HEALTH SCIENCES DIVISION

HSD-1

APPLICATION OF AN ALTERNATIVE ASSAY IN DETERMINING EYE IRRITATION POTENTIAL OF PHARMACEUTICAL PRODUCTS

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In-vitro assays which accurately assess safety of pharmaceutical products and replace, refine and reduce animal testing are currently undergoing international validation for regulatory acceptance. One of the *in-vitro* methods being adopted in UNILAB as an alternative to the Draize test is the HET-CAM*. It is simple, rapid, sensitive and inexpensive. The qualitative nature of this assay is compensated by the inclusion of positive standards and a comprehensive scheme for scoring irritant effects.

Seven-day old fertilized hen's eggs are rotated for 2 days in an incubator and candled next day to ascertain embryo viability. The shell around the air cell is pared off, exposing the chorioallantoic membrane. Test chemicals are instilled on the CAM for 5 minutes. Appearance of hemorrhage, lysis and/or coagulation of blood vessels and the time when these reactions occur are noted. Irritancy Scores (IS) are determined to evaluate irritation potential of the chemical.

A total of 43 chemicals were assayed. Out of 17 ZEBET** reference chemicals tested, 16 resulted in similar classifications. Sixteen compounds with published EU and OECD classifications yielded comparable results. Of these, 11 chemicals obtained the same irritation potential as those classified by EU and OECD, while 5 chemicals were classified differently. Another 15 chemicals, previously unclassified, were included to establish internal reference standards. Finally, 21 UL products including 8 samples of feminine wash, 5 facial lotions and 8 lotions with sunscreen were tested and classified as non- to slight irritants using the HET-CAM test. These confirm results of earlier experiments using the *in-vivo* Draize test.

While most chemicals tested using HET-CAM agreed with classifications published by ZEBET, EU and OECD***, a more comprehensive database of different pharmaceutical and dermatological products is needed.

For current purposes, employing the GHS**** stepwise approach, HET-CAM could be used to identify severe irritants without need for animal testing.

* Hen's Egg Test – Chorioallantoic Membrane
** ZEBET: Test protocol used in Phase II of German Validation Study for Replacement of Draize Eye Test
*** Organization for Economic Cooperation and Development
**** Globally Harmonized System

Keywords: hemorrhage, lysis, coagulation, chorioallantoic membrane, irritation potential

HSD-2

ANTITRICHOMONAL ACTIVITY OF PLANT EXTRACTS FROM FAMILY LAMIACEAE (*COLEUS BLUMEI*, *OREGANUM VULGARE*, AND *VITEX NEGUNDO*): IN VITRO STUDIES

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Trichomoniasis is an infection caused by the protozoan *Trichomonas vaginalis*, and ranked as the most common nonviral sexually transmitted disease (STD) in the world. A recent Philippine study on the prevalence of trichomoniasis estimates an infection rate of 37% among Filipino women. Efforts to contain and alleviate this problem remain a continuing initiative of government health institutions. As an alternative to conventional therapeutic regimen utilizing endemic natural products, this study was conducted to determine the activity of three crude ethanolic plant extracts from family Lamiaceae (i.e., *Coleus blumei*, *Vitex negundo*, and *Origanum vulgare*) against *T. vaginalis*. The crude extract from *C. blumei* exhibited the highest activity among the treatments, with a minimum inhibitory concentration (MIC) of 2 mg/mL, and an activity not significantly different from metronidazole, the drug of choice for the treatment of

trichomoniasis. Phytochemical analysis of the *C. blumei* extract indicated the presence of glycosides, plant acids, reducing agents, and alkaloids. Some of these substances have been reported to possess antitrichomonal or antiprotozoal activity, and would likely account for the observed activity of the *C. blumei* extract. The mice toxicity assay revealed minimal side effects even at several magnitudes of concentration (250-500X) higher than the MIC, suggesting a wide margin of safety for the crude extract. These initial findings warrant further studies to isolate, purify, and elucidate the active antitrichomonal natural product present in the *C. blumei* extract, and to explore its potential as an alternative STD medication for human use.

Keywords: Trichomoniasis, *Trichomonas vaginalis*, Lamiaceae, *Coleus blumei*, *Vitex negundo*, *Oreganum vulgare*, minimum inhibitory concentration, herbal medication

HSD-3

EVALUATION OF ANTIMICROBIAL ACTIVITIES OF *E. CAMALDULENSIS* DEHNH LEAF OIL AGAINST SCABIES

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The study was designed to evaluate the leaf oil extracted from *E. camaldulensis* by steam distillation for the treatment of scabies. The study was done in a depressed area in Barangay Del Remedio, San Pablo City where scabies is prevalent. Twelve subjects were selected for treatment. Six were treated with eucalyptus oil and six were treated with guava leaf extract, both in the form of ointment, for comparison.

The leaf oil was clear yellow in color, sp. gr. (25°C) 0.9518 and refractive index (25°C) 1.454. Gas chromatograph and GC-MS analysis revealed it has 25 monoterpene components, eight of these compounds were identified based on authentic samples, to wit: ?-pinene, ?-pinene, cineole, citronellol, citronellal, eugenol, terpineole, phellandrene. The methanolic fraction of the oil indicated bioactivity against some microorganism. A positive response against scabies was observed after 7 days treatment with eucalyptus oil ointment unlike guava leaf extract which took 12 days of treatment.

This result has significance in that the oil is a potential alternative medicine for the treatment of scabies which is a common disease suffered by the disenfranchised members of our society.

Keywords: leaf oil, monoterpenes, *E. camaldulensis*, GC, GC-MS, scabies

HSD-4

ANTI-INFLAMMATORY COMPONENT FROM THE EXTRACT LEAVES OF *AMARANTUS VIRIDIS LINN* (KOLITIS)

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Amaranthus viridis Linn is a common roadside weed on lowlands and low altitudes throughout the Philippines. It is an erect, smooth, branched unarmed herb growing from 30 to 80 cm. tall. While the plant may be at times be purple-reddish in color, it is generally green The aim of this study was to isolate the bioactive components from the leaves of Amaranthus viridis and examine its anti-inflammatory property. One thousand grams of air dried leaves were soaked in 5 li of ethyl acetate for 72 hours, the crude extract was concentrated with arotary evaporator, constituents separated by column chromatography using petroleum ether-ethyl acetate.

