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Effects of *Pleurotus ostreatus* extract against biofilms caused by *Stenotrophomonas maltophilia*

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Abstract
This study aims to determine the effects of *Pleurotus ostreatus* extract on Biofilms caused by *Stenotrophomonas maltophilia* and its significance on susceptibility and motility. Ethyl acetate method was performed for extraction procedure. Six (6) groups were assigned as positive group, negative group, and experimental group. Bacterial suspension was prepared on BHI medium. Experimental group were treated with different concentrations of the extract, Sulfamethoxazole (SXT) was used for the positive group and the negative group remained untreated. In the Biofilm assay, samples were dispensed on the wells of microtiter plates and was incubated then stained with crystal violet. In Motility assay, 1% of MHA was prepared, samples were dispensed on the surface of the 1% MHA for swimming motility. While the samples were pin inoculated at the bottom of the twitching plates. After 24 hours of incubation, growth was measured for swimming, and twitching plates were stained with Coomassie brilliant blue. For Susceptibility testing, the bacteria were inoculated on MHA, 10 ul of the extract and SXT were then dispensed onto the agar well. After 24 hours of incubation, zone of inhibition was measured. Results showed that *Pleurotus ostreatus* extract could not inhibit the biofilms, motility and multi-drug resistance. It was then concluded that *Pleurotus ostreatus* was considered as not effective in modulation of biofilms, motility and susceptibility of *Stenotrophomonas maltophilia*.

Keywords: analgesic, *Diplazium esculentum*, flavonoids, pteridophytes, aspirin

Introduction
Philippines is a tropical country with great biodiversity reason why many plants were discovered and cultivated until today. In ancient times, Filipinos utilize folklore herbal medicines in treating various illnesses such as pain. The availability of plants is not a problem to consider, the nature itself of the country supports the needs of its people in searching for unconventional medicines. However because of the changing society, people ascertained different technologies and ways on how to obtain drugs in a convenient way. Nowadays, several drugs of chemical source emerged in the market and one of these are the analgesics. Large number of the population prefers chemically obtained drugs rather than the natural or plant sources in the remedy of pain.

Pain is a tormenting unpleasant sensation that can lead from general to localized discomfort. The sensation is characterized as both physical and emotional. Patients can now select their treatment from a wide range of analgesic drugs in the market and some are available over the counter or those which are dispensed without a prescription. Aside from chemical origin, plants are also a basis of analgesic properties in which the researchers are interested to.

"Pako" or *Diplazium esculentum* is an edible fern popularly used in homemade salads in the Philippines and the other form is used as an ornamental plant. It belongs to the family Athyriaceae and widely distributed in Asia and to other parts of France, North America, Hawaii and Polynesia. The plant usually originates on stony bars and near banks of streams. The stipe grows 20-50 centimeters long and green in color (Quisumbing,). They are rich in calcium, phosphorus and a good source of iron and vitamin B. This plant has a lot of beneficial uses and the researchers focused on identifying its medicinal use.

According to the recent findings of Chawla *et al*., 2015. *D. esculentum* is a potential analgesic drug in the treatment of pain and further studies should be conducted to develop the property of this plant. It concluded that the active constituents responsible for the protective action were the flavonoids and sterols. Therefore, the researchers of this study decided to continue the determination of the constituent that elicit the desirable effect and flavonoids from the ethanolic extract of *D. esculentum* was subjected for evaluation of its analgesic property.

Review of related literature
Multi drug resistance of *Stenotrophomonas maltophilia*
Stenotrophomonas maltophilia is the third most common gram-negative, non fermentive, motile obligate aerobe and a rod-shaped bacteria (Brooke, 2012; Crossman *et al*., 2008) [4, 7]. A gram negative nosocomial pathogen that can cause infection to patients that are immunocompromised including those with cystic fibrosis. (Karunanidhi, Thomas, van Belkum, & Neela, 2013; Crossman *et al*., 2008) [7]. *Stenotrophomonas maltophilia* is readily isolated in aqueous sources of both outside and inside hospital and clinical setting and recovered from soil, plant roots and animals, water treatment and distribution, invertebrates, lakes rivers, hemodialysis and dialysate samples.

A characteristic of *Stenotrophomonas maltophilia* is its ability to attach to plastic. Increasing reports of acquired nosocomial infection caused by Stenotrophomas maltophilia has been alarming to the clinical setting (Brooke, 2012) [4]. Growing

resistant for other classes of antibiotics makes the *Stenotrophomonas maltophilia* a multi drug resistant organism but a combination of trimethoprim/sulfamethoxazole is mostly use drug for this organism. Growing resistant to this combined drug may cause some problems in treating the *Stenotrophomonas maltophilia*. (Karunanidhi, Thomas, van Belkum, &Neela, 2013).

Biofilm Formation

Stenotrophomonas maltophilia was most frequently seen on patients with cystic fibrosis for the past decade. It is mainly regulated by a RNA binding protein called Hfq gene. It adheres to theta the bronchial epitehelial and for replication of the microbe (Roscetto *et al*. 2012) [25]. However, its virulent factor remains unclear and little is known. Nosocomial outbreaks of this microorganism have recognizable characteristic which includes biofilm formation, sessile structured bacterial communities exhibiting recalcitrance to antimicrobial compounds and persistence despite sustained host defenses that contributed to disease pathogenesis in cystic fibrosis and other respiratory tract diseases associated with chronic bacterial infections (Pompilio *et al*., 2008) [21].

