

In Vitro Activity of Pandan (*Pandanus amaryllifolius*) Leaves Crude Extract Against Selected Bacterial Isolates

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Abstract - Nowadays, there is a demand for novel drugs to prevent these infections and the emergence from mutation of microorganisms. Given the rising incidence of resistance to synthetic antibiotics and in light of the rising costs of medicines it is well-timed to search for natural products such as plant derived antimicrobial drugs to reduce the resistance of microorganisms. Pandan (*Pandanus amaryllifolius*), in addition to synthetic alternatives, has the potential of antibacterial activity. The antibacterial properties of pandan crude extract however are not yet scientifically established. For that reason, the research proponents of this study aims to assess the antibacterial properties of pandan with the end view of providing low cost of medications and the prevention of resistance. This

research analyzed the in vitro activity of pandan leaves crude extract against bacterial isolates such as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. About 1 kilogram of freshly collected pandan leaves was subjected to water distillation and the filtrate was concentrated using rotary evaporator. The crude extract was then used for the phytochemical analysis. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of pandan against the said microorganisms were examined. This study also determined the stability of pandan as to pH and temperature.

Keywords - Pandan, *Pandanus amaryllifolius*, bacterial isolates

INTRODUCTION

Microbiology is the study of very small living microorganisms which are called microorganisms or microbes. The various categories of microorganisms include viruses, bacteria, archaeans, some algae, protozoa and some fungi. These are ubiquitous in nature, and found everywhere that live on and in various parts of our body are called our indigenous microflora. Opportunistic pathogens do not cause disease under ordinary circumstances; however, they have the potential to cause disease if they gain access to the wrong place at the wrong time (Engelkirk and Burton, 2007).

Bacteria are extremely small, prokaryotic microorganisms. Etiologic bacterial agents such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* are the common cause of nosocomial infections (Engelkirk and Burton, 2007). These infections include urinary tract infections (35%), the most prevalent type of nosocomial infection; surgical-incision-site infections (20%); lower respiratory tract infection including pneumonia (15%) and bloodstream infections (15%) in the USA. Studies show that 70% of bacteria that cause nosocomial infections are resistant to at least one antibiotic commonly used to treat

them. The increased incidence of resistance is a common concern in health care. According to the CDC's National Nosocomial Infection Surveillance System (NNIS System), multidrug-resistant pathogens have become increasingly problematic in recent years, especially in the critical care setting (Pharmacytimes [Online], 2009).

Etiologic bacterial agents such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* are the common cause of nosocomial infections (Engelkirk and Burton, 2007). These infections include urinary-tract infections, the most prevalent type of nosocomial infection, account for 35%; surgical-incision-site infections account for 20%; lower respiratory tract infections including pneumonia that accounts for 15% and bloodstream infections account for another 15% the USA. Studies show that 70% of bacteria that cause nosocomial infections are resistant to at least one antibiotic commonly used to treat them. The increased incidence of resistance is a common concern in health care. According to the CDC's National Nosocomial Infection Surveillance System (NNIS System), multidrug-resistant pathogens have become increasingly problematic in recent years, especially in the critical care setting (Pharmacytimes [Online], 2009).

Staphylococcus aureus normally resides in the nose, but also the throat, armpit, groin and intestinal tract. It can also cause toxin-mediated disease, such as toxic shock syndrome associated with surgery or tampon use, acute-onset food poisoning and scalded skin syndrome in babies (Lindsay, 2008). *S. aureus* is the most common cause of hospital acquired infection. *S. aureus* enter at surgical incision sites or at vascular access sites such as catheters and injection sites. Patients who are immunocompromised and elderly are at most risk, probably as they have greater trouble mounting a routine immune response to *S. aureus*. However, young and healthy patients can also be affected. The bacteria often cause localized infections affecting multiple tissues such as blood (bacteremia), heart (endocarditis), lungs (pneumonia), muscle (abscess), bone (osteomyelitis), eyes (conjunctivitis), and joints (bacterial arthritis). The infection may develop rapidly (acute) or may be long and drawn out (chronic); most will be minor others will be severe, even resulting in death. Some infections do not require treatment, others respond well to treatment, and some respond poorly to treatment (Lindsay, 2008).