The purified isolate was elucidated by infrared spectra, ultraviolet spectra and gas chromatography-mass spectra analysis. Anti-inflammatory assay was done with the crude extract. The percentage yield in the extraction process was 2.42%. The compound was insoluble in water and soluble in non-polar solvents. The crude extract was positive for tannins using ferric chloride solution.

Infrared spectra of the compound K_1 gave the following functional group: C-H aromatic stretch, C-H stretch, C=0 stretch and OH bend. The ultraviolet spectrum gave a maximum absorption was at 237nm with an absorbance of 2.035 which indicated the presence of an aromatic and carbonyl groups. The gas chromatography-mass spectra of K_1 showed the presence of 2-thiopheneacetic acid, heptyl ester.

The IR spectrum of K_2 isolate showed a broad peak at 3353.81cm^{-1} indicating the presence of OH group. Sharp signals at 2923.21 cm^{-1} and 2852.43 cm^{-1} were detected indicating C-H stretching for methyl and methylene groups respectively. Sharp peaks at 1640.28 cm^{-1} showed C=0 stretch indicating a carbonyl group which falls in the range of carboxylic stretching. Medium signals detected at 1451.52 cm^{-1} indicated a C=C bending and a signal at 1259.82cm^{-1} for Ar-OR stretch. The λ max for K_2 was at 245 nm. The gas chromatography-mass spectra for K_2 matched the chromatogram of phytol with a molecular weight of 296 and a molecular formula of $C_{20}H_{40}O$.

The crude extract was tested for possible anti-inflammatory activity with carrageenan-induced edema assay was used. Percentage inhibition or protection of the extract was obtained and compared to the positive control of aspirin. In 250-500mg/kg dose of the crude extract injected to the rats gave negative percent protection but in 1000 mg/kg dose gave positive result of 15.87%

Keywords: anti-inflammatory, carrageenan-induced edema, infrared spectra, ultraviolet spectra, gc-mass spectra

HSD-5

PROXIMATE ANALYSIS AND CHROMATOGRAPHIC CHARACTERIZATION OF ANTIMICROBIAL COMPOUNDS OF INDIGENOUS MEDICINAL FUNGI (*Ganoderma lucidum* Karst.)

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Increasing fungal and bacterial resistance have already made many anti-fungal drugs and antibiotics obsolete. Thus, the quest for effective and cheaper alternatives never ceases. The role of fungi, *Ganoderma lucidum* in particular, as a source of medicine has always been considered, but research pertaining to it seems to be less extensive.

The supernate was used in proximate analysis following AOAC (1980) procedure. Characterization of antimicrobial components was investigated using TSK-G 4000 PWXL column with 0.7% Na2SO4 solution as the mobile phase and RI for detection. Recently, the feasibility of employing high performance thin-layer chromatography (HPTLC) for the fingerprint profiling of polysaccharides extracted from the fruiting bodies of the medicinal fungi. HPTLC chromatogram of acid hydrolyzates of polysaccharides from water extracts of three different *Ganoderma* species were obtained under total hydrolysis conditions.

Proximate analysis of three medicinal fungi species were determined in terms of general food analysis, inorganic matter, vitamins and antimicrobial components. Average protein and carbohydrate contents of the three isolates were moderately high, equivalent to 29.30 and 43.60 %, respectively. Low amounts of fiber, fat and ash were noted. Dried basidiocarp contains high amounts of potassium, phosphorous and magnesium and fair amounts of calcium, iron and sodium. High amounts of choline (1,274 mg/100g) and inositol (319.91mg/100g) were noted. Antimicrobial component like polysaccharide G (PSG) was identified at an average of 16.93%.

Hydrolysis with total free acids (TFA) and determination by high-pH anion-exchange chromatography revealed that a bioactive proteoglycan isolated from *G. lucidum* was composed of eight different monosaccharides, predominantly d-glucose, d-galactose and d-mannose in the molar ratio of 3:1:1. Other components include galacturonic acid, glucorunic acid, galactose, arabinose, xylose and fructose. Rhamnose was detected only in water extracts of BQY002 basidiocarp.

An antimicrobial component like Polysaccharide-G (PSG) was detected and identified indicating that this medicinal fungus contains bioactive properties.

Keywords: proximate analysis, high performance thin-layer chromatography (HPTLC), *Ganoderma lucidum*, Polysaccharide-G (PSG), antimicrobial compounds

HSD-6

ACTIVE COMPOUND(S) FROM CRUDE EXTRACTS OF *Euphorbia milli var splendens*: A POTENT ANTIBIOTIC

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Search for drugs for mutant bacteria are the major problem in the field of medicine. The prevalence of people getting sick from disease causing bacteria has increased year by year due to continuous environmental destruction and pollution. Vancomycin is the only cure to fight mutant bacteria like Methicillin-resistant Staphylococcus aureus (MRSA) but it cannot be denied that these drugs have been already resisted and developed immunity by most bacteria. This study is found timely and relevant to test the potential use of flavanoids and milliamines from commonly available plants like *Euphorbia*.

Hexane, ethyl acetate and butanol extracts of *Euphorbia* milli var. splendens leaves and stem were tested against MRSA, *Salmonella* sp. #47 P^r, Str^r, Cn^r, Er^r and *Escherichia coli* B-1195 Str^r, Km^r, $Spec^r$, Ap^r by paper disc method. Zones of inhibition (dia. mm.) were measured and compared with the standard antibiotics. Bioautography (Marfori et al,2003) was done to determine the active spot against the test organisms.

Ethyl acetate extract of *Euphorbia* leaves has the highest zone of inhibition against MRSA #1 (15.5mm), MRSA #4 (17.6mm), *Salmonella* sp (13.4mm) and *E. coli* (18.9mm). However, the hexane extract of leaves greatly inhibited MRSA # 2 (13.3mm) while butanol extract of leaves inhibited MRSA #3 (16.5mm). The fractionated crude extract from the leaves and stem of *Euphorbia*, has a remarkable activity against Methicillin-resistant *Staphylococcus aureus* The active compound possess a broad spectrum antibiotic. These findings confirmed that *Euphorbia* can be an abundant source bioactive compounds.

