Sensitivity and Specificity of Maternal Salivary Glucose in comparison with Serum Glucose Levels in Screening for Gestational Diabetes Mellitus among Pregnant Women Visiting a Secondary Hospital in Angeles City Pampanga

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Introduction: Gestational diabetes mellitus (GDM), is one of most common complication of pregnancy and has been associated with adverse outcomes such as high risk for pregnancy and delivery complications. Gestational Diabetes Mellitus would pose health risks for both neonates and the gravid mother. Neonates of women with GDM are at increased risk of macrosomia, which is defined as a birthweight over 4000 g, as well as neonatal hypoglycemia, hyperbilirubinemia, birth trauma, respiratory distress syndrome, and shoulder dystocia. Maternal complications associated with GDM include gestational hypertension, preeclampsia, and non-elective cesarean delivery.

Approximately 7% of pregnancy worldwide is complicated with gestational diabetes. According to the Philippine Obstetrics and Gynecology Society Clinical Practice Guidelines on Gestational Diabetes Mellitus, 1.9% of pregnant women admitted in the last 5 years had GDM.

However, there is a lack of agreement with how women are screened and diagnosed with GDM in pregnancy. Guidelines differ in the following issues: selective or universal screening, optimal time for screening, appropriate test, cut-off values. Based on the Hyperglycemia and Adverse Pregnancy Outcome study, ACE, ADA, IADPSG, WHO, FIGO, and POGS used FBS as a screening tool with a cut of value of =92mg/dL. Based on the Clinical Practive Guidelines 2011 of Philippine Obstetrics and Gynecology Society (POGS) a mandatory screening for GDM is during the first prenatal visit of a pregnant woman

While the early diagnosis of diabetes is essential to prevent its devastating complications, the current method of investigation requires frequent invasive pricking process which causes unnecessary discomfort and is psychologically traumatic to the patients. Therefore, a much simpler and noninvasive technique for the diagnosis and monitoring of diabetes is very desirable

Objective: The study aims to determine the sensitivity and specificity of salivary glucose in comparison with the serum glucose in screening for GDM. The present study compared the serum and salivary glucose levels among pregnant women with GDM and non- diabetic women. Furthermore, the study aims to determine if a significant correlation exist between GDM and variables – age, BMI, number of cups of rice and history of GDM in previous pregnancies.

Materials and Methods: Ethical permission for conducting this study was obtained from Institutional Ethics Review Committee of a private institution. The study was designed as an observational study. Inclusion criteria includes pregnant women at any age who had their first prenatal visit and subjected for serum glucose level determination, non-diabetic pregnant women who met the criteria of Philippine Obstetrics and Gynecology Society (POGS) (FBS of >92mg/dL). Exclusion criteria include pregnant women with any systemic disease, history of diabetes before, history of smoking and using drugs that impairs the balance of antioxidant defense system. Subjects with any other pathology/disease that could affect salivary glands function or with gingivitis at the time of the study were not included. This information was obtained by studying medical records and clinical examination.

A total of 40 pregnant women visiting a secondary hospital in Angeles City Pampanga who met the inclusion criteria were included in the study, 13 of them were diagnosed with GDM and classified as study group while 27 of them are non-GDM and classified as control group. Saliva was collected on the same day of their serum glucose determination. The researchers and the registered medical technologists who determine the serum glucose and salivary glucose respectively were blinded to avoid the possible biases. Allocation concealment was done by placing the results in a sealed envelope. Salivary glucose determination was performed with the colorimetric kit Glucose (GO) Assay (Sigma-Aldrich, Inc.) based on glucose-oxidase reaction. Absorbance values were measured at 540 nm. Data was analysed using statistical software STATA.

Results: Linear regression analysis was done to show the correlation between the serum and salivary glucose. An upward linear regression line was obtained indicating that as the values serum and salivary glucose are directly proportional (Figure 1). A significant correlation between the two parameters was observed with p-value of 0.0000, correlation coefficient (r-value) of 0.9408 and r-squared (r2) of 0.9020, these estimations are very close to actual serum glucose values of the subjects. The correlation coefficient of r=9408 shows that the salivary glucose is capable of estimating 94% of serum glucose level.

From the total number of participants (n=40), 13 of them were true positive, 26 were true negative, 1 false positive and 0 false negative was obtained.