Bacterial adherence is the first step in the pathogenesis of infections of mucosal surfaces or prostheses showing that this microorganism colonization and biofilm formation on synthetic materials represent the top morbidity of patients undergoing prosthetic implantation. *Stenotrophomonas maltophilia* strains of both clinical and environmental origin have been reported to adhere to abiotic and living surfaces (Di Bonaventura, Spedicato, D'Antonio, Robuffo, &Piccolomini, 2004) [9].

Clinical *Stenotrophomonas maltophilia* isolates have been observed to form more biofilms at 32°C than at 37°C and 18°C. The level of biofilm production was higher under aerobic conditions and in a 6% CO2 atmosphere than the level of biofilm production under anaerobic conditions. The *Stenotrophomonas maltophilia* isolates produced comparable biofilms at pH 8.5 and 7.5 but larger amounts of biofilm than those produced at pH 5.5 (Brooke, 2012) [4].

Diseases caused by *Stenotrophomonas maltophilia*

Infections associated with *Stenotrophomonas maltophilia* has high fatal rate especially to those who are immunocompromised (Karunanidhi, Thomas, van Belkum, & Neela, 2013) and patients having direct contact to infectious materials (Garcia C.A., *et al*. 2015) which causes an increasing significant in human diseases. Nevertheless, *Stenotrophomonas maltophilia* nosocomial clinical strain may cause mortality and morbidity ratio (Alavi *et al*., 2013) [2].

Stenotrophomonas maltophilia mutation rate and high production of biofilm on clinical setting frequently seen in catheterized patients (Karunanidhi, Thomas, van Belkum, & Neela, 2013). *Stenotrophomonas maltophilia* has been associated with wide variety of infection including bacteremia, skin, soft tissue infection urinary tract infection, respiratory infection, meningitis, endocarditis and especially to patients with cystic fibrosis (Navarro, Karunanidhi, Thomas, van Belkum, & Neela, 2013; Navarro F,M., *et al*. 2013).

Virulence of *Stenotrophomonas maltophilia*

Virulence factor of the *Stenotrophomonas maltophilia* has many factors to consider in clinical settings (Adamek *et al*., 2011) [1] including siderophores, exopolysaccharide, lipopolysachharide, Smf1-fimbrial operon and protease StmPr1. (de Oliveira-Garcia *et al*, 2003, (Windhorst *et al*., 2002; Nicoletti *et al*., 2011, Huang *et al*., 2006, Garcia *et al*., 2012) [30, 13]. It has the importance of embedding the extra polymeric substance for the microbial cells communities which grows to biologic and abiologic surfaces. The biofilm of *Stenotrophomonas maltophilia* has a similar identification to a planktonic bacterias which increases the antibiotic resistant. (Huang *et al*., 2006; Passerini de Rossi *et al*., 2007; Pompilio *et al*., 2008) [13, 21]. (Di Bonaventura *et al*., 2004; Passerini de Rossie *et al*., 2009, 2012, Pompilio *et al*., 2010) [9, 21, 24].

Treatment: Sulfamethoxazole/Trimethoprim

The susceptibility and effectivity level result of antibiotics is best when the concentration is above the minimum inhibitory concentration (Pompilio *et al*., 2008) [21]. Infections associated to Stenotrophomas maltophilia is difficult to eradicate for it is known to be a multi resistant microbe usually to drugs like with lactam antibiotics, aminoglycosides chlorampenicol and tetracyclines (Brooke, 2012; Pompilio *et al*., 2008) [4, 21]. Selection of antimicrobial drugs against the infection of *Stenotrophomonas maltophilia* represents a major challenge for all clinicians testing the bacteria because of the problems associated with the in vitro susceptibility testing and the limited clinical trials for the determination of its optimal therapy (Al-Jasser, 2006) [3]. Trimethoprim/Sulfamethoxazole is the most use drug for the *Stenotrophomonas maltophilia* having a high susceptibility rate of 90%. The mode of action of this combined drug is usually controlled either by class I integrons or plasmids. Other strains of this *Stenotrophomonas maltophilia* has been described to be resistant to Co-trimoxazole. (Falagas, Valkimadi, Huang, Matthaiou, & Hsueh, 2008) [10]. TMP-SXT has also been one of the most recommended for the treatment of *Stenotrophomonas maltophilia* for it has high activity and favorable outcomes observe red in patients treated with this agent (Al-Jasser, 2006) [3]. Patients with infection of *Stenotrophomonas maltophilia* causing bacterimia which are resistant to Co-trimoxazole can use other dugs mostly ciprofloxacin with the correlation of caftazidine or ticarcillin-clavulanate. Respiratory infection like pneumonia caused by Stenotrophomonasmaltophiliacan be treat with the combination of drug doxycycline and colistin. (Brooke, 2012) [4].

Essence of active compounds used as alternative

For years, researchers used phytochemicals of edible plant extract as a method of alternatives in antibiotics. (Kalia, 2013). Capabilities of the phytochemicals of edible plant extract can block the communication of cells that can inhibit the virulence factor and biofilm formation of a microbes. Edible plants are the ideal alternative use for the inhibition of the biofilm formation and virulence factor of the microbes, choosing the right edible plant extract must not harm the patient health, having a low molecular mass and chemically