In the last 15 years, situation in hospitals has changed quite dramatically due to the evolution of *S. aureus* that are resistant to the most useful antibiotics, the beta-lactamase resistant penicillins such as flucloxacillin, oxacillin, and methicillin. This methicillin-resistant *S. aureus* (MRSA) account for 40-80% of all hospital; in some countries. The only remaining class of antibiotics that are *S. aureus* is resistant to are the glycopeptides such as vancomycin. These antibiotics are more expensive, must be administered intravenously requiring patient hospitalization, have more side-effects and are less effective. However the first fully resistant VRSA are now being described. New antibiotics have recently licensed, but they are significantly more expensive and resistance is already reported (Lindsay, 2008).

In 1885, Theodore Escherich first described *Escherichia coli* that was initially considered a harmless member of the colon resident flora. *E. coli* are the significant species in the genus *Escherichia*, a gram negative bacillus that is used as a primary marker of fecal contamination in water purification. *E. coli* is widely recognized as the most common cause of UTIs in humans that usually originate in the large intestine as resident or transient flora and may exist either as the predominant *E. coli* population or as a small part of the *E. coli* strains in the large intestine. Conversely, isolates from immunocompromised hosts consist of a wide variety of strains (Mahon et al., 2007).

Forbes et al. (2007) cited the several different gastrointestinal syndromes caused by *E. coli*. These are categorized as enterotoxigenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC) and enteroadherent, which includes the diffusely adherent (DAEC) and enteroaggregative (EaggEC) strains. These are associated with different gastrointestinal diseases.

Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *E. coli* vary widely. As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms. Antibiotics which may be used to treat *E. coli* infection include amoxicillin as well as other semi-synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and the aminoglycosides. Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as

S. aureus. *E. coli* often carry multidrug resistant plasmids and under stress readily transfer those plasmids to other species. Indeed, *E. coli* is a frequent member of biofilms, where many species of bacteria exist in close proximity to each other. This mixing of species allows *E. coli* strains that are pilated to accept and transfer plasmids from and to other bacteria. Thus *E. coli* and the other enterobacteria are important reservoirs of transferable antibiotic resistance (Wikipedia [Online], 2009).

Pseudomonas aeruginosa is the most commonly encountered gram-negative species that is not a member of the family Enterobacteriaceae. This organism survives in various environments in nature and in homes and hospitals (Forbes et al., 2007). It is also inherently resistant to a number of antimicrobial agents. A large variety of clinical diseases have been documented as caused by *Pseudomonas aeruginosa*, including bacteremia, often presenting with ecthyma gangrenosum of the skin; wound infection; pulmonary disease, especially among individuals with cystic fibrosis; nosocomial urinary tract infections; endocarditis; infections following burns or trauma; and, in rare cases; central nervous system infections, including meningitis (Mahon et al., 2007). *P. aeruginosa* is intrinsically resistant to various antimicrobial agents. However, *P. aeruginosa* readily acquires resistance to the potentially active agents. Antimicrobial agents used for *P. aeruginosa* are often considered for use against the other species; however, before proceeding with the development of treatment strategies, the first critical step should be to establish the clinical significance of the organism (Forbes et al., 2007).

Medical intervention in an infection primarily involves attempts to eradicate the infecting pathogen using substances that actively inhibit or kill it. Depending on the type of organisms targeted, these substances are also known as antibacterial, antifungal, antiparasitic, or antiviral agents. Because antimicrobial agents play a central role in the control and management of infectious diseases, some understanding of their mode of action and the mechanisms of microorganisms deployed to circumvent antimicrobial activity is important, especially because diagnostic laboratories are expected to design and implement tests that measure a pathogen's response to antimicrobial activity (Forbes et al., 2007).

Antimicrobial agents are antibiotics originated from microorganisms. Therefore, antibiotic resistance mechanisms always have been part of the evolution of bacteria as a means of survival among antibiotic-producing competitors. However, with the introduction of antibiotics into medical practice, clinically relevant bacteria have had to adopt resistance mechanisms as part of their survival strategy. With our use of antimicrobial agents, a survival of the fittest strategy has been used by bacteria to adapt to the pressures of antimicrobial attack (Forbes et al., 2007).

