

HEALTH SCIENCES

HS - 01

**ISOLATION AND IDENTIFICATION OF CONSTITUENTS
FROM THE ANTITUBERCULAR DCM FRACTION FROM
THE LEAF EXTRACT *Premna odorata* BLANCO**

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In recent years, the resurgence of multi- and extensively-drug resistant strains has prompted the need to develop safe drugs as quickly and efficiently as possible. In a previous study, the dichloromethane sub-extract (PoMD) obtained from the leaf extract of the Philippine medicinal plant *Premna odorata* Blanco was found to be active against *Mycobacterium tuberculosis* H₃₇Rv, exhibiting 99% inhibition at 128 µg/mL and 64 µg/mL concentrations. This study aims to purify the dichloromethane sub-extract and identify the constituents present therein. The PoMD extract was subjected to vacuum liquid chromatography (VLC) with gradient elution using hexane/dichloromethane and dichloromethane/methanol. Thin layer chromatographic monitoring resulted in 20 pooled fractions which were bioassayed using Microplate Alamar Blue Assay (MABA). The fractions PoMD.4,5,7,8,9 and 15 were found to be active, having a minimum inhibitory concentration (MIC) of 53.87, 119.71, 117.12, 113.12, 82.80 and 108.91 µg/mL, respectively. The high yielding PoMD.8 was further subjected to VLC as above and gave 7 pooled fractions. PoMD.8.2 and PoMD.8.4 were further purified by column chromatography with isocratic elution using hexane to obtain PoMD.8.2.1 and PoMD.8.4.1 as white flakes. Both isolates were characterized by spectroscopic methods and were found to be a mixture of β-sitosterol and stigmasterol. Purification of the other fractions is ongoing.

Keywords: *Premna odorata* Blanco, *Mycobacterium tuberculosis* H₃₇Rv, Tuberculosis, Philippine Medicinal Plant, Steroids

HS - 02

GENETIC POLYMORPHISM OF CYP2D6*10 GENE AMONG FILIPINOS

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Cytochrome P450 (CYP2D6) is one of the major drug metabolizing enzymes involved in the biotransformation of many clinically important medications including opioid analgesics. CYP2D6 enzyme activity varies considerably within a population. Particularly, CYP2D6*10 allele is more common among Orientals than among Caucasians. A recent study among Chinese individuals showed that the allele frequency of CYP2D6*10 (C188T) was about 37-70%. Phenotypic expressions include individuals with ultrarapid, extensive, intermediate, and poor metabolizer status. It has been shown that the various phenotypes have profound effects on the efficacy of drugs as well as its adverse reactions.

In this study, we determined the allele and genotype frequencies of CYP2D6*10 among Filipinos using PCR-RFLP and sequence analysis. Blood samples were obtained from healthy study participants and patients with chronic pain, with diagnosed cancer pathology stages 1 to 4.

The calculated allele frequencies in Hardy Weinberg equilibrium were 0.49 and 0.51 for the CYP2D6*10 dominant and recessive alleles, respectively. Out of the seventy seven samples, 18 (23%) were identified as homozygous for the wild type, 40 (52%) were identified as heterozygous, and 19 (25%) were identified as homozygous for the mutant allele. Our results showed that majority of the Filipinos were heterozygous for the intermediate allele (52%).

PCR-RFLP and sequence analysis provides a useful tool for CYP2D6*10 genotyping. The allele frequency of CYP2D6*10 was comparable with other Asian populations. Individuals heterozygous for the intermediate allele were found to be the predominant genotype among Filipinos.

Keywords: allele frequency, CYP2D6*10, PCR-RFLP, polymorphism, sequencing

HS - 03

**SINGLE NUCLEOTIDE POLYMORPHISM GENOTYPING
OF ANTITHROMBOTIC THERAPY RESISTANCE
MARKERS USING HIGH RESOLUTION MELT ANALYSIS**