stable. (Rasmussen &Givskov, 2006) [23].
Other active compounds were being tested to Burkholderia species. like cinnamaldehyde, baicalin hydrate and hamamelitannin. To calculate the biofilm of the organism, Staphylococcus aureus and Pseudomonas aeruginosa are used. Burkholderia cepaciawas the one being treated in measuring the viable counts in biofilms by adding 2-log that may reduce the viable cells which is much more effective compared to tobramycin alone. However, the combination Staphylococcus aureus and Pseudomonas aeruginosa biofilms having a combination therapy has the ability to increase the bactericidal activity but it has low capability of killing the Burkholderia specie. (LaSarre & Federle, 2013) [18].
A polyphenolic natural compound like chlorogenic acid is an anti-microbial agent which is harmless and ecological friendly can be usually seen in fruits like grapes, apples and pulp which is usually an effective bactericidal having the power of 4x minimum inhibitory concentration decreasing 2-long viable cell counts in just 10 hours. Effective in vitro anti biofilm shows 4-fold decrease to viable cell counts compared to 1x minimum inhibitory values of cholorogenic acid. Minimum inhibitory concentration of chlorogenic acid can decrease the adhesion of *Stenotrophomonas maltophilia* to plastic surfaces. Mostly 1X, 2X and 4X are usually used doses. (Karunanidhi, Thomas, van Belkum, &Neela, 2013). To *Stenotrophomonas maltophilia*, cholorogenic acid may be a promising anti-stenotrophomonas activity for the viable cell counts of biofilm forms like planktonic.

Active compounds extracted from mushrooms
One of the major problems and threats we may face in public health is the increasing resistance and tolerance of the microorganisms to respond against the available broad spectrum of antibiotics and the occurrence of mutation (Alves *et al*., 2013) [2]. Since antimicrobial resistance is a world-wide emergence, many research studies were focused on using nontoxic substances such as plant food extracts, natural products and phytochemical compounds which are harmless to human health (Soković, Ćirić, Glamočlija, Nikolić, & van Griensven, 2014) [27]. These substances were already used as medicament against microbial infection and it has been reported as success in search in of new medicine. (Truchado, Larrosa, Castro-Ibáñez, & Allende, 2015) [28].
Back then, one good source of a natural plant food extract may be found on edible mushrooms containing antioxidant activity and property which includes the phenolic content such as benzoic and cinnamic acid derivatives (Alves *et al*., 2013, lkayKoca & Gençcelep, 2011) [2, 15]. In fact, these mushrooms are good natural antibiotics for phenols and natural phenolic compounds showed significant cause of biofilm reduction formation (Alves *et al*., 2013) [2].
Recently due to many health benefits pharmaceuticals use of natural mushrooms are less expensive and involves minimal side effects. *Pleurotus ostreatus*, Coprinus comatus and Agaricus bisporus has a phenolic compounds that shows valuable antioxidant activity in their extract (Parihar, *et al*. 2014) [19]. Since this plant food extracts can be eaten by human active compounds that may inhibit the biofilm formation of microbes which can be used as an alternative because plants are harmless and nonhazardous to human cells (Koh *et al*., 2013) [17]

Research Methodology
Crude Extraction of *Pleurotus ostreatus*
Crude extraction of *Pleurotus ostreatus* is used as the extract to be treated to *Stenotrophomonas maltophilia* and see what happened to its biofilms.
Fruiting bodies of *Pleurotus ostreatus* was air dried for 15 days. After air drying, the mushrooms were put to hot oven at 45°C for 1 hour then after drying we use blender to powdered the mushrooms.
Powdered mushrooms were brought to the Department of Science and Technology (General Santos Ave., Bicutan, Taguig, Metro Manila) and subjected to extraction.

Agar well diffusion assay
The significance of this procedure is to determine the effects in the viability of the organism treated with different concentrations of the extracted mushroom.
Agar plates of Mueller-Hinton Agar were prepared and allowed to solidify, then the hand generated wells were made using sterile pipette. Test substance were labelled as Untreated, treatment with Cotrimoxazole, treatment with 25%, 50%, 75%, and 100% of mushroom extract and a total of 10uL were added to the hand generated wells (Wang *et al.*, 2011) [29]. The plates were subjected to incubation for 24 hours at 37□C.Susceptibility of *Stenotrophomonas maltophilia* treatments were measured by its zone of inhibition using caliper.

Biofilm Assay
The significance of this procedure is to confirm if the effectiveness of the crude extract of *Pleurotus ostreatus* to the Biofilms caused by *Stenotrophomonas maltophilia*.
Biofilm formation of *Stenotrophomonas maltophilia*is measured using polysterene microtiter plates having 200 ul of overnight cultures of *Stenotrophomonas maltophilia* on Nutrient broth (NB) untreated and treated with co-trimoxazole, 25% mushhroom extract, 50% mushroom, 75% mushroom, 100% mushroom were put on each well labelled as Untreated, CTX, 25%, 50%, 75%, and 100%, respectively. The plates were incubated for 24 hours and stained using crystal violet. Determination of biofilm formation will be based on staining reaction within the surface of the microtiter plates. Violet color around the surface of the wells signifies presence of biofilms.

Motility Assay
This procedure is for the demonstration of the effects of the mushroom extract in *Stenotrophomonas maltophilia* swimming and twitching motility.
Bacterial suspensions with treatment and no treatment used on previous procedures will be subjected in this assay. Whereas, Swimming and Twitching assay was demonstrated using 1% Mueller-Hinton Agar Plates. Swarming assay was performed using Mueller-Hinton Agar Plates. 5 ul of bacteria which were previously prepared and were incubated for 24 hours at 37°C were dispensed on the surface of the swim plates. After inoculating the bacteria for swim plates, the plates were then subjected for twitching motility by inoculating the bacteria at

the bottom at the bottom of the twitch plates using sterilized toothpicks. After incubation, the zone of motility will be determined by staining with Coomassie brilliant blue. (Roscetto *et al*., 2012) [25].