Keywords: Euphorbia, MRSA, Salmonella, E. coli, zone of inhibition

HSD-7

MANGOSTEEN (*Garcinia mangostana*) EXTRACT...POTENTIAL AGENT AGAINST METHICILLIN- RESISTANT *Staphylococcus aureus* (MRSA)

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Methicillin-resistant *Staphylococcus aureus* (MRSA) was considered a major clinical and epidemiological pathogen in hospitals worldwide. The drug of last resort is vancomycin but there is a fear that *Staphylococci* are now resistant to vancomycin. With the emergence of resistant organisms against antibiotics, it is high time to continue search for bioactive compounds that inhibit the growth of pathogens resistant to other antibiotics.

Mangosteen (*Garcinia mangostana*), the queen of fruits, was used by tribes in Southeast Asia as early as 600 AD as a general remedy and healing agent. This preliminary study search the bioactive compounds in mangosteen that is effective against MRSA.

The crown, skin, rind and seed of mangosteen were extracted with water and 95% ethanol by blending equal parts (1:1) with water or ethanol and assayed by paper disc method against 2 strains of MRSA. Ethanol extract of rind has the highest zone of inhibition (zoi) against MRSA 1 (13.1mm.dia.) while the ethanol extract of skin produced the highest zoi (15.8mm. dia.) against MRSA 8 which is not significantly different than the control antibiotic (12.3 and 17.7, respectively). Results showed that the water extract produced smaller zoi which ranges from 6.6 mm to 7.4mm against both test organisms. The ethanol extract of the crown of mangosteen inhibited MRSA 1 and 8 (11.0mm and 13.5mm, respectively). However, the ethanol seed extract has lower zoi (8.7 mm and 10.9mm) against MRSA 1 and MRSA 8, respectively. These findings revealed that active compounds from mangosteen are also effective against MRSA.

Keywords: Mangosteen, MRSA, paper disc assay, zone of inhibition, antibiotic resistant

HSD-8

NUTRITIVE VALUE OF CAMARO

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Mole crickets (*Gryllotalpa* sp.) or aro-aro in Tagalog, are known insect pests of rice in Central Luzon which local residents have resourcefully turned into a seasonal spicy delicacy called camaro. In order to assess the potential of this native delicacy as an alternative energy and protein source for Filipinos, the mean nutritive values of the camaro were determined through proximate analysis.

Energy content of this food item was estimated based on the known percentage of protein fat and carbohydrates. The carbohydrate content was calculated as the difference between 100 and the sum of the percentages of protein, moisture, fat and ash. Conversion factors used were 16.74 kilojoules (4.0 kilocalories per gram) for protein and carbohydrates and 37.66 kilojoules (9.0 kilocalories per gram) for fat.

Results of the analysis showed that this stir-fried cricket dish is a good source of both energy and protein. Based on the 2002 recommended energy and nutrient intake of Filipinos, a single serving of camaro or 150 grams of this dish will meet 28% and 74% of the daily energy and protein requirements of adult Filipinos between the ages 19-49.

Comparing the energy and protein content of camaro with another common protein source like a 150 grams fried chicken leg showed that camaro had higher energy and protein content. Fried chicken leg weighing 150 grams will provide around 20% and 63% of the recommended daily requirement of 19-49 year old Filipinos for energy and protein, respectively. Camaro therefore, can serve as a cheap alternative energy and protein source for Filipinos.

Keywords: alternative, ash, camaro, fat, grams, insect, kilojoules, kilocalories, nutrition, protein, proximate analysis, rice

HSD-9

MULTIVARIATE ANALYSIS OF SELECTED BIOMETRIC AND SOCIOECONOMIC FACTORS AND INTESTINAL HELMINTHIASIS IN CHILDREN IN TUBOD, LANAO DEL NORTE

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Helminthiasis has long been known to be attributed to ecological and socioeconomic factors. However, rather than focusing primarily on chemotherapeutic intervention, many recent studies emphasize the importance of site-specific conditions in the proper management of infection. Guided by this framework, a study was conducted to determine levels of helminthiasis among children ages 3 to 10 years old in Barangay Poblacion, Tubod, Lanao del Norte, and apply a multivariate method in establishing relationships between helminth abundance and selected biometric and socioeconomic indicators. Fresh stools from 123 randomly selected volunteer children were screened. Biometric and socioeconomic data were obtained using the questionnaire method. Respondents showed 27% infection with *Ascaris lumbricoides* alone, 7% with *Trichuris trichura*, 2% double infection with *A. lumbricoides* and *T. trichura*, and 1% with *A. lumbricoides* and *Ancylostoma duodenale*. Canonical correspondence analysis revealed highly significant relationships between ascariasis with low body mass index, low income, slum area neighborhood, high number of household members, more people sleeping in one room, and attendance in schools. On the other hand, trichuriasis is strongly associated with the presence of cockroaches in the house, religion, and absence of toilets, while hookworm infection was significantly associated with the female gender. Our data confirmed that soil transmitted helminthiasis is very common in the sampling site in spite of government deworming program. We recommend a more comprehensive but practical approach in the design and implementation of management interventions to lessen morbidity due to soil transmitted helminths.

Keywords: ascariasis, trichuriasis, helminths, socioeconomic factors, parasitism, hookworms

HSD-10

HER2 EXPRESSION PROFILE OF A POPULATION OF INVASIVE DUCTAL CARCINOMA OF THE BREAST: CLINICO-PATHOLOGIC AND CYTOLOGIC CORRELATES

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The HER2 profile of a population of 44 randomly picked women diagnosed with invasive ductal carcinoma (IDC) of the breast was assessed using immunohistochemistry (IHC). The youngest patient was 29 years old, and the oldest was 83 years old, with a median of 56 years. Clinico-pathologic and cytologic characteristics obtained from the final pathology reports and HER2 expression scores, were correlated using the *chi square test* and Spearman correlation (StataV9). HER2 overexpression used as marker for cancer aggressiveness, was expressed in five (5) patients, which accounted for 11.36% of the total sample size. Among the five patients with overexpression of HER2, three fell within the age range of 36 to 50 years old.

Correlation analysis revealed that tumor size, one of the clinico-pathologic characteristics considered, was significantly associated with HER2 overexpression. Observed necrosis, one of the cytologic variables considered, also revealed significant correlation with HER2 overexpression. Although the Spearman correlation did not show highly significant results, careful analysis of the clinico-pathologic data revealed a positive correlation between HER2 overexpression, cancer stage, tumor size, and proportion of positive nodes or nodal status. Furthermore, two cytologic variables, namely; presence of prominent nucleus and observed necrosis also had positive correlation with HER2 overexpression.