We observed 1 false positive result with salivary glucose, this may be due to the risk classification of the subject which was categorized as high risk patient and a further test is recommended. For the diagnostic accuracy of salivary glucose, a sensitivity of 100% and specificity of 96.30% were obtained. PPV and NNV were 92.86% and 100% respectively.

A significant correlation between GDM and previous history of GDM was also established (pvalue= 0.002). No significant relationship was observed between salivary glucose levels and age, BMI and number of cups of rice in both control and study group.

Conclusion: We therefore concluded that saliva can be used as a potential tool in the assessment of the blood glucose concentration in GDM patients. Nevertheless, further studies on larger populations and in different geographic areas are recommended to establish salivary glucose estimation as a diagnostic tool in screening for gestational diabetes.

Keywords: Gestational Diabetes Mellitus, saliva hyperglycemia

The Prevalence of Soil-transmitted Helminths in Aeta Children

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The Aeta children are considered to have a high risk of acquiring soil transmitted helminths. Due to their age, they tend to play with soil more which makes them susceptible to the infection. Eighty-one (81) Aeta children participated to be the subject for the study. The laboratory methods used in identification of parasites are Direct Fecal Smear (DFS), Kato-Katz, and worm burden. Stool analysis was initially done by DFS and then confirmed with Kato-Katz. The result showed that 44.4% were positive for *Ascaris lumbricoides,* 1.2% were positive for *Trichuris trichiura* and 49.4% were positive for both Ascaris and Trichuris in children ages 1-12 years old. In worm burden, it showed that 41.9% of the children had light intensity, 1.2% has moderate intensity and none for the heavy intensity of the *Ascaris lumbricoides*. On the other hand, for *Trichuris trichiura,* 1.2% had light intensity and none for both moderate and heavy intensity. While 45.6% had light intensity and 4.9% had moderate intensity for both *Ascaris lumbricoides* and *Trichuris trichiura.* The results of the study were reported to the municipal health office which became their basis for the upcoming deworming program on November 2018. Also the researchers gave a seminar and tarpaulins about proper hygiene and ways to avoid acquiring the infection as part of the community service to the locality where they conducted their research study.

Keywords: Ascaris lumbricoide, Trichuris trichiura, Hookworm, Direct Fecal Smear, KatoKatz Technique

Inhibition of A549 Lung Cancer Cell Proliferation using Semi-Purified Flavonoids from the peel of *Hylocereus undatus Haw.* Family Cactaceae (White Dragon Fruit)

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Hylocereus undatus, locally known as pitaya in Tagalog, and known commonly in English as white dragon fruit, is widely cultivated in tropical regions like the Philippines. According to the findings of Kim et al. (2011), *H. undatus* peel extract has a greater potential anti-proliferative activity against cancer cells than flesh extracts and the active constituents found responsible for the effect were flavonoids. The present study was done to evaluate the inhibition of A549 lung cancer cell proliferation using semi-purified flavonoids of *H. undatus* peel extract. Physical, chemical and instrumental analysis showed the presence of flavonoids. The evaluation used MTT assay of A549 lung cancer cells treated with different concentrations of plant extract serially diluted compared to the positive control (Methotrexate). The study used One Way ANOVA and Tukey's Multiple Comparison test in the analysis of data. The computed F which is 142.258 is greater than the F-critical which is 2.85 therefore there is a significant difference between the groups. The comparison used 95% level of confidence with a P value of 0.000. These data may prove that semi-purified flavonoids of H. undatus has showed inhibition on lung cancer cell proliferation as the concentration increases.

Keywords: inhibition, proliferation, semi-purified flavonoids, white dragon fruit, lung cancer cells

Cytotoxicity and Teratogenicity of *Cassia fistula* ethanolic leaf extract in Zebrafish (*Danio rerio*) embryo

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Cassia fistula is a tree native to Asian regions and known for its medicinal properties. Among its known activities are anti-inflammatory, antitussive, laxative, antitumor, hypocholesteroleimic, and hepatoprotective. However, plants also produce phytochemicals which may also cause harm when ingested. This study assesses the cytotoxicity and teratogenicity of the ethanolic leaf extract of *C. fistula* using *Danio rerio* embryo as model.