Results and Discussion

Concentration of *Pleurotus ostreatus* extract that take effect on *Stenotrophomonas maltophilia* using Agar well Diffusion Assay

Table 1 Summarizes the different zone of inhibition Results for Agar Well Diffusion Assay of *Stenotrophomonas maltophilia*. Treatment using Cotrimoxazole antibiotic resulted positive for zone of inhibition around the hand generated well, untreated *Stenotrophomonas maltophilia* resulted no zone of inhibition around the hand generated well. The concentrations of the mushroom extract (25%, 50%, 75%, and 100%) also resulted to no zone of inhibition around the well for Agar Well Diffusion Assay.

Table 1: Summarization of Zone of Inhibition for Agar well Diffusion Assay

Treatment	Result	Interpretation
CTX	Presence of zone of inhibition	Susceptible
Untreated	No zone of inhibition	Resistant
25	No zone of inhibition	Resistant
50	No zone of inhibition	Resistant
75	No zone of inhibition	Resistant
100	No zone of inhibition	Resistant

Figure 2:shows that the upper hand generated well having no treatment to *Stenotrophomonas maltophilia* resulted to no zone of inhibition on the Mueller-Hinton agar plate; the lower hand generated well having *Stenotrophomonas maltophilia* treated with Cotrimoxazole antibiotic resulted to 32.3, 28.7, 31.8, 30.6 and 31.5 mm zone of inhibition on the Mueller-Hinton agar plate.

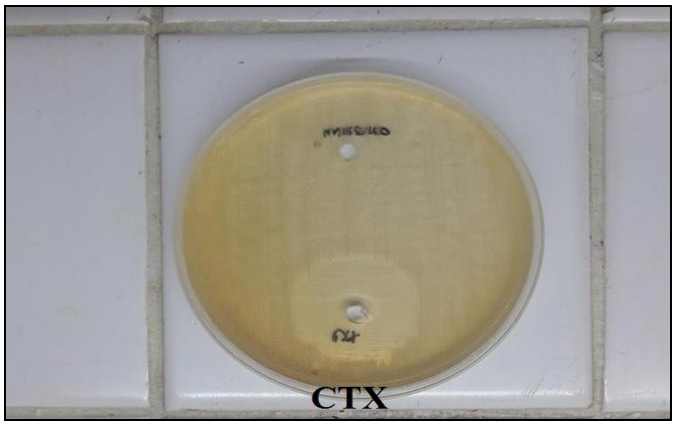

Fig 2: Untreated *Stenotrophomonas maltophilia* on the Upper hand generated well and *Stenotrophomonas maltophilia* treated with Cotrimoxazole on the lower hand generated well.

The lower hand generated well having *Stenotrophomonas maltophilia* treated with Cotrimoxazole antibiotic resulting to an average of 30.98 mm zone of inhibition on the Mueller-Hinton agar plate is used as the Positive control for the susceptibility testing of *Stenotrophomonas maltophilia*. Cotrimoxazole is the antibiotic of choice for patients having *Stenotrophomonas maltophilia* infection especially in patients with cystic fibrosis. (Falagas, 2008) [10]. There may be instances that thisantibiotic may not be effective for patients with Multi-Drug Resistant*Stenotrophomonas maltophilia*, According to Falagas,. *et al.*, 2008 [10], that if this bacterium is not inhibited by CTX, then no other antibiotics will be effective in treating *Stenotrophomonas maltophilia*.

Figure 3 shows that the upper hand generated well having *Stenotrophomonas maltophilia* treated with 25% of *Pleurotus ostreatus* extract resulted to no zone of inhibition on the Mueller-Hinton agar plate; the lower hand generated well having *Stenotrophomonas maltophilia* treated with 50% of *Pleurotus ostreatus* extract resulted to no zone of inhibition on the Mueller-Hinton agar plate.

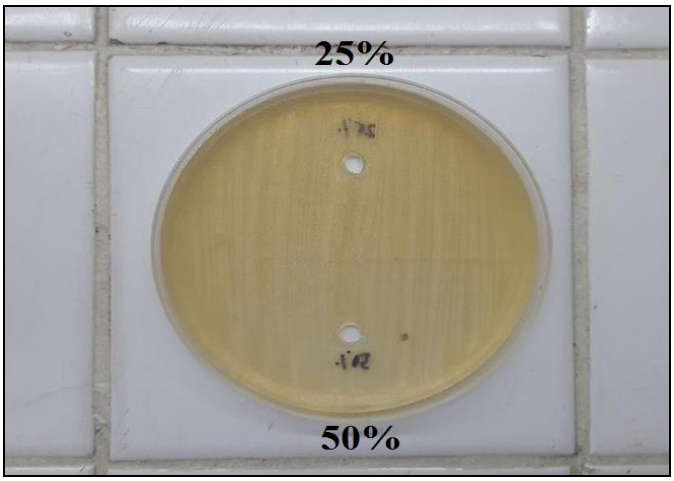

Fig 3: *Stenotrophomonas maltophilia* treated with 25% of mushroom extract on the Upper hand Generated well and Stentrophomonas maltophilia treated with 50% of mushroom extract on the lower hand generated well