Over the centuries, man made use of medicinal plants thus unable to find a rational explanation for their effects. It was not until the 19th century and the rapid development of organic chemistry and pharmacology, that man determined which active principles of group of principles are responsible for a given therapeutic effect (Ciocan and Bara, 2007). Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is twofold in the development of new drugs: first: they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or; second: a phytomedicine to be used for the treatment of disease.

The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Plants produce a diverse array of secondary metabolites, many of which have antimicrobial activity. Some of these compounds are constitutive, existing in healthy plants in their biologically active forms. Others such as cyanogenic glycosides and glucosinolates, occur as inactive precursors and are activated in response to tissue damage or pathogen attack.

Worldwide, there has been a renewed interest in natural products. The potential for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phytomedicines. The immediate source of financial benefit from plants based antimicrobials is from the herbal products market. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large

contributions to human health and well-being. Their role is twofold in the development of new drugs: first: they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or; second: a phytomedicine to be used for the treatment of disease. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Ciocan and Bara, 2007).

Given the rising incidence of resistance to synthetic antibiotics and in light of the rising costs of medicines coupled with the lack of scientific data on the antibacterial properties of *P. amaryllifolius*, the research proponents of this study aims to assess the antibacterial properties of pandan with the end view of providing low cost of medications and the prevention of resistance.

The pandan known as *Pandanus amaryllifolius* is one of such plants with promising antibacterial potential. It is an evergreen perennial aromatic plant, a cultivated plant now found worldwide due to importation and human migration (Igardendigest [Online], 2009). It is also a tropical plant in the screw pine genus which is known commonly as pandan and used widely in Southeast Asian cooking (Wikipedia [Online], 2009). It contains an aromatic volatile oil that is being used in other countries as a purgative, cardiogenic and cephalic (for headaches) as well as for wounds, with one writer claiming that the oil has antiseptic properties comparable to that of eucalyptus oil. The Philippine National Formulary suggests the use of the roots in decoction as a diuretic besides for headache and rheumatism (Tan, 1980).

Studies on pandan-derived compounds and fractions revealed its antioxidant potential (Fatihanim et al., 2007) and anticancer activity (Kumar et al., 2007). Various pandan species also demonstrate selective antibacterial and antiviral activity (Tan et al., 2007; Ooi et al., 2005; Ooi et al., 2004). It also has potential as a pest management tool (Ho and Li, 2003). The antibacterial properties however of *P. amaryllifolius* are not yet scientifically established particularly on its crude extract.

However, only one percent of the known species were chemically investigated. The Pandanaceae is commonly focused on their traditional uses e.g. in cooking and for some medicinal purposes (Takayama et al., 2000 as cited by Mohsin et al., 2008).

The botanical name of this plant is *Pandanus amaryllifolius* Roxb.

(Igardendigest [Online], 2009). This is synonym to *Pandanus hasskarlii* Merr., *Pandanus latifolius* Hask., *Pandanus odoratus* Ridley. The etymologies of this plant are fragrant pandan, fragrant screw pine, pandan; Hindi: flower: kewra, keora; Indonesian: pandan, pandan wangi, pandan rampe, Malaysian: daun pandan, pandan, pandan rampai, pandan wangi; Pilipino: pandan, pandan mabango, Thai: toey hom, panae-wonging; and Vietnamese: ladua (Seidemann, 2005).



Figure 1. Pandan (*Pandanus amaryllifolius*) plant

Pandang mabango (*Pandanus amaryllifolius* Roxb.) resembles pinya (pineapple) more than it resembles pandan (which is a tree). Pandang mabango, as its name implies, contains an aromatic volatile oil. The oil is used in other countries as a purgative, cardiotoxic and cephalic (for headaches) as well as for wounds, with one writer claiming that the oil has antiseptic properties comparable to that of eucalyptus oil. The Philippine National Formulary suggests the use of the roots in decoction as a diuretic. For headache and rheumatism, the leaves can be crushed, mixed with oil and applied on the affected areas. Either pandan or pandang mabango can be used (Tan, 1980).