Various plant parts were collected and submitted to the Bureau of Plant Industry in Malate, Manila for identification. Leaves were air-dried for five days and submitted to Saint Mary's University in Bayombong, Nueva Vizcaya for ethanolic extraction and phytochemical analysis. The latter reveals the presence of essential oils, triterpenes, phenols, fatty acid, sugars, anthraquinones, coumarines, anthrones, tannins, flavanoids, and steroids. For the bioassay, six treatments were prepared, a control composed solely of embryo water, and five concentrations of the extract in embryo water as follows: 10,000 µg/mL, 1,000 µg/mL, 100 µg/mL, 10 µg/mL and 1 µg/mL. Three zebrafish embryos at their segmentation phase were placed in each well of the 15-well ELISA plate, with 3mL of the treatment solutions. The plate was incubated at 26°C ± 1°C. The embryos were examined in a dissecting microscope after 12, 24, 36, and 48 hours of incubation for toxic and teratogenic activity.

Coagulated embryo was the basis of determining the toxic effects of the extract. Based on the results, the extract exhibited toxicity toward the embryo at a concentration of 10,000 µg/mL. Studies have shown that flavonoids, which are present in the extract, have coagulating properties which can alter the development of embryos. On the other hand, teratogenicity was determined through the eggs' hatchability and delayed growth. After 48 hours, eggs treated with 10,000 µg/mL extract did not hatch. While some eggs did not hatch in the lower concentrations at this time, the numbers were not significantly differently with that of the control. Prolonged observations however show that the eggs hatched at a later time, indicating delayed growth. A number of researches attribute delayed growth to triterpenes, which were detected in the *C. fistula* leaf ethanolic extract used in this investigation. These findings suggest that 10,000 µg/mL of the extract exerts cytotoxic and teratogenic activity toward zebrafish.

It is recommended that cytotoxic and teratogenic effects of the extract to higher forms of vertebrate (e.g., rats) are explored in future studies.

Cytotoxic Activity of *Cocos nucifera Linn.*Husk Fiber Extracts against MCF 7 Breast Cancer Cell Line

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Breast cancer, one of the most prevalent and leading cause of death in women, is responsible for half of gestational malignancies, alongside cervical cancer. Gestational breast cancer is defined as breast cancer that is diagnosed during pregnancy until first postpartum year, or any time during lactation (Litton, 2017). The thickening of the breast during pregnancy makes it difficult to notice small masses, accounting for late-stage diagnosis (Hughes, 2017). Breast cancer alone is not a threat topregnancy,however, the need for prompt treatment may complicate it. The primary treatment for breast cancer is mastectomy with either adjuvant chemotherapy, radiotherapy, or both, depending on the stage upon diagnosis. There is great potential for research on the cytotoxic activity of *Cocos nucifera* against gestational malignancies as previously proven to possess cytotoxic activity against HeLa cervical cancer cell line and antiproliferative activity against MCF7 breast cancer cell line.

Cytotoxicity is the quality of compound to cause dangerous effects on cells. Cytotoxic compounds prevent cell growth and division, causing death. Testing the effects of compounds on the viability of cells is widely used as a predictor of potential toxic effects in animals.

Coconut is one of the most useful plants in the world with multiple uses, from arrack to food staple, lumber and oil among many others (Philippine Alternative Medicine, 2016). The aqueous extracts from the husk fibers of *Cocos nucifera* present antibacterial, antiviral, antinociceptive and free radical scavenging properties. Defatted coconut kernel methanolic extract bysoxhlet methodshow significant antioxidant and anti-proliferative properties against MCF-7 cell line by inducing nuclear damage, free radical production and cell death (Mantena et. al., 2003). The cytotoxic activity of lyophilized aqueous shell extracts against Henrietta Lax (HeLa) cervical cancer cell lines demonstrates high cytotoxic activity determined by 3,4,5dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) assay (Nasimun et. al., 2014).

The objective of this study is to determine the lowest concentration at which methanolic and ethyl acetate extracts elicit cytotoxic activity against MCF7 breast cancer cell line and to determine which among the two extract elicits a more significant cytotoxic activity against MCF7 breast cancer cell line.

The methanolic and ethyl acetate husk fiber extracts were prepared according to the method of Adebayo et al (2013). The fibers were washed with distilled water, cut into smaller pieces then subjected to air-drying

under shade at 28°C (room temperature) for 21days, then were ground to powder form. Four hundred grams (400g) of the sample was divided into 2 then fully soaked on each of the following given solvents: ethyl acetate and methanol. The two preparations were stored in a tightly stoppered glass container for 72 hours.