The upper hand generated well having *Stenotrophomonas maltophilia* treated with 25% crude extract of *Pleurotus ostreatus* resulting to no zone of inhibition on the Mueller-Hinton agar plate denotes the resistance of the bacteria to the crude extract of *Pleurotus ostreatus*. The 25% crude extract of *Pleurotus ostreatus* did not take effect on the growth of *Stenotrophomonas maltophilia*. The lower hand generated well having *Stenotrophomonas maltophilia* treated with 50% crude extract of *Pleurotus ostreatus* resulting to no zone of inhibition on the Mueller-Hinton agar plate denotes the resistance of the bacteria to the crude extract of *Pleurotus ostreatus*. The 50% crude extract of *Pleurotus ostreatus* did not also take effect on the growth of *Stenotrophomonas maltophilia*

Figure 4 shows that the upper hand generated well having *Stenotrophomonas maltophilia* treatedwith 75% of *Pleurotus ostreatus* extract resulted to no zone of inhibition on the Mueller-Hinton agar plate; the lower hand generated well having *Stenotrophomonas maltophilia* treated with 100%of *Pleurotus ostreatus* extract resulted to no zone of inhibition on the Mueller-Hinton agar plate.

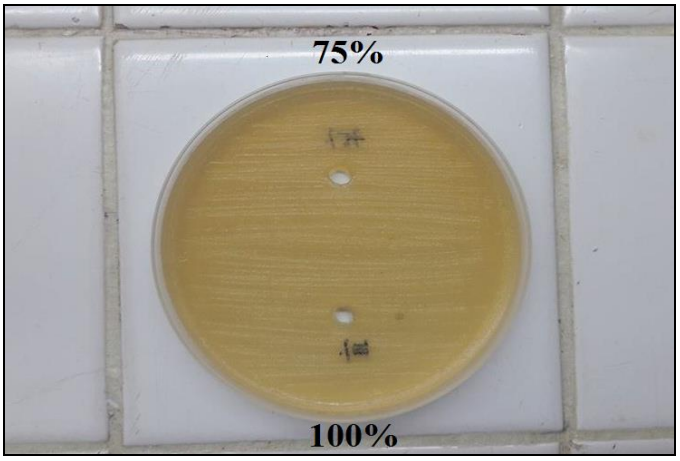

Fig 4: *Stenotrophomonas maltophilia* treated with 75% of mushroom extract on the upper hand generated well and Stenotropomonas maltophilia treated with 100% of mushroom extract on the lower hand generated well

The upper hand generated well having *Stenotrophomonas maltophilia* treated with 75% crude extract of *Pleurotus ostreatus* resulting to no zone of inhibition on the Mueller-Hinton agar plate denotes the resistance of the bacteria to the crude extract of *Pleurotus ostreatus*. The 75% crude extract of *Pleurotus ostreatus* did not take effect on the growth of *Stenotrophomonas maltophilia*. The lower hand generated well having *Stenotrophomonas maltophilia* treated with 100% crude extract of *Pleurotus ostreatus* resulting to no zone of inhibition on the Mueller-Hinton agar plate denotes the resistance of the bacteria to the crude extract of *Pleurotus ostreatus*. The 100% crude extract of *Pleurotus ostreatus* did not also take effect on the growth of *Stenotrophomonas maltophilia*.

As seen on the figures (Figure 3 and Figure 4), the extract didn't inhibit the growth of *Stenotrophomonas maltophilia* as compared to the inhibition made by the treatment with Cotrimoxazole antibiotic. According to Krishnamoorthy *et al*. 2014, *Pleurotus ostreatus* is composed of mainly organic component such as Proteins (aspartic acid, threonine, serine, and glutamic acid), Carbohydrates (glucans), Vitamins (thiamin, riboflavin, niacin, folate and ascorbic acid), Minerals (potassium, calcium, sodium, and magnesium) and Fibers. *Stenotrophomonas maltophilia*is usually resistant to metals based on (Brooke 2014) [4] like silver, cadmium, copper, gold, mercury, and selenium. Selenium having the highestmetal resistance in O2 which has a value 40 (mM)a and Sm777 having a value of 50.0 (mM)a.

Biofilm formation reaction on biofilm assay

Table 2 illustrates the summarization of results for Biofilm Formation of *Stenotrophomonas maltophilia* using no treatment to the bacteria, treatment with Cotrimoxazole (CTX), 25%, 50%, 75% and 100% of mushroom extract. The Untreated *Stenotrophomonas maltophilia* reacted Positive on the Biofilm Formation of *Stenotrophomonas maltophilia*, as well as the 25%, 50%, 75% and 100% of *Pleurotus ostreatus* extract in treatement on *Stenotrophomonas maltophilia* using Biofilm Assay while Treatment with CTX on the Biofilms of *Stenotrophomonas maltophilia* reacted Negative on the Assay.

Table 2: Summarization of Results for Biofilm Formation of *Stenotrophomonas maltophilia*

Treatment	Result	Interpretation
CTX	Negative	No Biofilm Formation
Untreated	Positive	Biofilm Formation
25% P. ostreatus extract	Positive	Biofilm Formation
50% P. ostreatus extract	Positive	Biofilm Formation
75% P. ostreatus extract	Positive	Biofilm Formation
100% P. ostreatus extract	Positive	Biofilm Formation

Figure 5 shows the color reaction of the dye to the different test substances used in Biofilm Assay for *Stenotrophomonas maltophilia*. Test substances are as follows: Untreated *Stenotrophomonas maltophilia*, *Stenotrophomonas maltophilia* treated with Cotrimoxazole antibiotic, *Stenotrophomonas maltophilia* treated with 25% of mushroom extract, *Stenotrophomonas maltophilia* treated with 50% of mushroom extract, *Stenotrophomonas maltophilia* treated with 75% mushroom extract and *Stenotrophomonas maltophilia* treated with 100% of mushroom extract.