According to Li and Ho (2003), an analysis of the volatile essential oils of *P. amaryllifolius* by MacLeod and Pieris (1982) revealed that this species contains mainly terpenes and sesquiterpene hydrocarbons (6-42%), as well as a major aroma component – 2-acetyl-1-pyrroline (2AP) (Yoshihashi, 2002). The presence of essential oils, i.e. mixtures consisting predominantly of mono- and sesquiterpene derivatives,

accounts for the insect-repellent and attractant properties associated with some aromatic plants (Herout, 1970; Rice, 1983). Previous studies have established significant repellent activity of *P. amaryllifolius* against American cockroaches (*Periplaneta americana* L.) (Ahmad et al, 1995), but similar effects against other species of cockroaches have not yet been looked into.

Mohsin et al. (2008) reported the Pandanaceae research in support for industrial plantation. In the said study, they have included conservation proposals, phytochemical work, chemosynthetic, bioinformatic, biogenetic and taxonomic researches, mycological, zoological and entomology observation plus ethnobotany and social studies. They have reported the procedure of both neutral and acid-base extractions of leaves sample from *Pandanus* species. From the sample of *P. sanderi* roots added with non-aqueous solvents such as hexane, chloroform and methanol, consecutively, then subjected to High Performance Liquid Chromatography (HPLC), important signals which corresponded to a number of phytochemical compounds, were observed.

The study of Fatihanim et al. (2007) evaluated that *Pandanus amaryllifolius* leaf extract as a natural antioxidant in refined, bleached and deodorized (RBD) palm olein, using accelerated oxidation and deep frying studies at 180°C from 0 to 40 h. In sensory evaluation studies, different batches of French fries were not significantly different from one another for oiliness, crispiness, taste and overall acceptability when the same oil was used for up to the 40th hour of frying. The study reported that *P. amaryllifolius* leaf extract had polyphenol content and exhibits an excellent heat-stable antioxidant property and may be a good natural alternative to existing synthetic antioxidants in the food industry.

Kumar et al. (2007) conducted an experiment to the anticancer activity of ethanol extract of *Pandanus fascicularis* Lam. (EPF) in mice. It was evaluated against Ehrlich Ascites Carcinoma (EAC) tumor model on dose dependent manner. The activity was assessed using survival time, average increase in body weight, hematological parameters and solid tumor volume. Oral administration of EPF increased the survival time and decreased the average body weight of the tumor bearing mice. After 14 days, EPF is able to reverse the changes in the hematological

parameters, protein and PCV consequent to tumor inoculation. By this, it was stated that the oral administration of EPF was effective in reducing solid tumor mass development induced by EAC cells. The results shows EPF possess significant anticancer activity on dose dependent manner.

Tan et al. (2007) assessed the chloroform extract of *Pandanus tectorius* Soland. Var *laevis* leaves having essential chemical constituents mixed by phytosterols, stigmasterol and beta-sitosterol. In the said experiment, the Microplate Alamar Blue Assay showed inhibitory activity against *Mycobacterium tuberculosis*. The isolation of new tirucallene-triterpene squalene, and a mixture of sterols and their antitubercular activity was reported in the said study.

The research of Ooi et al. (2005) focused the purification and characterization of non-specific lipid transfer proteins from the saline extract of mature leaves of *Pandanus amaryllifolius*. The saline extract was isolated using affinity chromatography on fetuin-agarose and Affigel Blue gel, anion exchange chromatography as well as gel filtration. The proteins were demonstrated as non-glycoproteins. All of them have similar N-terminal amino acid sequences with only minor variations and are matched to non-specific lipid transfer proteins (nsLTPs) of the other plants such as wheat LTP using NCBI Blast searching for short, nearly exact matches.

Ooi et al. (2004) studied the antiviral activities of a lectin, designated Pandanin from the saline extract of the leaves of *Pandanus amaryllifolius*. Pandanin was isolated using ammonium sulfate precipitation, affinity chromatography on mannose-agarose and molecular size exclusion by gel filtration.