After each solvent extraction, contents were filtered with Whatmanfilter paper no. 1. Filtrates were placed in labeled 120ml-amber bottles. The filtrates were then concentrated using rotary evaporator at 40°C. Crude extracts of 0.15g and 0.26g were obtained for ethyl acetate and methanol respectively.

The crude extracts were brought to UP Diliman Marine Sciences Laboratory for in-vitro MTT cell viability assay against MCF7 breast cancer cell line. In-vitro MTT cell viability assay is a colorimetric assay based on assessing the cell metabolic activity. The biochemical mechanism behind the MTT assay involves NADPH-dependent cellular oxidoreductase (mitochondrial dehydrogenase) enzyme that converts the yellow tetrazolium MTT into insoluble (E,Z)-5-(4,5-dimethylthiazol-2-yl)-1,3- diphenylformazan (formazan). The formazan can be dissolved with dimethyl sulfoxide (DMSO) to give a purple color absorbed at 540 nm. Intensity of purple color is directly proportional and indicative of cell viability.

The assay will yield IC50 (inhibitory concentration) values for each extract: ethyl acetate and methanolic. The IC50 represents the concentration of a drug that is required for 50% inhibition of a specific biological or biochemical function in vitro. According to the U.S. National Cancer Institute (NCI) and Geran protocol, cytotoxicity fractions against mammalian cell lines are classified as follows: IC50 </=20mcg/mL = highly cytotoxic; IC50 21-200mcg/mL = moderately cytotoxic; IC50 201-500 mcg/mL =weakly cytotoxic; and >/=501mcg/mL = no cytotoxicity (17-20).

From the data gathered, analyzed and interpreted, the researchers conclude that *Cocos nucifera* Linn. ethyl acetate and methanolic extracts possess moderate cytotoxic activity. MTT assay demonstrates decreasing cell viability with increasing increments of both extract concentrations. IC50 yields values fell into the range of “moderately cytotoxic” (21-200ug/mL) based from the U.S. National Cancer Institute (NCI), indicating potential for anti-cancer activity. Necessity of this research focuses on the current epidemiologic evidence of breast cancer prevalence among pregnant women (Litton, 2017) and providing possible alternatives for chemotherapy-based treatment due to their high transmittance in breastmilk (Kayser et. al., 2012).

Evaluation of the Antioxidant Activity of the Ethanolic Extract of *Erythrina crista-galli* Flowers

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Antioxidant activity of the ethanolic extract of Erythrina crista-galli flowers was evaluated using the DPPH assay and ferric chloride test. The DPPH assay of the ethanolic extract was compared to a standard antioxidant ascorbic acid and revealed effective free radical scavenging activity. There was a decrease of DPPH absorbance which indicates that it has antioxidant property. Also, through Ferric chloride test, it showed evident results that it contains a phenolic compound by producing black color reaction confirming presence of antioxidant activity of the ethanolic extract. And the researchers were able to evaluate the IC50 and AEAC of the extract which is 48. 9826 µg/ml and 4.8595 µg/mL.

Keywords: Erythrina crista-galli, DPPH assay, DPPH, Ascorbic Acid (Vitamin C), IC50, AEAC

Aspergillus Contaminated Smoked-cured Milk Fish Sold in Bataan Fish Market

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Introduction: Food safety is everybody's concern and food borne illnesses may result from the consumption of food contaminated by microbial pathogens, fungi, toxic chemicals or radioactive materials. Smoked milkfish production is well-known industry in province of Bataan, in the last decade modern food packaging methods have been employed to extend the shelf life of products, as well as lessen, if not to prevent, microbial contamination. Nevertheless, for quality control monitoring there is a need to evaluate the efficiency of these packaging methods. We report here the initial results showing the presence of fungal contamination found in smoked milk fish (*Chanos chanos*) sold in two markets in Bataan.