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High resolution melt (HRM) analysis is a powerful technique for detecting DNA sequence variations such as mutations, polymorphisms and epigenetic differences. Single Nucleotide Polymorphisms (SNPs) in the CYP3A4 and CYP2C19 genes coding for Cytochrome P450 enzymes involved in the metabolism of nearly all drugs have been reported to be associated with resistance to clopidogrel, an antiplatelet aggregation drug. Moreover, SNPs in the P2Y₁₂ gene coding for the target receptor of the active metabolite of clopidogrel have been determined to be associated with high platelet reactivity, thus possibly lowering response to clopidogrel as well. In this study, we employed HRM analysis as a diagnostic test to detect these SNPs. Primers were designed to amplify short regions containing the CYP3A4*1G (G>A), CYP2C19*2 (G>A), P2Y12 G36T (T>G) and P2Y12 C18T (C>T) SNPs. Blood samples were blotted on Whatman FTA® Elute membrane, from which DNA was extracted. Eleven samples from volunteers were used for screening the three representative genotypes, which we termed homozygous mutant, heterozygous, and homozygous wild-type. Melting temperatures and melting curves generated after fluorescence normalization, temperature adjustment, and difference analysis of the samples revealed distinct genotypes. Larger amplicons were generated by PCR for samples representative of the genotypes and sent for sequencing. Sequencing confirmed the heterozygous genotype for all SNPs, and one homozygous genotype for CYP3A4*G (mutant), P2Y12 G36T (mutant), and P2Y12 C18T (wild-type). We are awaiting the completion of sequencing for the remaining homozygous genotypes. Current results nonetheless suggest a reliable protocol for detecting the heterozygote genotype of CYP3A4*1G, CYP2C19*2, P2Y12 G36T, and P2Y12 C18T using HRM analysis.

Keywords: polymerase chain reaction, high resolution melting analysis, single nucleotide polymorphism, genotyping, clopidogrel, cytochrome P450, P2Y₁₂ platelet receptor

HS - 04

A PRELIMINARY STUDY ON THE EFFECT OF RED GRAPE EXTRACT (*Vitis vinifera*) ON THE INHIBITION OF ANGIOGENESIS ON A NINE-DAY OLD CHICK EMBRYO

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Cancer and tumor growth is the second leading cause of mortality worldwide and currently the third leading cause of death in the Philippines. Angiogenesis, or new blood vessel formation, is one of the major pathogenesis common in cancer formation and metastasis.

Studies have shown that phytonutrients found in a variety of fruits and vegetables offer many ways to offset cancer. One phytonutrient, called Resveratrol (3,5,4-trihydroxystilbene) found in red grapes, is believed to impede and prevent angiogenesis in tumor cells. Hence, the aim of this study is to determine the effect of red grape juice extract on the angiogenesis, utilizing nine days-old chicken embryos.

The experimental study employed a static group comparison where embryo egg assay was done on 50 nine-day old live embryonated chicken eggs, utilizing a modified procedure of yolk sac inoculation. Several preparations of the test agent was prepared using fresh red grapes as well as commercial grape extract and controlled amounts were administered in vitro to the each subgroup of the experimental group. The control group received same amounts of isotonic saline solution while blanks received none. All eggs were then incubated at 36-38°C and were examined after five days for any observable change.

Results showed that all three subgroups given with the test agent demonstrated angiogenesis inhibition. However, only the subgroup given with concentrated grape pulp extract showed statistically significant results at 95% confidence level. It is then concluded that red grape extracts demonstrated inhibition of angiogenesis on nine-day old chick embryos, but further studies are needed to demonstrate the said effect quantitatively, and to determine if this is specifically due to the phytonutrient resveratrol and/or other extrinsic/intrinsic factors.

Keywords: cancer, tumor, tumor cells, angiogenesis, phytonutrient, resveratrol, red grapes, embryo egg assay