Ho and Li (2003) studied about seven compounds and fractions prepared from leaves of *P. amaryllifolius*. The leaves were evaluated for repellent activity against *Blattella germanica* using a modification of the linear tract olfactometer. The study showed that Pandan essence and the hexane-pandan extract and the acetone-pandan extract was attractive at increasing concentrations minimum of 62 % attractancy; artificial pandan flavouring and the dichloromethane-pandan extract gave erratic results. The undiluted crude aqueous pandan extract displayed an attractancy of 62%. This study discussed the potency of *P. amaryllifolius* as a natural and environmentally friendly pest management tool.

OBJECTIVES OF THE STUDY

This research aims to assess the antibacterial properties of pandan. Moreover, the study intends to determine the chemical compounds present in pandan through phyto-chemical analysis, as well as to describe the in vitro activity, minimum inhibitory concentration, minimum bacterial concentration, and stability as to pH and temperature of pandan crude extract.

MATERIALS AND METHODS

This research study analyzed the in vitro activity of pandan (*Pandanus amaryllifolius*) leaves crude extract against bacterial isolates such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

About 1 kilogram of fresh pandan leaves collected from Pinagtungulan, San Jose, Batangas was subjected to water distillation. The crude extract that was collected using rotary evaporator was subjected to phytochemical analysis. The biological activity of pandan leaves crude extract to the test microorganisms was determined using the paper disc diffusion method. In this method, different concentration of oil extract from 1.25 % to 40% were incorporated onto 6 mm paper discs were gently placed on the seeded assay plates and the zone of inhibition was measured after incubation. The before proceeding to the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of pandan against the said microorganisms were also examined. This study also determined the stability of pandan to varying pH of 2, 4, 6 and 8 and at varying temperatures of 40°C, 50°C, 80°C and 100°C

RESULTS AND DISCUSSION

Phytochemical Analysis

The researchers conducted the study of phytochemical analysis of pandan crude extract to determine the chemical compounds present in the said plant. The researchers performed the phytochemical analysis

of the pandan at Department of Science and Technology, analysis of pandan includes the screening alkaloids, saponins, flavinoids and tannins. The results of the experiments from the different tests are shown in Table1.

Table 1. Phytochemical Analysis of Pandan Crude Extract

Tests	Results	Indication
1. Mayer's Test	Formation of white precipitate	Presence of alkaloids
2. Dragendorff's Test	Formation of orange precipitate	Presence of alkaloids
3. Dragendorff's and Mayer's Tests	Formation of orange precipitate with Dragendorff's and Mayer's reagents respectively	Presence of quaternary bases and/or amine oxides
4. Froth test	did not form honeycomb froth greater than 3 cm	
5. Sodium carbonate Test	Formation of dense and stable froth	Absence of saponins
6. Keller Killiani Test	Formation of reddish-brown color	Presence of free fatty acids
7. Kedde Test	Formation of Blue-violet color	Presence of 2-deoxysugars
8. Bate-Smith & Metcalf	Did not form red or violet color	Presence of unsaturated lactones
9. Wilstatter "Cyanidin" Test	Formation of orange color	Absence of Leucoanthocyanin
10.Ferric Chloride Test	Formation of brownish-green precipitate	Presence of flavonoids
11.Modified Borntrager	No reaction	Presence of tannins
12.Spot test	Formation of greasy/oily appearance	Absence of anthraquinone Presence of fats and oils
13.Liebermann-Burchard Test	Formation of green color	Presence of steroids

Table 1 presents the phytochemical analysis of pandan. The chemical compounds present in the pandan crude extract are alkaloids, quaternary bases and/or amine oxides, free fatty acids, 2-deoxysugars, unsaturated lactones, flavonoids, fats and oils, and steroids. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being (Ciocan and Bara, 2007). These results indicate

that pandan leaves crude extract contained phytochemical compounds owing to be an effective alternative antibacterial agent that will reduce the occurrence of the ever increasing antibiotic resistance and other life threatening diseases.