Methodology and Materials: A total of 10 samples of smoked-cured bangus (*Chanos chanos*) samples were bought from a vendor each in two markets in Bataan. Two of the samples were vacuum-packed fishes (only from Market1), four samples were freshly-smoked, newspaper-wrapped fishes (from Market1), and the other 4 were 1-day-old-smoked newspaper-wrapped refrigerated fishes (from Market2). All of the samples were processed, aseptically, at the same day they were obtain. Abdominal muscles from each of the specimens were macerated and fractions of the mixture were placed in PDA plates (in triplicate; with Streptomycin to inhibit bacterial growth). After three days, the fungi colonies were classified based on their cultural, morphological and molecular characteristics.

For molecular characterization, two colonies with contrasting morphologies (coming from the cultures of fishes from Market2; 1-day-old-smoked newspaper-wrapped refrigerated fishes), were brought to The Philippine Genome Center (PGC) at UP Diliman, for further DNA sequencing using ITS1 and ITS4 marker. We then subjected the raw DNA sequence from PGC to sequence-alignment using BioEdit 7.2 software. The resulting aligned sequences were then compared to the NCBI database using BLASTn software.

Results: Cultures from all of the ten (10) samples yielded colonies which were identified as belonging to the genus *Aspergillus*, based on the fungi colony and hyphae morphology. Cultures from the vacuum-packed smoked milk fish, presented the least amount of fungi growth compared to the other samples. Cultures from the 1-day-old-smoked newspaper-wrapped refrigerated fishes (from Market2) presented the largest amount of fungal growth.

Molecular characterization done in revealed that the aligned DNA sequences, when compared to the NCBI database using BLASTn yielded the following: The first sample sequence showed the 99% similarity to *Aspergillus flavus.* The second sample sequence has a 95% similarity to *Aspergillus fumigatus.*

As 97% sequence similarity is the consensus acceptable for identification using DNA sequence, to the species level, the specific identity of the second colony sample is still in question. The DNA sequences of the second sample finds credence to our identification of it belonging to the genus *Aspergillus* based on colony morphology. Type cultures of the above are currently stored in the laboratory at AUF.

Conclusion: We were able to identify fungi belong to the genus *Aspergillus* in all of the fish samples bought in the two major markets in Bataan. *Aspergillus flavus* has been identified. The other species may be *Aspergillus fumigatus* but this still has to be ascertained.

As the cultures from the vacuum-packed smoked milk fish yielded the least amount of fungi growth compared to the other samples, then it appears that vacuum-packing may help mitigate though not guarantee that the fishes will be free from fungi contamination.

Aspergillus is capable of growing rapidly at 37°C and their conidia known to tolerate temperatures about 70°C. *Aspergillus* is the major producer of Aflatoxins. Aflatoxins are heat-resistant and can withstand exposure to normal cooking temperatures. Thus, it is recommended that there should be thorough review of the fish processing protocols toward the improvement of QA/QC modalities in assuring food safety.

Comparative Evaluation of *In-vitro* Antiurolithiatic Potential of *Allium sativum* (Garlic) Bulb and *Lactuca sativa* (Lettuce) Leaf Aqueous Extracts on Calcium Oxalate and Calcium Phosphate Crystallization