The result of this study is valuable in improving the treatment course and prognosis of IDC by clinicians. As the first study of its kind in the Philippines, this can serve as a benchmark for similar molecular marker studies on breast cancer in women across age groups, ethnic categories, and other social clustering.

Keywords: HER2, invasive ductal carcinoma, immunohistochemistry, cancer aggressiveness, correlation analysis

HSD-11

BONE MINERAL DENSITY (BMD) AND FRACTURE RISK ASSESSMENT TOOL

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A bone mineral density (BMD) test is used to measure bone density of minerals such as calcium, determine fracture risk for osteoporosis, and monitor the effectiveness of treatments for osteoporosis. A BMD test can be performed using a special X-ray, computed tomography (CT) scan, or ultrasound. BMD results are generally scored by two measures, the T-score and the Z-score. The World Health Organization uses T-scores to define normal bone mass, low bone mass (or osteopenia), and osteoporosis. The T-score compares a person's bone density to the average bone density of young healthy adults of the same gender. A Z-score compares his bone density to the average values for a person of his age and gender. A low Z-score (below –2.0) is a warning sign that he has less bone mass (and/or may be losing bone more rapidly) than expected for someone his age.

The Bone Mineral Density and Fracture Risk Assessment Tool is an online system that aims to empower physicians with treatment management tools for effective interpretation of osteoporosis risk factors based on Kanis' Method, Black's Method, and the Canadian Osteoporotic Society Method. The risk assessment tool incorporates not only bone density, but other, important risk factors, including age, body mass index, smoking habit, corticosteroid usage, and other factors. It also classifies patients as either normal, osteopenic, or osteoporotic.

Keywords: Bone Mineral Density (BMD), osteoporosis, Kanis' Method, Black's Method, Canadian Osteoporotic Society Method, fracture risk assessment

HSD-12

MOLECULAR DESIGN OF SUNFLOWER (*Helianthus annuus*) TRYPSIN INHIBITOR-1 (SFTI-1) PEPTIDE ANALOGUES AS DENGUE VIRUS NS2B-NS3 SERINE PROTEASE INHIBITOR

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The dengue NS2B (non-structural protein 2B) cofactor and NS3 (non-structural protein 3) protease forms a complex that functions in polyprotein processing, thus representing an attractive target for the development of anti-viral drugs. The sunflower trypsin inhibitor-1 (SFTI-1) was considered as a good template for the design of inhibitors of the dengue NS3 protease and dengue NS2B-NS3 protease complex. In the absence of complete crystal structures of the four NS3 protease and NS2B-NS3 protease corresponding to the four serotypes of the dengue virus, homology modeling was performed. Structural analysis of the models revealed a conformational change upon binding of the NS2B cofactor to the NS3 protease. Trp61 of the NS2B cofactor provided Π-cation interaction with the protease residue 142 of dengue 1, dengue 2 and dengue 4. The loss of this interaction for dengue 3 was compensated by the formation of a salt-bridge between Glu63 of the cofactor with Arg142 of the protease. Docking experiments of the SFTI-1 peptide analogues with the homology models was able to identify an analogue with the sequence GNleCRRSGSGHCFPD as the most potent inhibitor of any of the four serotypes of the dengue virus. This peptide analogue also exhibited a structural homology with the template with an RMSD of 0.82Å and 0.76Å for the backbone and α-carbon atoms respectively. Langevin dynamics simulation of the docked structures of this peptide analogue showed increased distance with respect to the Ser135 γ oxygen of the NS2B-NS3 protease and Arg5 carbonyl atom of the peptide analogue compared to the NS3 protease only. The results indicate that the peptide analogue can inhibit any serotype of the dengue virus with the NS2B-NS3 protease having a longer time to cleave the scissile bond of the analogue, thus it can slow down or totally inhibit the proliferation of the virus inside the human body.

Keywords: homology modeling, SFTI-1 analogue, NS3 protease, NS2B-NS3 protease

HSD-13

PCR-RFLP DETECTS CLARITHROMYCIN-RESISTANT *Helicobacter pyolri* STRAINS ISOLATED FROM BIOPSY SAMPLES

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Helicobacter pylori is a human pathogen associated with chronic gastritis, peptic ulcer disease and in cases of adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Eradication of this organism has become a medical challenge due to increasing incidence of antibiotic resistance. However, due to difficulty of growing the organism, detecting its antibiotic susceptibility is another problem. In this study, clarithromycin- resistant *H. pylori* isolated from Filipino patients was determined using *23s rRNA gene* mutation and sensitivity testing.

Nineteen strains of *H. pylori* from gastric biopsies of patients with gastroduodenal diseases were cultured. DNA was extracted (QIAGEN DNA Mini Kit). Point mutation (AG) on the *23s rRNA gene* at positions 2143 and 2142 was associated with clarithromycin resistance. The presence of mutation was determined using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The product, which shows a band of approximately 1.4kb, was digested using *Bsa I* and *Mbo II* enzymes. The digested fragments were separated on 2% agarose and analyzed by UV transilluminator.

The Epsilometer (E-Test) was used to determine the antimicrobial susceptibility of the 19 isolates. The strip was placed onto the surface of the agar plate inoculated with bacteria and incubated for 3 days at 37C under microaerophilic condition. The elliptical zone of inhibited growth was interpreted.

E-Test results showed 16 of the 19 strains were clarithromycin-sensitive (0.25ug/ml) while 3 strains **(153c, 189c, 193)** were resistant (1.0ug/ml). Using PCR-RFLP, 4 out of the 19 strains were resistant **(38a, 153c, 189c, 193)**. Three of these have A2142G mutation as detected using *Mbo II* and one has A2143G mutation using *Bsa I*.

In this study, we found that PCR-RFLP is an efficient tool for detection of clarithromycin-resistant *H. pylori*. It can also be used in identifying the mutation type, which will be helpful in establishing treatment regimens.