In vitro Activity of Pandan crude extract

The in vitro activity of pandan crude extract against the bacterial isolates such as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 is determined consecutively to 5%, 10%, 20% and 40 % of pandan crude extract which were incorporated in 6mm paper disc and tested using paper disc diffusion method. The results of the experiments are presented in the following tables and figures.

Table 2. In vitro Activity of Varying Concentration of Pandan Crude Extract Suspension against *Staphylococcus aureus* ATCC 25923

Varying Concentration of Pandan crude extract	Mean Zone of Inhibition*	Interpretation
5%	6 mm	Inactive
10%	6 mm	Inactive
20%	6 mm	Inactive
40%	13 mm	Partially active

* assays performed in triplicates

Table 2 presents the in vitro activity of varying concentration of pandan crude extract against *Staphylococcus aureus* ATCC 25923. The lowest concentration of pandan crude extract which is 5% suspension has no in vitro activity against *S. aureus* as indicated by its 6mm mean of inhibition against the said microorganism. This indicates that the said concentration is not suitable as a starting concentration for the serial dilution of pandan for MIC and MBC determination as it yielded a very minimal zone of inhibition. Correspondingly, regardless of the two-fold increase in concentration, 10% pandan suspension and

three-fold concentration which is 20% pandan suspension are yet still have no in vitro activity by 6mm mean of inhibition against the said microorganism. The said concentrations of pandan are maybe too low to produce marked zones of inhibition against the *S. aureus*. Pandan crude extract however in its pure concentration, 40% suspension has a partially active in vitro activity against *S. aureus* as shown by its 13mm mean zone of inhibition. This is the greatest mean of inhibition of pandan crude extract indicating that that it has in vitro activity against the said test isolate which is the most common cause of hospital acquired infection (Lindsay, 2008). Thus this result entails the antibacterial potential of pandan crude extract as an additional basis to one percent of the known species that were chemically investigated (Takayama et al., 2000 as cited by Mohsin et al., 2008). *Pandanus amaryllifolius* contains an aromatic volatile oil. The oil is used in other countries as a purgative, cardiogenic and cephalic (for headaches) as well as for wounds, with one writer claiming that the oil has antiseptic properties comparable to that of eucalyptus oil (Tan, 1980). Since *S. aureus* is a common cause of minor skin infections, particularly when introduced into a wound or skin incision (Lindsay, 2008), this may also simplify the susceptibility of pandan crude extract.

Table 3. In vitro activity of varying concentration of pandan crude extract suspension against *Escherichia coli* ATCC 25922

Varying Concentration of Pandan crude extract	Mean Zone of Inhibition*	Interpretation
5%	6 mm	Inactive
10%	6 mm	Inactive
20%	6 mm	Inactive
40%	6 mm	Inactive

* assays performed in triplicates

Table 3 shows the in vitro activity of varying concentration of pandan crude extract against *Escherichia coli* ATCC 25922. The least concentration of pandan crude extract which is 5% suspension has no in

in vitro activity against *E. coli* as indicated by its 6mm mean of inhibition against the said microorganism. This implies that 5 % pandan crude extract concentration is inappropriate as starting concentration for the serial dilution pandan crude extract of for MIC and MBC determination as it yielded no zone of inhibition. Respectively, the increasing concentrations such as 10%, 20% and 40% of pandan suspension as well have no in vitro activity by 6mm mean of inhibition against the said microorganism. Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *E. coli* vary widely. As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms (Wikipedia [Online], 2009). The concentration of pandan crude extract maybe too low to produce marked zones of inhibition against the test isolate.

Table 4. In vitro Activity of Varying Concentration of Pandan Crude Extract Suspension against *Pseudomonas aeruginosa* ATCC 27853

Varying Concentration of Pandan crude extract	Mean Zone of Inhibition*	Interpretation
5%	6 mm	Inactive
10%	6 mm	Inactive
20%	6 mm	Inactive
40%	6 mm	Inactive

* assays performed in triplicates

Table 4 presents the in vitro activity of varying concentration of pandan crude extract against *Pseudomonas aeruginosa* ATCC 27853. Similarly to *E. coli*, varying concentration of pandan suspension such as 5%, 10%, 20% and 40 % have no in vitro activity by 6mm mean of inhibition against the *P. aeruginosa*. All suspension are unseemly starting concentration for the serial dilution pandan crude extract of for MIC and MBC determination as it yielded a minimal zone of inhibition. An important note about the ubiquitous nature of *P. aeruginosa* is the

leading cause of nosocomial respiratory tract infections however the said isolate is also intrinsically resistant to various antimicrobial agents (Forbes et al., 2007). The concentration of pandan crude extract maybe too low to produce marked zones of inhibition against the test isolate.