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The kidneys are exceptionally important organs of the human body. They have numerous functions and are mainly known to contribute to the filtration, reabsorption, secretion, and excretion of the body's waste products. They also play a role in the regulation of the blood to keep it within physiological pH through a process called acid-base homeostasis. In more recent studies, it has been found out that among the various diseases affecting the urinary tract, urolithiasis is now considered to be the third most common disease with recurrence rate of 50% affecting approximately 4-15% of human population all over the globe with a peak incidence between 20 to 50 years of age. According to the Global Burden of Disease 2015 Disease and Injury Incidence and Prevalence, Collaborators (2016), for the year 2015 alone, 22 million cases of urolithiasis have been recorded. This has resulted to an estimate of 16,000 deaths. In this study, the researchers utilized Quantitative Approach Experimental Design to compare and evaluate the antiurolithiatic activity of *Allium sativum* bulb and *Lactuca sativa* leaf aqueous extract on synthesized calcium oxalate and calcium phosphate crystals in comparison to a well-known *Ayurveda* – Cystone which served as the positive control in search for an alternative treatment for urolithiasis which is cost-effective and confer less harmful side-effects. Specifically, the researchers measured the significant differences between the degrees of dissolution and inhibition exemplified by the plant materials in three assays conducted all *in vitro* – Dissolution Assay using a semi-permeable membrane, Nucleation Assay, and Aggregation Assay. These three assays were done to mimic the pathogenesis of stone formation involving the successive physiochemical events of supersaturation of urine, nucleation, growth, and aggregation of calculi within the renal tubules. The crystallization of the calcium oxalate begins with the increased concentration of urine with the subsequent formation of the solid crystalline particles within the urinary tract followed by nucleation wherein stone-forming salts in supersaturated urinary solution coalesce into clusters that gradually increase in size. These crystals grow and aggregate with other crystals in solution, and are retained and accumulated by the kidney. Therefore, an important protective factor in the prevention of the development of the disease is the inhibition of the agglomeration of calcium oxalate crystals. Since the treatment of urolithiasis is concerned in dissolving the existing stones and preventing the recurrence therefore, the study aimed to arrest the stone on its physiochemical stages of formation. In order to meet the objectives of the study, the researchers have analyzed the data gathered from the three assays by employing quantitative means of analysis utilizing *ANOVA* and *Tukey's*. Upon measuring the optical densities of the treatment groups spectrophotometrically against 620 nm, there were significant differences observed between the absorbances in Calcium oxalate and Calcium phosphate groups. This however, is not conclusive and does not provide sufficient data to establish the antiurolithiatic activity of the plant extracts – *Allium sativum* bulb and *Lactuca sativa* leaf. To further evaluate, the researchers conducted Nucleation and Aggregation Assays to measure the percentage inhibition against Calcium oxalate crystals. For the Nucleation Assay, Two-way ANOVA showed significant differences between Treatment groups and Concentration groups. Post-hoc analysis revealed that there is a significant difference between the percentage inhibition of Cystone-Garlic, and Cystone-Lettuce. Based on the computed values of the Percentage Inhibition, Cystone still proved to be the most potent among the three treatments. *A. sativum* and *L. sativa* still showed comparable antiurolithiatic properties. As reviewed from the journals, this is due to the high saponin content of the plant materials. Significant differences observed between Concentration groups were also analysed under Post-hoc between 50%-10% and 50%-20% concentrations. This is conclusive that increasing the concentration of the plant extracts is directly proportional to the amount of the crystals inhibited from clustering. The inhibitory properties of the two plant materials however, did not show significant difference implying that their inhibitory effects are almost similar. Lastly, Aggregation Assay showed significant differences between the optical densities of the treatment groups – Cystone-Garlic, and Cystone-Lettuce. Two-way ANOVA showed significant differences between the Treatment groups and Concentration groups. Post-hoc analysis revealed that there is a significant difference between the percentage inhibition of Cystone-Garlic, and Cystone-Lettuce. Based on the computed values of the Percentage Inhibition, Cystone still proved to be the most potent among the three treatments. *A. sativum* showed comparable antiurolithiatic property with Cystone as there is no significant difference observed between the two treatments upon Post-hoc analysis. As reviewed from the journals, this is due to the higher saponin content of the *A. sativum* compared to *L. sativa.* Significant differences were observed between Concentration groups under Two-way ANOVA but Post-hoc analysis did not reveal which specific concentrations have significant differences at $p=0.05$. However, the 50-10% concentration actually had a significant value near the accepted p-value of the research study ($p=0.068$). Based on the foregoing, significant differences were observed between the degrees of dissolution in terms of optical density for both the Calcium oxalate and Calcium phosphate groups. Nucleation Assay and Aggregation Assay were conducted to further evaluate the antiurolithiatic properties of the plant extracts compared to Cystone. Both extracts had significant inhibitory effects on Calcium oxalate nucleation comparable to Cystone. However, Cystone still proved to be the most potent among the three treatments. Increasing the concentration of the extracts also increases the percentage inhibition on Calcium oxalate crystals as established by the Nucleation and Aggregation Assays. In terms of clustering, both *Allium sativum* and *Lactuca sativa* have similar inhibitory effects. On the other hand, *Allium sativum* has greater inhibitory effects on crystal growth. Therefore, both extracts are potent antiurolithiatic agents. In light with the result of the study and the antiurolithiatic potential of the plant materials utilized – *Allium sativum* and *Lactuca sativa*, several *in-vivo* studies of antiurolithiasis conducted confer the activity to saponin, which is the bioactive component of the plants on study, to have stimulating effects on renal ATPases and reduction on urine ANP levels on which was not yet to be proved and covered by this study.