Keywords: *H. pylori*, Clarithromycin, *Bsa I, Mbo II*, PCR-RFLP

HSD-12

MOLECULAR DESIGN OF SUNFLOWER (*Helianthus annuus*) TRYPSIN INHIBITOR-1 (SFTI-1) PEPTIDE ANALOGUES AS DENGUE VIRUS NS2B-NS3 SERINE PROTEASE INHIBITOR

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The dengue NS2B (non-structural protein 2B) cofactor and NS3 (non-structural protein 3) protease forms a complex that functions in polyprotein processing, thus representing an attractive target for the development of anti-viral drugs. The sunflower trypsin inhibitor-1 (SFTI-1) was considered as a good template for the design of inhibitors of the dengue NS3 protease and dengue NS2B-NS3 protease complex. In the absence of complete crystal structures of the four NS3 protease and NS2B-NS3 protease corresponding to the four serotypes of the dengue virus, homology modeling was performed. Structural analysis of the models revealed a conformational change upon binding of the NS2B cofactor to the NS3 protease. Trp61 of the NS2B cofactor provided Π-cation interaction with the protease residue 142 of dengue 1, dengue 2 and dengue 4. The loss of this interaction for dengue 3 was compensated by the formation of a salt-bridge between Glu63 of the cofactor with Arg142 of the protease. Docking experiments of the SFTI-1 peptide analogues with the homology models was able to identify an analogue with the sequence GNleCRRSGSGHCFPD as the most potent inhibitor of any of the four serotypes of the dengue virus. This peptide analogue also exhibited a structural homology with the template with an RMSD of 0.82Å and 0.76Å for the backbone and α-carbon atoms respectively. Langevin dynamics simulation of the docked structures of this peptide analogue showed increased distance with respect to the Ser135 γ oxygen of the NS2B-NS3 protease and Arg5 carbonyl atom of the peptide analogue compared to the NS3 protease only. The results indicate that the peptide analogue can inhibit any serotype of the dengue virus with the NS2B-NS3 protease having a longer time to cleave the scissile bond of the analogue, thus it can slow down or totally inhibit the proliferation of the virus inside the human body.

Keywords: homology modeling, SFTI-1 analogue, NS3 protease, NS2B-NS3 protease

HSD-13

PCR-RFLP DETECTS CLARITHROMYCIN-RESISTANT *Helicobacter pyolri* STRAINS ISOLATED FROM BIOPSY SAMPLES

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Helicobacter pylori is a human pathogen associated with chronic gastritis, peptic ulcer disease and in cases of adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Eradication of this organism has become a medical challenge due to increasing incidence of antibiotic resistance. However, due to difficulty of growing the organism, detecting its antibiotic susceptibility is another problem. In this study, clarithromycin- resistant *H. pylori* isolated from Filipino patients was determined using *23s rRNA gene* mutation and sensitivity testing.

Nineteen strains of *H. pylori* from gastric biopsies of patients with gastroduodenal diseases were cultured. DNA was extracted (QIAGEN DNA Mini Kit). Point mutation (AG) on the *23s rRNA gene* at positions 2143 and 2142 was associated with clarithromycin resistance. The presence of mutation was determined using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The product, which shows a band of approximately 1.4kb, was digested using *Bsa I* and *Mbo II* enzymes. The digested fragments were separated on 2% agarose and analyzed by UV transilluminator.

The Epsilometer (E-Test) was used to determine the antimicrobial susceptibility of the 19 isolates. The strip was placed onto the surface of the agar plate inoculated with bacteria and incubated for 3 days at 37C under microaerophilic condition. The elliptical zone of inhibited growth was interpreted.

E-Test results showed 16 of the 19 strains were clarithromycin-sensitive (0.25ug/ml) while 3 strains (**153c, 189c, 193**) were resistant (1.0ug/ml). Using PCR-RFLP, 4 out of the 19 strains were resistant (**38a, 153c, 189c, 193**). Three of these have A2142G mutation as detected using *Mbo II* and one has A2143G mutation using *Bsa I*.

In this study, we found that PCR-RFLP is an efficient tool for detection of clarithromycin-resistant *H. pylori*. It can also be used in identifying the mutation type, which will be helpful in establishing treatment regimens.

Keywords: *H. pylori*, Clarithromycin, *Bsa I, Mbo II*, PCR-RFLP

HSD-14

CHARACTERIZATION OF HEPATITIS C VIRUS GENOTYPE 1 ISOLATES USING PCR-RFLP AND SEQUENCE ANALYSIS

Michael O. Baclig,[1] May M. Rivera,[1] Rey Z. Predicala,[1] Mark Pierre S. Dimamay,[1] Ronald R. Matias,[1] Filipinas F. Natividad,[1] Juliet Gopez Cervantes,[2] and the Liver Diseases Study Group

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Introduction: Hepatitis C virus (HCV) can be classified into six major genotypes, HCV-1 to HCV-6, and many subtypes with the predominant genotype in most areas of the world being genotype 1. Studies have shown that patients with HCV-1 infection are more likely to develop liver cirrhosis and hepatocellular carcinoma than patients with other HCV genotypes. Furthermore, variations at the subtype level may play a role in the progression of the disease. Therefore, accurate genotyping and subtyping is important in the clinical management and epidemiological studies of Hepatitis C infection.

Methodology: HCV genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the 5' non-coding region (5'-NCR) using five restriction enzymes. Subtypes of HCV-1 isolates were identified by sequence analysis of the non-structural protein 5A (NS5A) gene. Sequence data were analyzed using BioEdit software. Nucleotide sequences were compared for homology with sequences in the National Center for Biotechnology Information GenBank using basic local alignment search tool (BLAST) program.

Results: Out of the 15 isolates, 4 (27%) and 11 (73%) were classified as HCV-1a and HCV-1b, respectively using PCR-RFLP. Sequence analysis disclosed that all of the isolates were subtype 1b.

Conclusion: HCV-1b is the predominant genotype in this study. Sequence-based analysis of the NS5A region can be used for accurate identification of Hepatitis C virus subtypes.

