity of antimicrobial peptide from *Bacillus subtilis* SCK-2 against *Bacillus cereus*. *J. Biosci. Bioeng*, (in press)

Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agri. Food Chem.*, 47: 3954-3962.

Karaman, I., F. Sahin, M. Gulluce, H. Ogutcu, M. Sengul and A. Adiguzel. 2003. Antimicrobial activity of aqueous and methanol extract of *Juniperus oxycedrus* L. *J. Ethnopharmacol*, 85: 231-235.

Kipnis, E., T. Sawa and J.W. Kronish. 2006. Targeting mechanisms of *Pseudomonas aeruginosa* pathogenesis. *Médecine et maladies infectieuses*, 36:78-91.

Kortiranta, A., K. Lounatmaa and M. Haapasalo. 2000. Epidemiology and pathogenesis of *Bacillus cereus* infections. *Microb. Infect.*, 2(2): 189-198.

Kotloff, K.L., J.P. Winickoff, B. Ivanoff, J.D. Clemens, D.L. Swerdlow, J .Sansonetti, G.K. Adak and M.M. Levine. 1999. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *B World Health Organ*, 77: 651-666.

Lebleau, N., C. Roquesb, P. Aimara and C. Causseranda. 2009. Role of the cell-wall structure in the retention of bacteria by microfiltration membranes. *J. Membrane Sci.*, 326: 178-185.

Madsen, H.L. and G. Bertelsen. 1995. Spices as antioxidants. *Trends Food Sci. Tech.*, 6: 271-277.

Mehmooda, M., R. Qureshi, M. Arshad and M. Gulfaraz. 2012. Antibacterial activity of root and fruit extracts of *leptadeniaPyrotechnica* (Asclepiadaceae) from Pakistan. *Pak. J. Bot.*, 44(4): 1209-1213.

Miller, J.M., J.G. Hair, M. Hebert, L. Heber, F.J. Robert and R.S. Weyant. 1997. Fulminating bacteremia and pneumonia due to *Bacillus cereus*. *J. Clin. Microbiol.*, 35(2): 504-507.

Morrison, D.C. and J.L. Ryan. 1987. Endotoxins and disease mechanisms. *Ann. Rev. Med.*, 38: 417-32.

Perez, A.R., M.A. Abanes-De and K. Pogliano. 2000. SpoIIB localizes to active sites of septal biogenesis and spatially regulates septal thinning during engulfment in *Bacillus subtilis*. *J. Bacteriol.*, 182(4): 1096-1108.

Raaijmakers, J.M., I. De. Bruijn, O. Nybroe and M. Ongena. 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS. Microbiol. Rev.*, 34(6): 1037-1062.

Rachel, J.G. and D.L. Franklin. 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. *Clin. Infect Dis.*, 46(5): 350-359.

Rajeev, B. and A.A. Karim. 2010. Tongkat Ali (*Eurycoma longifolia* Jack): A review on its ethnobotany and pharmacological importance. *Fitoterapia*, 81: 669-679.

Rodríguez, V.M.J., S.L.R. Tomassini, N.M.C. Manca and S.A.M. Strasser. 2010. Antioxidant capacity and antibacterial activity of phenolic compounds from Argentinean herbs infusions. *Food Control*, 21: 779-785.

Ryan, K.J. and S. Falkow. 1994. Enterobacteriaceae. In: *Microbiology. An introduction to infectious diseases*, (Ed.): K.J. Ryan, Medical 3rd edition, Appleton and Lange, Norwalk, Connecticut, 328-332.

Sadr, G.S.B., S.S. Madaeni, A.G. Fane and R.P. Schneider. 1999. Aspects of microfiltration and reverse osmosis in municipal wastewater reuse. *Desalination,* 106: 25-29.

Sahgal, G., S. Ramanathan, S. Sasidharan, M.N. Mordi, S. Ismail and S.M. Mansor. 2009. Phytochemical and antimicrobial activity of *Swietenia mahagoni* crude methanolic seed extract. *Trop. Biomed*, 26(3): 274-279.

Stein, T. 2005. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Mole Microbiol.*, 56(4): 845-857.

Tariq, H., M. Arshad, S. Khan, H. Sattar and M.S. Qureshi. 2011. *In vitro* screening of methanol plant extracts for their antibacterial activity. *Pak. J. Bot.*, 43(1): 531-538.

Tzar, M., Y. Hamidah, S. Hartini, M. Marianayati and A. Nazrun. 2011. The antibacterial or antifungal effects of *Eurycoma longifolia* root extract. *Int. J. Herbal Plant Med.*, 1(1): ISSN 2158-0413.

Uren, N.C. 2000. Types, amounts and possible functions of compounds released into the rhizosphere by soil grown plants. In: *The rhizosphere: biochemistry and organic substances at the soil interface edition*, New York. 19-40.

Veckman, V., M. Miettinen, J. Pirhonen, J. Siren, S. Matikainen and I. Julkunen. 2004. *Streptococcus* pyogenes and *Lactobacillus rhamnosus* differentially induce maturation and production of Th1-type cytokines and chemokines in human monocyte-derived dendritic cells. *J. Leukocyte Biol.*, 75(5): 764-771.

Yao, X., M. Jericho, D. Pink and T. Beveridge. 1999. Thickness and elasticity of gram negative murein sacculi measured by atomic force microscopy. *J. Bacteriol.*, 181(22): 6865-6875.

Yen, G.C., P.D. Duh and H.L. Tsai. 2002. Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chem.*, 79: 307-313.

Young, H.R., E.B. Jung, S.Y. Jae, S.K. Seok, I. Jintaek, H.Y. Cheol, W.K. Dong, K.C. Kangseok Lee, Dae, R.J. Hyang and H.H. Seung. 2009. Differential immunostimulatory effects of Gram-positive bacteria due to their lipoteichoic acids. *Internat Immunopharmacol*, 9: 127-133.

Zakrzewski, P.A. 2002. Bioprospecting or biopiracy? The pharmaceutical industry's use of indigenous medicinal plants as a source of potential drug candidates. *Univ. Toronto Med. J.*, 79(3): 252-254.

Zdenek, Z., F. Hassan and K. Eva. 2010. Intrinsic nitric oxide-stimulatory activity of lipoteichoic acids from different Gram-positive bacteria. *Nitric Oxide*, 23: 300-310.

(Received for publication 15 April 2012)