Keyword: Antiurolithiasis, Dissolution Assay, Nucleation Assay, Aggregation Assay

Characterization of Lytic Bacteriophage Infecting Antibiotic Resistant *Pseudomonas aeruginosa*

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Antibiotic treatment against infections caused by Gram-negative bacteria has become a major concern in the health care setting due to the emergence of drug resistant strains. Bacteriophages has been suggested as a more potent alternative remedy that could eliminate many distinctive forms of bacteria. Bacteriophages are viruses that targets one definite type of bacteria. Accordingly, the specificity of bacteriophages can be an interest because it diminishes the unwanted side-effects of antibiotics unlike treatments with phages in which they do not affect the normal microbiota of an individual since it directly targets the bacteria itself. Since phages are the most numbered microorganisms, humans are exposed to bacteriophages from the very moment of birth, and this explains the favorable tolerance for bacteriophage-based treatments (Hill, et. al, 2017). However, articles and studies related to this matter reflect little knowledge of extensive research and clinical utilization of the phage therapy in our country. The objectives of this study is to isolate and characterize a bacteriophage infecting an antibiotic resistant *Pseudomonas aeruginosa*.

The bacteriophage cocktail was isolated from sewage water in San Fernando Pampanga. Characterization was done by examining the morphology using electron microscope, pH stability, temperature stability and gene detection. Only plaque producing bacteriophages when exposed to *Pseudomonas aeruginosa* was characterized. Upon examination using electron microscopy, the isolated bacteriophages belongs to Myoviridae with long, rigid, contractile tails, the Siphoviridae with long, flexible, noncontractile tails and the Podoviridae with short, noncontractile tails. All belonging to order Caudovirales which are tailed phages having dsDNA genome. When tested for pH stability, the phage cocktail is stable at pH 5.0, 9.0 and 12.0. In terms of temperature, it is stable at 22°C, 37°C and 50°C. Stability was observed upon production of plaque forming units when the phage cocktail was exposed to the said conditions, indicating that it was able to enter a lytic cycle and lysed the bacterial host. Stability test was done to asses the conditions that are favorable for the phages to exhibit a lytic cycle indicated by plaque formation. The stability results of the phage cocktail suggests that it can be stable at conditions that are found in the human body such as the temperature and pH tolerance. Therefore further studies must be conducted to assess if the isolated phages can be use for phage therapy to treat drug resistant *Pseudomonas aeruginosa*. In conclusion, the isolated phage cocktail is effective in inhibiting antibiotic resistant *Pseudomonas aeruginosa.*

Keywords: phage cocktail, Caudovirales, plaque formation, Myoviridae, Podoviridae, Siphoviridae

Hepatoprotective Effects of *Lagerstroemia speciosa* (Banaba) Leaf Extract against CCL_4-induced Liver Damaged Rats

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Introduction: The liver is considered to be one of the most vital organs that functions as a centre of metabolism of nutrients such as carbohydrates, proteins, and lipids. Additionally, it is also handling the metabolism and excretion of drugs, waste metabolites and other xenobiotics from the body thereby providing protection against foreign substances by detoxifying and eliminating them.

Liver disease refers to any disorder of the liver. Liver disease includes the conditions like cirrhosis, or scarring of the liver, inflammation (hepatitis) from infectious (hepatitis B, hepatitis C) or non-infectious causes (chemical or autoimmune hepatitis), tumors, benign and malignant (liver cancer), metabolic disorders. Cirrhosis is an end result of a variety of liver diseases characterized by fibrosis and architectural distortion of the liver with the formation of regenerative nodules and can have varied clinical manifestations and complications.

Liver cell injury caused by various toxic chemicals (certain antibiotic, chemotherapeutic agents, carbon tetrachloride (CCl_4), thioacetamide, etc.), excessive alcohol consumption, and microbes is well-studied. The available synthetic drugs to treat liver disorders in this condition also cause further damage to the liver. Hence, herbal drugs have become increasingly popular and their use is widespread. Herbal drugs are more widely used than allopathic drugs as hepatoprotective because they are inexpensive, better culturally acceptable, better compatible with the human body and have minimal side effects. These herbal drugs have shown the ability to maintain the normal functional statues of the liver with fewer side effects.