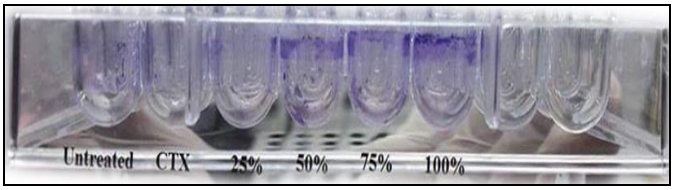

Fig 5: Biofilm Formation Reaction of Untreated, Treatment with CTX, 25%, 50%, 75%, and 100% of mushroom extract on Biofilms caused by *Stenotrophomonas maltophilia*

The microtiter plate containing *Stenotrophomonas maltophilia* untreated resulting to purplish color on the surface of the well shows that there is a Biofilm formed by *Stenotrophomonas maltophilia*. The Untreated *Stenotrophomonas maltophilia* became the Negative Control for the Biofilm Assay. While on the other hand, *Stenotrophomonas maltophilia*treated with Cotrimoxazole antibiotic resulting to clear or no presence of the purplish coloration on the surface of the wall states that Cotrimoxazole antibiotic inhibited the Biofilm Formation caused by *Stenotrophomonas maltophilia*, (c) treated with 25% of mushroom extractresulting to bluing of the surface of the well shows that the Biofilm formation caused by *Stenotrophomonas maltophilia* is not inhibited by 25% mushroom extract, (d) treated with 50% of mushroom extractresulting to bluing of the surface of the well shows that the Biofilm formation caused by *Stenotrophomonas maltophilia* is not inhibited by 50% mushroom extract, (e) treated with 75% mushroom extractresulting to bluing of the surface of the well shows that the Biofilm formation caused by *Stenotrophomonas maltophilia* is not inhibited by 75% mushroom extract and (f) treated with 100% of mushroom extract resulting to bluing of the surface of the well shows that the Biofilm formation caused by *Stenotrophomonas maltophilia* is not inhibited by 100% mushroom extract. The mushroom extract did not take effect because biotic support for the establishment of a bacterial biofilm may be provided by the mushroom. (Fre-Klette, 2011) Resulting to the presence of the Hfq genes (Roscetto, 2012) [25]. *Pleurotus ostreatus* has the ability to inhibit the biofilm formation of the

Stenotrophomonas maltophilia, it has active compounds like tannin and phenolic acid that promote the antibacterial effectivity of the mushroom (Krishnamoorthy *et al.*, 2014) but there are some enzymes being produce to the *Stenotrophomonas maltophilia* which may cause the resistance to the *Pleurotus ostreatus*, esterase, DNase, fibrinolysin, proteases, and RNase are some of the enzyme that may take effect in being resistant to the mushroom (Brooke 2012) [4].

Growth of *Stenotrophomonas maltophilia* in treatment with *Pleurotus ostreatus* extract using Motility Assays

Table 3 Illustrates the raw data of the results measured using the Swimming Assay. The motility of the positive control measurement are: 9cm, 6cm, 6cm, 6cm, 9cm and has a mean deviation of 7.2 cm. The motility of the negative control measurements are: 23cm, 17cm, 26cm, 28cm, 24cm and has a mean deviation of 23.6 cm. The motility of the 25% mushroom extract measurement are: 24cm, 31cm, 22cm, 22cm, 12cm and has a mean deviation of 22.2 cm. The motility of the 50%mushroom extract measurements are: 26cm, 22cm, 21cm, 22cm, and 10cm and has a mean deviation of 20.2 cm. The motility of the 75% mushroom extract measurements are: cm, 24cm, 24cm, 21cm, 23cm, 19cm and has a mean deviation of 22.2cm. The motility of the 100% mushroom extract measurements are: 52cm, 28cm, 32cm, 29cm, 30cm and has a mean deviation of 34.2 cm

Table 3: Summarization of Results of Swimming Assay

	Positive	Negative	25%	50%	75%	100%
	9	23	24	26	24	52
	6	17	31	22	24	28
	6	26	22	21	21	32
	6	28	22	22	23	29
	9	24	12	10	19	30
Mean	7.2	23.6	22.2	20.2	22.2	34.2

The Statistical Analysis as shown in Table 3 of the Swimming Assay which represents the mean and standard deviation of the positive control, negative control, 25%, 50%, 75% and 100% concentration of the *Pleurotus ostreatus* resulting to a closeness of results of the negative control to the different concentrations of the *Pleurotus ostreatus* having a negative result in different concentration of the mushroom. *Pleurotus ostreatus* has active compound which has a beta-d-glucan which promotes antimicrobial activity that didn't take effect in inhibiting the *Stenotrophomonas maltophilia* (Krishnamoorthy *et al*.. 2014).

Table 4 Illustrates the Statistical Analysis of Swimming Assay (Negative vs Experimental) using one way ANOVA. Negative Control (Untreated *Stenotrophomonas maltophilia*) is compared to the Mushroom extracts of *Pleurotus ostreatus* (25%, 50%, 75% and 100%) resulting to Treatment (between columns): SS of 619.8, DF of 4, MS of 155; Residual (within columns): SS of 822.4, DF of 20, MS of 41.12 with a Total 1442 SS, and 24 DF. The statistical data gives a result of F (DFn, DFd) resulted to F(4,20) = 3.768 and a P value of 0.0193.