Minimum Inhibitory Concentration of Pandan Crude Extract

The extent of the in vitro activity of pandan crude extract against bacterial isolates showing at least a partially active activity is confined by the determination of minimum inhibitory concentration against *S. aureus*. The results of the experimentation are indicated in the succeeding tables and figures.

Table 5. MIC in tubes of Pandan Crude Extract against *Staphylococcus aureus* ATCC 25923

Tube Number	Pandan Concentration(%)	Result*
1	40%	Turbid
2	20%	Turbid
3	10%	Turbid
4	5%	Turbid
5	2.5%	Turbid
6	1.25%	Turbid
7	0.63%	Turbid
8	0.31%	Turbid
9	0.16%	Turbid
10	0.08%	Turbid

* assays performed in triplicates

Table 5 presents the MIC of pandan crude extract against *S. aureus* resulted as partially active in vitro activity in the screening for its in vitro activity. The presence of turbidity in the tubes showed growth. From the table, turbidity was observed from all tubes. The results indicate that the pandan crude extract has a minimum inhibitory concentration of between 20% - 40% and cannot inhibit the growth of *S. aureus* at a concentration below 40%. Results of this study indicated

by the presence of turbidity are due to the consistency of pandan or the bacterial growth. The growth was confirmed through streaking in the assay plates with results in tabulated form below.

Table 6. MIC in plates of Pandan Crude Extract against *Staphylococcus. aureus* ATCC 25923

Plate Number	Pandan Concentration(%)	Result*
1	40%	No growth
2	20%	With growth
3	10%	With growth
4	5%	With growth
5	2.5%	With growth
6	1.25%	With growth
7	0.63%	With growth
8	0.31%	With growth
9	0.16%	With growth
10	0.08%	With growth

* assays performed in triplicates

Table 6 and figure 5 present the MIC of pandan suspension in assay plates. It can be seen from the table and figure that no growth was observed at tube 1 with 40% pandan suspension. Growth however was observed from the rest of the tubes. These findings indicate that the minimum inhibitory concentration of pandan that can inhibit the growth of *S. aureus* is between 20% to 40% concentration. The said test isolate is the most common cause of hospital acquired infection (Lindsay, 2008), this may explain its susceptibility to pandan.