Keywords: Hepatitis C virus, non-structural protein 5A, sequencing, genotyping, subtyping

HSD-15

TREATMENT OUTCOMES IN TB SYMPTOMATICS ENROLLED AT THE PTSI-QI DOTS, YEAR 2006

Lynore M. Sandoval[1,2], Donna Mae de la Cruz[1], Catherine Dupra[1], Ronela Puylong[1], Delia de Castro-Ontengco*[1], Claire Lanceta[2], Diana Mallari[2] and Violeta Perez[2]

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Detection and cure remains the cornerstone for tuberculosis (TB) control. Cure and prevention of drug resistance is dependent upon the adherence of patients to the recommended anti-TB treatment regimen. Directly observed therapy short course (DOTS) ensures adherence. This paper investigated the treatment outcomes and success rate of the Philippine Tuberculosis Society, Inc.-Quezon Institute DOTS Center among 113/176 (64.2%) TB symptomatics who were enrolled and initiated to treatment during the year 2006. Using the guidelines set by the National TB Control Program of the Department of Health, the patients' sputum smears were stained with Ziehl Neelsen and searched for the presence of acid fast bacilli (AFB) at recommended periods before and during treatment. Of the 113 TB symptomatics, 64 were new smear positives; 33, new smear negatives, 13 relapse cases, one failed, and two were unclassified and did not undergo chemotherapy. The treatment outcomes were: 61 (55%) cured, 32 (28.8%) completed treatment, 1 (0.9%) died, 3 (2.7%) failed treatment, 6 (5.4%) defaulted, and 8 (7,2%) transferred-out. Treatment success rate of the PTSI-QI DOTS Center was computed at 83.8%, a little below the WHO target of 85%. This study documented the achievement of the PTSI-QI DOTS Center in their implementation of the DOTS strategy. It further highlighted the critical role played by DOTS centers in TB control.

Keywords: tuberculosis, DOTS, TB control, PTSI-QI DOTS Center, treatment outcomes

HSD-14

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Results: Out of the 15 isolates, 4 (27%) and 11 (73%) were classified as HCV-1a and HCV-1b, respectively using PCR-RFLP. Sequence analysis disclosed that all of the isolates were subtype 1b.

Conclusion: HCV-1b is the predominant genotype in this study. Sequence-based analysis of the NS5A region can be used for accurate identification of Hepatitis C virus subtypes.

Keywords: Hepatitis C virus, non-structural protein 5A, sequencing, genotyping, subtyping

HSD-15

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Keywords: tuberculosis, DOTS, TB control, PTSI-QI DOTS Center, treatment outcomes

Number (MPN) per gram. Based on the microbiological analyses conducted by the microbiologist of the Bureau of Fisheries and Aquatic Resources 7 using AOAC and APHA methods, *Perna viridis* taken from the Lapulapu market had the highest weighted mean bacterial load of 2.90 x 10^4 cfu/g, while samples taken from Mandaue City market contained 5.10 x 10^3 cfu/g. However, green mussels obtained from the two Cities of Lapulapu and Mandaue markets were not safe for human consumption based on the MPN per gram of *Escherichia coli*, which contained 460 MPN/g and 93 MPN/g, respectively. The green mussels taken from the markets of the two cities are not safe for human consumption based on MPN/g of *E. coli*, if eaten fresh, however when this commodity be cooked thoroughly, the product are safe to eat since *E. coli* can easily be destroyed above boiling point.

Keywords: *Perna viridis*, microbial flora densities,. *Escherichia coli*

HSD-20

HAND HYGIENE COMPLIANCE IN A LOCAL TERTIARY HOSPITAL IN ILIGAN CITY

Romel F. Castillano[1], Katheryne P. Coronado[1], Diane Mae A. Sanchez[1] and Lady Jane C. Fanuncio[2*]

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One third of all hospital infections have been documented to be preventable. Majority of these cases has been associated with transmission of nosocomial pathogens by the hands of healthcare workers. Hand hygiene has been identified to be the simplest and most effective course of action for preventing hospital-acquired illnesses. Registered nurses and student nurses on duty at a government-run tertiary hospital in Iligan City were asked to participate in the study for the assessment of hand hygiene compliance. Structured observations of routine patient care were conducted and hand-swab samples of the healthcare worker's dominant hand were taken to quantify bacterial colony counts at the end of a defined period of patient care. All of the subjects reported to have had formal education and informal training on hand hygiene but this did not reflect on their actual usage patterns. Few of the registered nurses or student nurses performed hand hygiene procedure in every after patient contact and at any time in between seeing ten patients. Hand hygiene procedures were done after they were told that their hands will be swabbed.

It was observed that incorrect handwashing procedures and insufficient time for hand-rubs were done. The noncompliance to prescribed hand hygiene protocols was due to the belief of low risk in acquiring infection from patients and blatant disregard to guidelines and protocols. Bacterial counts from the hands of student nurses were significantly higher than the registered nurses (p=0.052895). More than 60% of both population harbored *Staphylococcus aureus* and all had gram-negative bacilli. S. *aureus* and gram- negative bacilli are considered important nosocomial pathogens causing a wide array of severe infections. This study highlights the need for stricter adherence to hand hygiene policies among healthcare workers which may in turn be crucial in lowering nosocomial infections.

Keywords: hand hygiene, nosocomial infections, handwashing, hand-rubs

HSD-21

MICROBIAL AIR QUALITY OF GREGORIO T. LLUCH MEMORIAL HOSPITAL, ILIGAN CITY

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The rising incidence of hospital acquired infections has been attributed to the lack of cleanliness in hospitals. The risk of acquiring nosocomial infections increases with elevated aerobic bacterial counts. The level of airborne microorganisms in Gregorio T. Lluch Memorial Hospital of Iligan City was unknown and the study was carried out to monitor the bacterial densities and distribution in different areas of the tertiary hospital. The settle plate method was employed using nutrient agar for heterotrophic bacterial counts and blood agar for hemolytic microorganisms. The hospital's lobby and obstetrics-gynecology ward exhibited the highest HPC count of which occupant density was identified as a contributing factor in influencing the level of airborne microorganisms. Predominant bacterial strains were *Staphyloccus, Streptoccus* and *Enteroccocus*, all of which are considered causative agents of severe nosocomial infections. Surveillance is essential in recognizing causative factors which may lead to increased nosocomial infections and results of constant monitoring will lead to formulation of recommendations as well as guidelines for effective preventive measures.