HS - 05

**CHITOSAN POLY(ACRYLIC ACID) SEMI-IPN
PATCH FOR DRUG ENCAPSULATION AND RELEASE**

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In recent years, the formulation of transdermal delivery patches using biopolymers has been given extensive attention. Chitosan, a derivative of chitin, has the ability to form strong films, hence forms a prime component of wound dressing patch. The research reports the fabrication of semi-interpenetrating network (semi-IPN) hydrogel patch from chitosan(Ch) and polyacrylic acid(PAA). PAA is a biocompatible, biodegradable, and bioadhesive and can absorb a large amount of water. Semi-IPN composed of PAA with Ch and Glycidyl Trimethyl Ammonium Chloride (Quat 188) modified Ch was synthesized by polymerizing AA and crosslinking it with N,N' methylene biscarylamide in the presence of unmodified and modified Ch in deionized water at 70°C using potassium persulfate as the initiator. Ch was modified to improve its antimicrobial property. The strength of the semi-IPNs improved with increase of the crosslink density, molecular weight of Ch and Ch to PAA ratio and by Quat 188 modification. Unmodified Ch semi-IPN swelled 400% in pH 7 and 325% in pH5.5 buffer in 2h. In 2h Quat 188 modified Ch semi-IPN swelled 1200% and 1000% in pH 5.5 and 7 buffer respectively. Two drugs AgNO₃ and mafenide acetate(MFC) were encapsulated in the semi-IPNs. The semi-IPNs could incorporate 100% of free AgNO₃ in 10 minutes from its aqueous solution but showed negligible release. In case of MFC, pH dependent encapsulation and release was observed. Modified Ch semi-IPN encapsulated 95% MFC from its solution in 2h and release 56% MFC in 10h at pH 5.5 whereas unmodified chitosan semi-IPN encapsulated 86% MFC from its solution in 6h and release 35% MFC in 10h at pH 7.

Keywords: chitosan, poly(acrylic acid), semi-ipn, AgNO₃, mafenide acetate

HS - 6

PREVALENCE OF ADIPONECTIN GENE POLYMORPHISMS AMONG FILIPINO PATIENTS WITH CORONARY ARTERY DISEASE EXAMINED AT ST. LUKE'S MEDICAL CENTER

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The adiponectin gene located on chromosome 3q27 has been previously identified as a susceptibility locus for metabolic disorders and coronary artery disease (CAD). More than ten polymorphisms in the gene have been found and two of these, the rs2241766 (SNP45T>G) at exon 2 and in intron 2, rs1501299 (SNP276G>T), have been related to low circulating adiponectin levels that has been associated with obesity, insulin resistance, and CAD. The allelic distribution of the SNP45 and SNP276 polymorphisms was studied in Filipino patients with CAD using polymerase chain reaction-restriction fragment length polymorphism method. The observed allelic distribution was 0.71 and 0.29 for the T and G alleles respectively in 1,012 patients genotyped for the SNP45 polymorphism and was in accordance with the Hardy-Weinberg law using X^2 test. Genotypic frequencies were 0.503, 0.41, and 0.10 for the TT, TG, and GG genotypes respectively. In comparison, the allelic distribution of the G and T alleles were 0.57 and 0.43 respectively in 754 patients genotyped for the SNP276 polymorphism. Genotypic frequencies of 0.32, 0.49 and 0.19 for the GG, TG and TT genotypes respectively, were obtained. Linkage between the two polymorphisms was determined. The association of these polymorphisms as a risk factor for the development of CAD and Type 2 diabetes is currently being determined.

Keywords: SNP, PCR-RFLP, cardiovascular genomics, coronary artery disease, type 2 diabetes

HS - 07

**HOUSEHOLD DAILY NITRITE CONSUMPTION LEVELS
FROM SOME COMMONLY CONSUMED PROCESSED
MEATS IN SELECTED SITES/AREAS IN THE PHILIPPINES**

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Ten nitrate- and nitrite-treated meat products rank among the top 20 processed meats consumed by Filipinos (6th National Nutrition Survey, 2003). Nitrate and nitrites when ingested in high concentrations have been implicated as factors in the etiology of methemoglobinemia, intestinal/colon cancer, and type I diabetes. The study determined the contents of nitrites and the potential nitrite intakes/exposure risks in ten commonly consumed processed meats collected from selected sites in nine study areas. Popular branded, canned and plastic-packaged processed meats (i.e. hotdog, corned beef, luncheon meat, Vienna sausage, and beef loaf) were purchased in SM Supermarket - Las Piñas. Commonly consumed cured meat products (i.e. *tocino*, *longganisa*, *tapa*, *chorizo*) were also purchased in selected markets of some major processed meat-consuming cities (i.e. Las Pinas, Muntinlupa, Baguio, Bacolod, and Butuan), and provinces (i.e. Bulacan, Iloilo, Southern Leyte, and Davao del Sur). Nitrite contents of the collected meat samples were compared against the BFAD-DOH Guidelines on Food Additives (2006). Nitrite contents of most test samples within the same sampling area were found to be highly variable but still within the BFAD maximum levels. Nitrite intakes from all the processed meat consumed in each study site/area were then computed using the low and high concentration range, and compared against the JECFA-WHO/FAO (2002), Acceptable Daily Intake (ADI) of 0.07 mg nitrite per kg body weight equivalent to 4.13 mg for a 59 kg adult Filipino male. Results indicated that household nitrite intakes from processed meats in selected sites pose potential exposure risks in the cities of Las Piñas (4.84-38.46 mg/capita) and Muntinlupa (6.53-7.59 mg/capita), and in the provinces of Bulacan (6.53-7.59 mg/capita) and Southern Leyte (7.13 mg/capita).