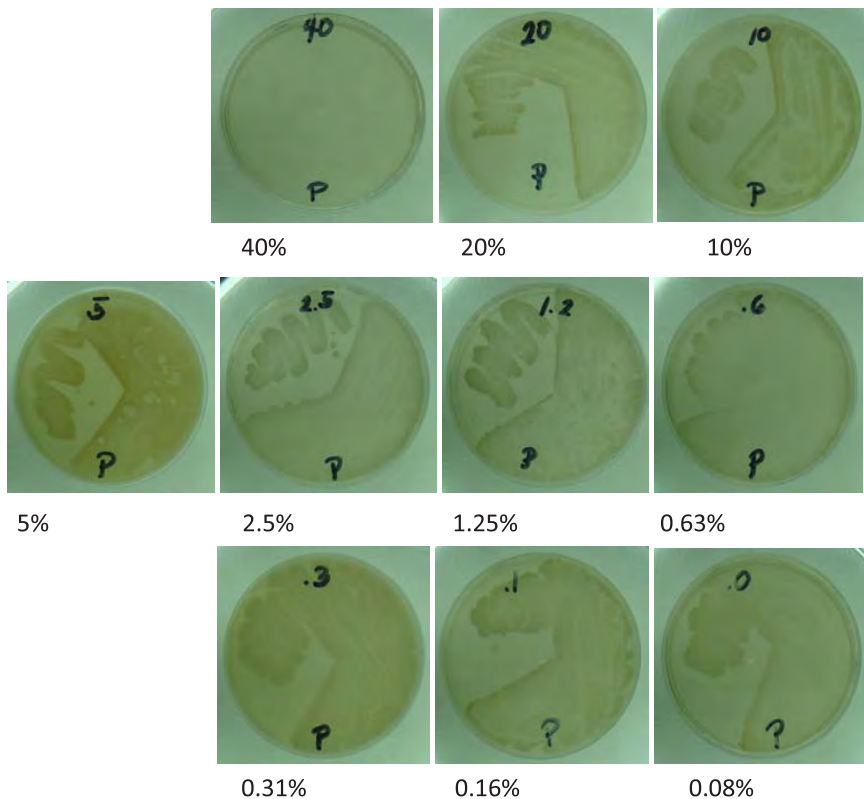


Figure 5. MIC of pandan crude extract against *Staphylococcus aureus* ATCC 25923

Minimum Bactericidal Concentration of Pandan Crude extract

Pandan crude extract killing activity against bacterial isolates particularly *S.aureus* is determined by minimum bacterial concentration. Tubes from the MIC determination resulting to the absence of growth were plated in agar plates and incubated at 37oC. The results of the experiments are shown in the succeeding tables.

Table 7. MBC of 40% Pandan Crude Extract against *Staphylococcus aureus* ATCC 25923

Tube Number	Pandan Concentration(%)	Result*
1	40%	No growth

This table 7 shows the MBC of 40% pandan crude extract against *S. aureus*. It can be seen from the table and figures were observed that there is no growth at tube 1, 40%, and growths were observed to the rest of the tubes. These findings revealed that the bacterial concentration of pandan crude extract is greater at concentration of 40%. Pandan crude extract can inhibit the growth of *S. aureus* resulted from the biological screening and can kill the said bacterial isolate. The study implied that the use of pure concentration may result in inhibition and in killing of the infecting organism. The pandan crude extracts concentration therefore of 20% or less maybe low to exert a bacterial effect.

Stability of Pandan Crude Extract as to pH

In addition to, the researchers also obtained the effect of varying pH on the antibacterial activity of pandan. A control of pH at room temperature was used to validate the results. The results of the experimentation are shown in the succeeding figures and table.

Table 8. Stability of 40% pandan crude extract in varying pH

pH	<i>S. aureus</i> *
5.4	No growth
2	No growth
4	No growth
6	With growth
8	With growth

* assays performed in triplicates

Table 8 shows the stability of 40% pandan crude extract in terms of pH. It can be seen from the table and figures, no growth was observed in pH 2.0 and 4.0 and growth was noted in pH 6.0 and 8.0. The actual pH of pandan crude extract at room temperature is 5.4 prior to the alteration of its pH. This indicates that the antibacterial activity of pandan was affected with varying pH. Most microorganisms prefer a neutral or slightly alkaline growth medium (Engelkirk and Burton, 2007). These results suggested that the effectiveness of pandan crude extract has a greater antibacterial activity at a lower pH.

Stability of Pandan Crude Extract as to Temperature

The researchers obtained to study the stability of pandan if subjected to varying temperature. This procedure was considered to determine the ability of pandan to preserve its antibacterial activity if introduced to varying temperatures. A control at room temperature was used to validate the results. The results of the experimentation are presented in the table below.

Table 8. Stability of 40% pandan crude extract in varying temperatures

Temperature	<i>S. aureus</i> *
RT	No growth
40	With growth
50	With growth
80	With growth
100	With growth

* assays performed in triplicates

Table 8 presents the stability of 40 % pandan crude extract in terms of temperature. From the table and figure, growth was observed in all tubes resulting to the varying concentration of pandan. The control at room temperature produced acceptable results. This indicates that the

antibacterial activity of pandan is affected with varying temperatures specifically at 40oC, 50oC, 80oC and 100oC. These results implied that the effectiveness of pandan crude extract is unstable and altered its bacterial action at varying temperatures.

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