Keywords: microbial air quality, nosocomial infections, settle plate method

HSD-16

A CROSSOVER COMPARISON OF ULTRAVIOLET IRRADIATION AND ALCOHOL DISINFECTION OF NOSOCOMIAL PATHOGENS

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The rising incidence of hospital acquired infections is compounded by the emergence of antibiotic resistance bacterial strains. These non-susceptible potentially pathogenic microorganisms associated with nosocomial infections may be transferred by person-to-person contact and can even be transmitted via airborne route. In order to reduce nosocomial infections, there is the need to employ constant monitoring of efficacy of disinfecting procedures as well as to identify of the predominant contaminant bacteria. Ultraviolet (UV) irradiation and isopropyl alcohol are commonly used for disinfecting air, various surfaces and personnel skins. Nosocomial pathogens were isolated using settle plate method for air microflora, swabs from a variety of hospital surfaces and hand cultures of healthcare workers after routine disinfection procedures. Twenty-four hour old bacterial cultures of the five predominant antibiotic resistant bacterial strains were exposed for 15 minutes at 30 watts of UV light to determine the UV resistance abilities and were mixed separately in varying concentrations of isopropyl alcohol for 30 minutes to identify resistance to alcohol disinfectants. *Enterococcus* had the highest UV resistance exhibiting growth of colonies even after nine minutes of exposure, while *Escherichia coli* had nine colonies after one minute of UV exposure. Sensitivity tests showed that 16% of *Streptococcus* remained viable in 10% alcohol and conversely *Enterococcus* were recovered in full at 25% alcohol concentration. The results have shown that all five antibiotic resistant nosocomial pathogens are still susceptible to the effects of UV and isopropyl alcohol. However, the efficacy of both disinfectants is species- specific. Strong negative relationship between the efficacy of UV and isopropyl was observed for *E. coli*, *Enterobacter* and *Enterococcus* and a moderately negative relationship was exhibited by *Staphylococcus* and *Streptococcus*. The importance of hospital microflora still remains largely ignored and epidemiology associated with local nosocomial infections remains poorly understood. Thus, there is a need of constant monitoring for formulation of effective infection control measures.

Keywords: nosocomial pathogens, UV irradiation, antibiotic resistance, disinfectants

HSD-17

SURVEILLANCE FOR METHICILLIN-RESISTANT *Staphylococcus aureus* AMONG MEDICAL PERSONNEL OF VETERANS MEMORIA MEDICAL CENTER

Juniper G. Germinal, Marie Grace B. Lavadia, Suzette R. Malapad, Cariene N. Tormon, Hermia Mae D. Villapando, and Delia C. Ontengco*

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Methicillin-Resistant *Staphylococcus aureus* (MRSA), also known as Oxacilin-Resistant *S. aureus* (ORSA), is a significant hospital-associated pathogen that resists multiple beta-lactam antibiotics. The spread of MRSA can be difficult to control because of asymptomatic nasopharyngeal carriage, which threatens the safety of patients when healthcare personnel carries them. This study determined the incidence of MRSA among hospital staff of Veterans Memorial Medical Center (VMMC). Forty medical personnel (10 physicians, 10 medical technologists, 10 nurses, and 10 nursing aides) were randomly selected, their forearms and external nares swabbed, which were then cultured for the presence of MRSA using Mannitol Salt Agar for isolation, amd the Oxacillin and Cefoxitin Disk Tests for susceptibility. There were reports that some of these MRSAs may have inducible clindamycin resistance, and the D test was also performed. Results showed that 17/40 (42%) and 29/40 (73/40) of the isolates were *S. aureus*, from the forearms and nares, respectively. From these, 5/17 (29%) from the forearm was identified as MRSAs (from two medical technologists, one nurse, and two nursing aides), and from the external nares, 5/29 or 17% (from two physicians, one medical technologist, and two nursing aides). One nursing aide was colonized with MRSA in both nares and forearm. Methicillin-sensitive *S. aureus* with inducible clindamycin resistance was identified from the forearm of one medical technologist. Our findings indicate the presence of MRSAs among healthcare workers that could facilitate unknowingly the spread of MRSAs in the hospital setting. Medical personnel, therefore, should use proper hygienic measures to prevent transfer of the organism from themselves to patients or among patients.

Keywords: Methicillin-Resistant *Staphylococcus aureus*, MRSA, nasopharyngeal carriage, healthcare workers, ORSA, inducible clindamycin resistance

HSD-17

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Keywords: Methicillin-Resistant *Staphylococcus aureus*, MRSA, nasopharyngeal carriage, healthcare workers, ORSA, inducible clindamycin resistance

On a daily basis, close to 6,000 individuals were served with 65% school children, 23% young adults, and 12% adults.

Although fish ball street vending has been shown to be a good livelihood requiring little investment and technical skill, a number of factors were identified as potential risk factors in terms of food safety and public health. Fly infestation, dirty fingernails of food handlers, lack of personal hygiene and garbage disposal, polluted surroundings, lack of clean water source, and use of recycled cooking oil, are the identified risks in fish ball street vending.

There is therefore an urgent need to address these risk factors through the formulation and implementation of pertinent ordinances for street food vending by the local government units. The academe will have an important role in information, education and training for street food vendors.

Keywords: street food, fish ball, food safety, public health, risk factors

HSD-19

MICROBIAL FLORA DENSITIES OF *Perna viridis* DISTRIBUTED IN THE CITIES OF MANDAUE AND LAPULAPU MARKETS, CEBU PROVINCE

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Green mussels *Perna viridis* is commonly distributed in the province of Cebu particularly in the Cities of Lapulapu and Mandaue markets and an outbreak on gastroenteritis was felt by the Mandaue residents as published by BFAR personnel (2005) in the local newspaper. The science faculty member of Tabok National High School was so concerned on this problem thus, the Cebu State College of Science and Technology (CSCST), Main Campus, College of Industrial Technology and Engineering (CITE), major in Food Technology conducted a research to investigate the safety of green mussels, locally known "tahong" distributed in the two cities in collaboration with the Science Department of Tabok National High School, Mandaue City, based on the microbial flora densities and evaluate its potential hazard specifically on the *Escherichia coli* in Most Probable

Number (MPN) per gram. Based on the microbiological analyses conducted by the microbiologist of the Bureau of Fisheries and Aquatic Resources 7 using AOAC and APHA methods, *Perna viridis* taken from the Lapulapu market had the highest weighted mean bacterial load of 2.90×10^4 cfu/g, while samples taken from Mandaue City market contained 5.10×10^3 cfu/g. However, green mussels obtained from the two Cities of Lapulapu and Mandaue markets were not safe for human consumption based on the MPN per gram of *Escherichia coli*, which contained 460 MPN/g and 93 MPN/g, respectively. The green mussels taken from the markets of the two cities are not safe for human consumption based on MPN/g of *E. coli*, if eaten fresh, however when this commodity be cooked thoroughly, the product are safe to eat since *E. coli* can easily be destroyed above boiling point.