Keywords: processed meats, nitrite, household intakes, ADI, potential exposure risk

HS - 8

**TELENURSING THRU SMS: ITS EFFECT ON
KNOWLEDGE AND ADHERENCE TO PROPER
DIET AND PHYSICAL ACTIVITY**

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This study aims to develop and implement a telenursing intervention program to increase the knowledge and adherence to proper diet and physical activity for the prevention of weight-related diseases such as Hypertension, Type II *Diabetes mellitus*, and cardiovascular diseases among overweight college students aged 18-25 years old. 24 college students undergone the DeFit It! Program. They have a body mass index (BMI) between 25.00-25.99 kg/m². Subjects in the experimental group were subjected to telenursing by receiving 4 daily short messaging service (SMS) for 21 days regarding health education on proper diet and physical activity, and reminders to follow the DeFIT it! Program. There is a significant increase in the knowledge of telenursing users before and after the study. The mean post test score of the telenursing and nontelenursing users group is 15 and 16 respectively. There is no significant difference on the physical activity and diet adherence scores of telenursing users when compared to nontelenursing users. There is a significant decrease in the BMI of the nontelenursing users group which may be attributed to their low diet adherence scores since they ate in less than the recommended amount of diet.

Keywords: Telehealth, telenursing, diet, overweight, adherence, knowledge

HS – 09

**ORAL MICROBIAL DIVERSITY ANALYSIS OF
CARIES-FREE AND CARIES-ACTIVE HEALTHY FILIPINO
ADULTS THROUGH THE 16S rRNA GENE**

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The oral cavity is inhabited by hundreds of bacterial species that play vital roles in maintaining oral health or in shifting to a diseased state like dental caries which is one of the most common oral problems that affect 92.4% of Filipinos. Due to the limitations that are posted by microbial identification through culture-dependent techniques, molecular techniques are currently being used to better understand the bacterial etiology of dental caries. This study aimed to assess the oral microbial diversity of caries-free and caries-active Filipino adults through the 16S ribosomal ribonucleic acid gene (16S rDNA).

Saliva, supragingival plaque samples from 6 caries-free and 6 caries-active adults together with carious teeth samples from the caries-active patients were used. Bacterial DNA from the samples were amplified using polymerase chain reaction. Diversity of the samples were assessed using denaturing gradient gel electrophoresis and the microorganisms were identified through sequencing of the 16S rDNA. Identified bacteria from saliva and plaque samples were compared between these groups as well as with carious teeth samples.

Based on the gathered data, the caries-free group exhibited a more diverse microflora compared with their caries-active counterpart. Several bacteria were found to be common to both groups comprising the resident microflora. *Neisseria subflava* and *Capnocytophaga* species were some of the identified potentially cariogenic bacteria since they were seen only among the caries-active adults. The partial clone library of the 16S rDNA included various species of *Leptotrichia*, *Streptococcus* and *Neisseria*.

This study showed the oral microbial diversity profile of caries-free and caries-active Filipino adults using culture-independent techniques. The saliva and plaque samples of the caries-free group exhibited greater biodiversity compared with the caries-active group. This result suggests that part of the microflora may be inhibited or absent in a caries-active state.

Keywords: dental caries, 16S rDNA, caries-free, caries-active

HS – 10

**ANTIBACTERIAL ACTIVITIES AND DIVERSITY
OF MARINE FUNGI ASSOCIATED WITH
SEAGRASSES COLLECTED FROM LUBANG ISLAND,
OCCIDENTAL MINDORO**