Table 4: Statistical Analysis of Swimming Assay I (Negative vs Experimental)

	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	619.8	4	155	F (4, 20) = 3.768	P=0.0193
Residual (within columns)	822.4	20	41.12		
Total	1442	24			

*SS-Sum of Squares, DF-,MS- Mean of Square

Table 5 Illustrated in the table are the results in Swimming Assay (Negative VS Experimental). The Mean Difference and Significance of the test experiments. Negative VS 25% has a mean difference of 1.4; non-significant, Negative VS 50% has a mean difference of 3.4; non-significant, Negative VS 75% has a mean difference of 1.4; non-significant, Negative VS 100% has a mean significant of 10.6; non-significant.

Table 5: Statistical Analysis of Swimming Assay II (Negative VS Experimental)

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Negative vs. 25	23.6	22.2	1.4	4.056	5	5	0.3452	20
Negative vs. 50	23.6	20.2	3.4	4.056	5	5	0.8383	20
Negative vs. 75	23.6	22.2	1.4	4.056	5	5	0.3452	20
Negative vs. 100	23.6	34.2	-10.6	4.056	5	5	2.614	20

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
Negative vs. 25	1.4	-9.352 to 12.15	No	ns	0.9902	B	25
Negative vs. 50	3.4	-7.352 to 14.15	No	ns	0.8169	C	50
Negative vs. 75	1.4	-9.352 to 12.15	No	ns	0.9902	D	75
Negative vs. 100	-10.6	-21.35 to 0.1525	No	ns	0.0540	E	100

Table 6 Illustrates the Statistical Analysis of the results in Swimming Assay (Positive vs Experimental) using one way ANOVA. Positive Control (*Stenotrophomonas maltophilia* treated with Cotrimoxazole antibiotic) compared to the Experimental test (25%, 50%, 75% and 100% of *Pleurotus ostreatus* extract) resulted to Treatment (between columns): SS of 1840, DF of 4, MS of 460 and Residual (within columns): SS of 764, DF of 20, MS of 38.2 with a Total 2604 SS and 24 DF. The statistical data gives a result of F(DFn, DFd) of F(4, 20) = 12.04 and a P value of <0.0001.

Table 6: Statistical analysis of swimming assay I (Positive VS Experimental)

	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	1840	4	460	F (4, 20) = 12.04	P<0.0001
Residual (within columns)	764	20	38.2		
Total	2604	24			

Table 7 Illustrates the results in Swimming Assay (Positive VS Experimental). The Mean Difference and Significance of the test experiments. Positive VS 25% has a mean difference of -15; significant, Positive VS 50% has a mean difference of -13; significant, Positive VS 75% has a mean difference of -15; significant, Positive VS 100% has a mean significant of -27; significant.

Table 7: Statistical analysis in swimming assay II (Positive VS Experimental)

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive vs. 25	7.2	22.2	-15	3.909	5	5	3.837	20
Positive vs. 50	7.2	20.2	-13	3.909	5	5	3.326	20
Positive vs. 75	7.2	22.2	-15	3.909	5	5	3.837	20
Positive vs. 100	7.2	34.2	-27	3.909	5	5	6.907	20

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	A-?	
Positive vs. 25	-15	-25.36 to -4.636	Yes	**	0.0037	B	25
Positive vs. 50	-13	-23.36 to -2.636	Yes	*	0.0117	C	50
Positive vs. 75	-15	-25.36 to -4.636	Yes	**	0.0037	D	75
Positive vs. 100	-27	-37.36 to -16.64	Yes	****	0.0001	E	100

Table 8 Illustrates the raw data of the results measured using the Twitching Assay. The motility of the positive control measurement are: 4cm, 4cm, 4cm, 3cm, 4cm and has a mean deviation of 3.8 cm. The motility of the negative control measurement are: 7cm, 5cm, 4cm, 5cm, 5cm and has a mean deviation of 5.2 cm. The motility of the 25% mushroom extract measurement are: 5cm, 6cm, 6cm, 5cm, 5cm and has a mean deviation of 5.6 cm. The motility of the 50% mushroom extract measurement are: 7cm, 6cm, 5cm, 6cm, and 6cm and has a mean deviation of 6 cm. The motility of the 75% mushroom extract measurement are: cm, 7cm, 6cm, 6cm, 6cm, 6cm and has a mean deviation of 6.2cm.The motility of the 100% mushroom extract measurement are: 5cm, 5cm, 7cm, 7cm, 6cm and has a mean deviation of 6 cm.

Table 8: Summarization of results of twitching assay

	Positive	Negative	25%	50%	75%	100%
	4	7	5	7	7	5
	4	5	6	6	6	5
	4	4	6	5	6	7
	3	5	5	6	6	7
	4	5	6	6	6	6
Mean	3.8	5.2	5.6	6	6.2	6

The significance of the Statistical Analysis shown in Table 8 given is the positive control and negative control to the different concentration of the *Pleurotus ostreatus* resulting to the significant of positive control vs the negative control. The closeness of the negative control to the different concentration of the mushroom shows the signifance of the 25%, 50%, 75% and 100% concentrations of mushroom to the positive control concluding it to be a negative result based on the mean and standard deviation which means that the *Stenotrophomonas maltophilia* has a no flagellar movement. There are some mutant gene that had been discovered having a deficiency in the motility of the *Stenotrophomonas maltophilia*. The fliI mutant gene is a deficiency in the flagella of the *Stenotrophomonas maltophilia* which do not take effect in the biofilm and virulence factor of the bacteria (Brooke 2012) [4].