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HSD-20

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HSD-21

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Keywords: microbial air quality, nosocomial infections, settle plate method

HSD-22

PROTECTING PATIENT IDENTITY THROUGH DEIDENTIFICATION OF PATIENT MEDICAL DATA: A CRUCIAL STEP TO MEDICAL RESEARCH

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One of the benefits of computerization of patient records is that it enables the researcher, if given permission by the hospital's Institutional Review Board (IRB), to access these data to answer a number of new and exciting research questions. However it is also important to maintain patient's anonymity to ensure his privacy. This paper discusses the techniques that can be employed to implement deidentification over two different database environments: 1) text-based pathology case report files, and 2) entire patient database in order to remove clues that would lead to the patient's identity. These techniques include substitution and encryption. Furthermore, data masking techniques that are not applicable for medical research are likewise discussed.

An overview of SPIN (Shared Pathology Informatics Network), an open-source, publicly accessible database of deidentifed patients with surgical pathology reports, is presented to illustrate how deidentification can be implemented in text-based pathology case report files. This is followed by a discussion of Data Masker, a proprietary deidentification tool that can operate on an entire database and whose degree of customizability, i.e. depth of cleansing required, is at the discretion of the user.

By using a database of deidentified patients provided by the institution, both the researcher and the institution can avoid possible litigation costs due to alleged violations of one's privacy or due to alleged misuse of identified patient information. Some researchers can make their deidentified patient database downloadable and hence can easily be loaded onto a number of statistical software to encourage others to do more research. Furthermore, other researchers will be able to validate claims made by the researcher or improve upon his conclusions. Without access to such data, readers are asked to just accept the findings as an act of faith, rather than as a scientific conclusion.

Keywords: deidentification, data masking, data scrubbing, anonymization, SPIN, Data Masker, medical research

HSD-23

DETECTION OF mecA GENE IN PHENOTYPICALLY-CONFIRMED METHICILLIN-RESISTANT Staphylococcus aureus (MRSA) AMONG LOCAL CLINICAL ISOLATES

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In the Philippines, the presence of the *mecA* gene on MRSA clinical isolates has not yet been established. A head-to-head comparison using the automated Vitek system, the Oxacillin and Cefoxitin Disk Tests, and polymerase chain reaction (PCR) was done to correlate the utility of the phenotypic and molecular assays in detecting oxacillin resistance in local staphylococcal clinical isolates. Using the PCR-amplified products of *mecA* gene as gold standard, MRSAs were confirmed in 32.9% or 23/70 clinical isolates: five (5) from the lower respiratory tract, one (1) from the upper respiratory tract, three (3) from the genito-urinary tract, eight (8) from the skin/soft tissues, four (4) from the blood, and two (2) from other sources. Results of the study indicated that Oxacillin (OXA) and Cefoxitin (FOX) Disk tests would detect oxacillin-resistance in *S. aureus* at a sensitivity of 82.6%, specificity of 100%, and efficiency of 94.3%. However, based on the results, the Vitek system would only be sensitive at 43.5% and efficient at 81.4%. It should be done in conjunction with a supplementary test that is more reliable.

Keywords: MRSA, Methicillin-Resistant *Staphylococcus aureus*, *mecA* gene, Oxacillin Disk test, Cefoxitin Disk test, Vitek

HSD-24

PRE-CLINICAL STUDY ON THE ANALGESIC EFFECT OF ESSENTIAL OILS FROM SOME PHILIPPINE PLANTS

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The analgesic activity of seven (7) locally produced essential oils of *Cymbopogon citratus* (DC) Stapf. (lemongrass), *C. winterianus* (citronella), *Eucalyptus camaldulensis* (eucalyptus), *Zingiber officinale* (ginger), *Psidium guajava* (guava) and *Cinnamomum mercadoi* Vidal (cinnamon) bark and leaves was evaluated using the Plantar test or Hargreaves method. Bio-assay of these oils involved the use of Sprague-Dawley rats with acetyl salicylic acid (aspirin) and normal saline solution (NSS) as the positive and negative controls, respectively. Three (3) increasing doses of the test material were given orally to the animals. Physico-chemical properties of these oils were also analyzed.

Among the essential oils studied, ginger oil exhibited the highest analgesic activity of 58.3% at 500 mg/kg dose. Lemongrass oil showed analgesic activity of 47.8% at the same dose, while essential oils of guava and cinnamon leaves exhibited slight % protection. Guava oil gave 36.8% while cinnamon leaf oil showed 40.0%. Low % protection was exhibited by eucalyptus oil.

The positive control aspirin showed % protection of 52.2% at 300 mg/kg dose.

The results obtained will serve as a basis in the development of analgesic products from natural essential oils

Keywords: analgesic, *Cymbopogon citrates, Zingiber officinale*

HSD-25

CAFFEINE: A NOVEL SLIMMING AGENT

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A fat-burner cream was developed using phytobioactives that include a citrus oil and caffeine. An oil-in-water emulsion was formulated incorporating the phytobioactive ingredients in concentrations of 3.0% and 5.0%. A placebo was also prepared for use as control. Clinical testing for the efficacy claim substantiation of the formulated product as fat-burner/slimming agent was conducted by dermatologists from Ospital ng Maynila Medical Center for 4 and 8 weeks of testing involving 63 subjects. Subjects were grouped into three (3) for 3.0%, and 5.0% concentration of phytobioactives; respectively; and placebo. Changes in weight, waist ((W) & hip (H) measurements, W-H ratio and body mass index (BMI)

were measured. Results showed that both concentrations (3% and 5%) of the fat-burner cream were statistically superior to placebo on all efficacy parameters. Reduction in weight, waist and hip measurements generally occurred during the 8-weeks of treatment. Of the groups analyzed, the highest reduction on efficacy parameters occurred in group 2, making the 5% concentration of the fat-burner cream more effective over the 3% preparation.

Keywords: phytobioactives, BMI, caffeine