Lagerstroemia speciosa is commonly called “Banaba” in the Philippines, while in India it is called “Pride of India”. It has proven antihyperglycemic effects hence it is used to treat Diabetes Mellitus type II and obesity. However, its hepatoprotective effects have yet to be established.

Objective: This study aims to determine the hepatoprotective effects of *Lagerstroemia speciosa* (Banaba) leaf extract against CCl_4-induced liver damage in Albino Wistar rats. Specifically, the study also aim to determine the effect of LLE on serum liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) as determinants of liver function.

Methodology: The study utilized an experimental research design. The mature leaves of *L. speciosa (Banaba plant)* were collected in Arayat, Pampanga. The leaves were pulverized and percolated and prepared into an ethanolic extract of 80% ethanol.

A total of twenty Albino Wistar rats weighing at an average of 150-200 grams each were purchased at an Animal Research and Laboratory, San Pedro, Laguna. After acclimatization for 1 week, baseline serum liver enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)) were measured. The rats were then randomly divided into five groups with four rats each (n=4) and for seven days they received treatment once per day. The group that received the normal saline was used as negative control and the group that received 1:1 (v/v) CCl_4 in olive oil with 100 mg/kg bodyweight of Silymarin was used as positive control group. Group 1 received 1:1 (v/v) CCl_4 in olive oil (CCL_4 group); Group 2 received 1:1 (v/v) CCl_4 in olive oil with 100mg/kg bodyweight of LLE (Low Dose (LD) group); Group 3 received 1:1 (v/v) CCl_4 in olive oil with 250 mg/kg bodyweight of LLE (High Dose (HD) group).

The extract was administered via oral gavage for 7 days, after which all rats were sacrificed by cardiac puncture.

Assays were carried out in triplicate for every liver function test. The results were expressed as mean and standard deviation values. Differences between means were determined by the analysis of variance (ANOVA) and Bonferroni Multiple Comparison Test which were analyzed with Stata 13.

Results and Discussion: The Control Group showed little change in the liver enzymes after seven days of normal diet and administration of normal saline only. There was a markedly increased levels of liver enzymes in the CCl_4 group as expected since no treatment was given to the animal subjects. The rats treated with the CCl_4 + 100mg/kg of Silymarin had a lower increase in their liver enzymes compared to those of the CCl_4 group. Also, both the Low and High Dose groups that were given CCl_4 + 100mg/kg and 250mg/kg LLE respectively, had a lower increase in their liver enzymes compared to the CCl_4 group.

The High Dose group gave a lower increase in their liver enzymes compared to the Low Dose group. However, both of the experimental groups still had higher levels of their liver enzymes compared to those of the Silymarin group. One-way ANOVA reveals that there is a significant difference in all the results of the experiment proper (with all $p\text{-values} < 0.05$). the results were also consistent with the Bonferroni Comparison Test, the researchers were able to compare all groups and were able determine that the mean differences of the serum liver enzymes of the rat samples with the Silymarin group having the lowest mean difference among the three, followed by the High Dose group then the Low Dose group.

Conclusion: This study was successful in showing the hepatoprotective effect of L. speciosa leaf extract in CCl_4 induced liver damage in Wistar albino rats by conducting in-vivo tests. These tests indicated that feeding of LLE to CCl_4 intoxicated rats prevented significant liver damage as evidenced by lower serum liver enzymes in the experimental groups compared to the negative control group. Moreover, there is an observed significant difference between the low dose and the high dose LLE as seen in the results. Although not as efficient as the positive Silymarin group, the high dose LLE proved to be of near comparison to the market-standard Silymarin. The researchers can also conclude from this information that LLE has antioxidant effects since the mode of action of CCl_4 in inducing liver damage is through the production of free radicals as a result of lipid peroxidation in the liver. Therefore, the hepatoprotective effects of LLE can be associated to its antioxidant effects.

Although the research had promising results, the researchers encourage to do phytochemical analysis in the future studies to confirm the active constituents of the plant specimen used in their locality. In addition, since there were only 2 extractions (pre and post induction), it can be worth studying the liver enzymes of the test subjects at mid-experiment to establish a trend or establish the time of onset of the hepatoprotective effects of LLE.

Keywords: hepatoprotective, Lagerstroemia speciosa, CCl_4-induced, liver damage