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Marine fungi are commonly tapped for the production of novel secondary metabolites. However, in the Philippines, very few studies explore marine fungi associated with seagrasses in spite of the numerous species of seagrasses found in the country. Our research study assessed the diversity and antibacterial activities of marine fungi (MF) associated with decaying and healthy seagrass leaves collected from Lubang Island, Occidental Mindoro. A total of 61 MF strains belonging to 15 morphospecies were isolated from surface-sterilized leaf explants inoculated on Potato Carrot Agar supplemented with 33 g/L marine salts and antibiotics. Morphocultural and molecular characterization identified the MF isolates as belonging to the genera *Aspergillus*, *Fusarium*, *Cladosporium* and *Penicillium*. Assessment of species diversity showed highest value in the host seagrass *Thalassia hemprichii*, though, highest species richness was noted in *Cymodocea rotundata*. Cluster analysis resulted in the grouping of MF based on their host seagrass. Then, 15 MF morphospecies were grown on PDA for the production of secondary metabolites. The crude culture extracts were tested for their antibacterial activities using paper disc diffusion assay. Our result showed that only one isolate, *Penicillium* sp. 1, exhibited inhibitory activities against extended spectrum beta-lactamase (ESBL)-producing and non-ESBL strains of *E. coli* and *K. pneumoniae* while five MF strains exhibited inhibitory activities against *S. aureus*. The antibacterial activities though were considered as weak. Five MF were then grown on five different media and their crude culture extracts tested against ESBL(+) strains of *K. pneumoniae* and *E. coli* using the microtiter plate assay. Results showed that two morphospecies of *Penicillium* grown on four media exhibited the lowest MIC and MBC values between 7.14 to 71.43 mg/mL. Interestingly, cultivation of MF isolates on a different culture medium resulted in the production of bioactive secondary metabolites as observed in one morphospecies of seagrass-associated *Penicillium*.

Keywords: marine fungi, fungal diversity, fungal natural products, secondary metabolites, antibacterial activities

HS – 11

HYDROGEOCHEMISTRY AND GROSS ALPHA-BETA ACTIVITIES OF AKLAN'S WATERCOURSES

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Neutron activation analysis (NAA) and gross alpha-beta measurement by Liquid Scintillation Spectrometry (LSC), were used to mark out regions with impending elevated concentrations of minerals/pollutants in the offshoot streams of the Aklan River. Five tributaries were selected as sampling points: Tinigao, Numancia, Badyangan, Mobo and Bakhaw Norte. Streambed sediments were collected from Tinigao, Numancia, Badyangan, and Mobo sites, and were analyzed for elemental composition using neutron activation analysis (NAA); surface water from all of the five sites were analyzed for bicarbonate ions by titrimetry ; pH and conductivity by selective electrodes; and radioactivity by Wallac 1414 Liquid Scintillation Counting. None of the watercourses exceeded the regulatory limits set by the Philippine National Standards for Drinking Water for conductivity, pH and bicarbonate parameters for surface waters. As far as radioactivity is concerned, each of the Tinigao, Numancia, Badyangan, Mobo and Bakhaw Norte water sample displayed total alpha activity of less than the detection limit, LLD, (LLD= 0.03 Bq/L) which was way below the drinking water regulatory limit of 0.1 Bq/L for alpha emitters; all the samples exhibited beta activities of less than LLD (LLD= 0.3 Bq/L), which were also way below the drinking water regulatory limit of 1.0 Bq/L for beta emitters. The determination of major riverbed components (Cl, Ti, Ca, Mg, V, Si, Al, Na, K, Mn) was accomplished using neutron activation analysis. All the measured parameters were put together to be able come up with a more accurate reconstruction of the ecological processes occurring across the whole watershed system.

Keywords: neutron activation analysis, NAA, gross alpha-beta, Liquid Scintillation Spectrometry (LSC), Aklan River

HS – 12

STABILITY OF BIOACTIVE COMPOUNDS AND SHELF LIFE OF RESVERATROL-ENHANCED PEANUTS

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Phenolic compounds including resveratrol, have antioxidant properties that delay aging and reduce the risk of cancer, cardiovascular and Alzheimer's diseases. Previously, we showed that combined ultrasound (US)-UV processes enhanced resveratrol in peanuts better than US or UV alone, to levels 2.8 times greater than that in red wine, the major food source. This study aims to determine shelf life of roasted resveratrol-enhanced peanuts (REP) and to investigate the stability of *trans*-resveratrol (RES), total phenolics (TP) and trolox equivalent antioxidant capacity (TEAC) during storage.