Table 9 Illustrates the Statistical Analysis of the results in Twitching Assay. Positive Control and Negative Control (*Stenotrophomonas maltophilia* untreated and treated with Cotrimoxazole antibiotic) were compared to the Experimental test (25%, 50%, 75% and 100% of *Pleurotus ostreatus* extract) resulted to Treatment (between columns): SS of 61.6, DF of 5, MS of 12.32 and Residual (within columns): SS of 17.2, DF of

24, MS of 0.7167 with a Total 78.8 SS and 29 DF. The statistical data gives a result of F (DFn, DFd) of F(5, 24) = 17.19 and a P value of <0.0001.

Table 9: Statistical analysis of twitching assay

	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	61.6	5	12.32	F (5, 24) = 17.19	P<0.0001
Residual (within columns)	17.2	24	0.7167		
Total	78.8	29			

Table 10: Multiple comparison test of twitching assay

Mean Diff.		95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
Positive vs. Negative	-4.4	-6.055 to -2.745	Yes	****	<0.0001	A-B		
Positive vs. 25%	-3.6	-5.255 to -1.945	Yes	****	<0.0001	A-C		
Positive vs. 50%	-3.4	-5.055 to -1.745	Yes	****	<0.0001	A-D		
Positive vs. 75%	-2.4	-4.055 to -0.7445	Yes	**	0.0019	A-E		
Positive vs. 100%	-1.8	-3.455 to -0.1445	Yes	*	0.0276	A-F		
Negative vs. 25%	0.8	-0.8555 to 2.455	No	ns	0.6710	B-C		
Negative vs. 50%	1	-0.6555 to 2.655	No	ns	0.4446	B-D		
Negative vs. 75%	2	0.3445 to 3.655	Yes	*	0.0116	B-E		
Negative vs. 100%	2.6	0.9445 to 4.255	Yes	***	0.0008	B-F		
25% vs. 50%	0.2	-1.455 to 1.855	No	ns	0.9989	C-D		
25% vs. 75%	1.2	-0.4555 to 2.855	No	ns	0.2565	C-E		
25% vs. 100%	1.8	0.1445 to 3.455	Yes	*	0.0276	C-F		
50% vs. 75%	1	-0.6555 to 2.655	No	ns	0.4446	D-E		
50% vs. 100%	1.6	-0.05546 to 3.255	No	ns	0.0623	D-F		
75% vs. 100%	0.6	-1.055 to 2.255	No	ns	0.8680	E-F		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Positive vs. Negative	3.6	8	-4.4	0.5354	5	5	11.62	24
Positive vs. 25%	3.6	7.2	-3.6	0.5354	5	5	9.509	24
Positive vs. 50%	3.6	7	-3.4	0.5354	5	5	8.981	24
Positive vs. 75%	3.6	6	-2.4	0.5354	5	5	6.339	24
Positive vs. 100%	3.6	5.4	-1.8	0.5354	5	5	4.754	24
Negative vs. 25%	8	7.2	0.8	0.5354	5	5	2.113	24
Negative vs. 50%	8	7	1	0.5354	5	5	2.641	24
Negative vs. 75%	8	6	2	0.5354	5	5	5.283	24
Negative vs. 100%	8	5.4	2.6	0.5354	5	5	6.868	24
25% vs. 50%	7.2	7	0.2	0.5354	5	5	0.5283	24
25% vs. 75%	7.2	6	1.2	0.5354	5	5	3.17	24
25% vs. 100%	7.2	5.4	1.8	0.5354	5	5	4.754	24
50% vs. 75%	7	6	1	0.5354	5	5	2.641	24
50% vs. 100%	7	5.4	1.6	0.5354	5	5	4.226	24
75% vs. 100%	6	5.4	0.6	0.5354	5	5	1.585	24

Table 10: Illustrates the results in Twitching Assay. Using one way ANOVA the researchers got the Mean Difference and Significance of the test experiments. Positive VS 25% has a mean difference of -15; significant, Positive VS 50% has a mean difference of -13; significant, Positive VS 75% has a mean difference of -15; significant, Positive VS 100% has a mean significant of -27; significant.

Conclusion and Recommendations

The agar well diffusion assay shows that in the different concentration of the mushroom used was not able to form any zone of inhibition which concludes that the *Pleurotus ostreatus*does not have the ability to inhibit the *Stenotrophomonas maltophilia* resulting it to be resistant and cannot inhibit the biofilm of the bacteria. Correlating the results of the biofilm assay which shows that the *Pleurotus ostreatus* did not take effect the *Stenotrophomonas maltophilia* biofilm because of the presence of the violet stain around the well indication the presence of the bacterium. For the viability of *Stenotrophomonas maltophilia* we used the motility assay which represents the movement of the bacteria to the different concentration resulting to a viable bacterium having a negative effect to the different concentration of the *Pleurotus ostreatus*.

The researches would like to recommend the following for the future researchers in improvement of this study. The researchers should supplement antibiotics for the inhibition of growth of other organisms to prevent contamination and avoid discrepancy of results. Additional test groups should be added to acquire more accurate and precise results. To quantify the results in biofilm assay and recommend the future researchers to use spectrophotometric analysis. The researchers should secure an ATCC or Biotech type of strain of microorganism for the integrity of the sampling. Additional tests/assay should be added to support and correlate to the pathogenicity and mechanism of the bacteria. A more specific and rich active compound from other plant sources must be used to ensure the effectiveness of the extract. Additionally the researchers recommend to use DSF Assays to the future researchers to demonstrate the quorum sensing inhibitory activity that could be significant in modulation of the bacteria's pathogenicity, mechanism and viability. It also recommended to use HPLC or TLC to check the presence or absence of the gene the codes for its quorum sensing system and to determine the other effects of the extracts.

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