Raw peanuts were washed, sanitized, imbibed, sliced, processed using optimum US-UV by exposing to US (70 mW/cm³ power density for 10 min) followed by 50 min exposure at 40 cm distance from UV light (254 nm), incubated for 36 h at 25°C, dried, roasted, and packaged in polyethylene bags. Untreated samples were prepared as controls. Packages were stored at 30, 35 and 40°C and at ambient, about 25°C. At pre-determined intervals, a total of six sampling times/temperature, samples were withdrawn from storage, and analyzed for RES, TP, TEAC, hexanal, descriptive sensory properties and consumer acceptance.

Lipid oxidation, critical to REP's shelf life, was due processing and storage effects. Initially, REP had higher hexanal and oxidized/off-flavors intensities but lower roasted peanutty flavor and overall acceptance (OA) than controls. During storage, oxidized/off-flavors increased as roasted peanutty flavor and OA decreased. REP's shelf life was 52 days at 25°C. Lipid oxidation in REP followed first-order reaction with 0.02/day rate constant at 25°C, Q₁₀ of 2.2, and activation energy of 300 cal/mol. At the end of shelf life, *trans*-resveratrol, TP, and TEAC were reduced by 13, 8, and 27% to 3.29 µg/g, 1.76 mg GAE/g, and 6.06 µMTE/g, respectively, suggesting that *trans*-resveratrol and TP but not TEAC were stable in REP based on d⁰ 80% retained required during shelf life.

Keywords: bioactive compounds, peanuts, resveratrol, antioxidant capacity, ultrasound, UV, shelf life, Q₁₀

HS – 13

CALAMANSI WASTES FOR THE PRODUCTION OF USP GRADE PECTIN AS SOURCE OF DIETARY FIBER

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Enormous amount of wastes are being generated by calamansi juice processors in the country. These wastes pose health and environment hazard in the environment, thus its utilization into high value products is the main objective of this project.

Pharmaceutical grade pectin was produced from the wastes of *Citrus microcarpa* Bungé (calamansi) obtained from calamansi juice processing plants. Percentage yield was 10-14% (dry weight basis) by alcoholic precipitation method. The physico-chemical properties of produced pectin were analyzed and compared with standard specifications of the United States Pharmacopeia for pharmaceutical grade pectin. Techno-economic assessment of producing USP grade pectin was undertaken.

ITDI produced pectin was off-white to beige in color, odorless and had a slightly acidulous taste. It had a methoxyl content of 8.6% to 10.20% and galacturonic acid content of 77.6% to 82.2%. Degree of esterification ranged between 73.0% and 83.2%. Moisture content was determined at 10.0%. The soluble dietary fiber content was 70.6% using the enzymatic/gravimetric method. The arsenic content and the lead content were 0.15 ug/g and 0.8 ug/g, respectively, using the ashing-acid digestion and atomic absorption spectrophotometry.

The produced pectin was utilized as a source of dietary fiber where it is helpful in maintaining good digestive balance. It is advocated for those suffering from ulcer and for regulating blood pressure.

The total project cost of producing 23 kgs./month of USP grade pectin is P735,235.00. Unit cost of production per kilogram is P13,557.75, with a proposed selling price of P15,500.00 only.

Keywords: Pectin, dietary fiber, *Citrus microcarpa*

HS - 14

**CYTOTOXIC CARDENOLIDE AND STEROLS
FROM *Calotropis gigantea***

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The dichloromethane extract from the leaves of *Calotropis gigantea* Linn. was strongly cytotoxic against non-small cell lung carcinoma (A549), colon carcinoma (HCT 116) and hepatocellular carcinoma (Hep G 2) and non toxic to non cancer Chinese hamster ovary cells (AA8). The extract afforded uscharin (**1**), 3,5,8-trihydroxy-24-methylcholest-6,22-diene (**2**), a mixture of (24R)-3-hydroxy-24-ethylcholest-5-en-7-one (**3a**) and (24S)-6-hydroxy-24-ethylcholest-4,22-dien-3-one (**3b**), and another mixture of (24R)-24-ethylcholest-4-en-3-one (**4a**) and (24S)-24-ethylcholest-4,22-dien-3-one (**4b**). Compound **1** exhibited extreme toxicity to A549, HCT 116 and Hep G 2 with IC₅₀ of 0.003 µg/mL, 0.013 µg/mL, and 0.018 µg/mL, respectively, while sample **3** exhibited IC₅₀ of 1.35 µg/mL, 4.46 µg/mL, and 3.83 µg/mL, respectively.

Keywords: *Calotropis gigantea* Linn., Asclepiadaceae, uscharin, cytotoxic, MTT