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Formulation of a *Garcinia zeylanica* herbal aqueous cream as a potential antiseptic and determination of the antimicrobial activity *in vitro*

Senevirathne MAL[1], Anuradha UK[1], Peiris MMK[2], Hewageegana HGSP[3], Arawwawala LDAM[4], Siriwardhene MA[1], Dabare PRL[1], Weerasekera MM[2], Fernando SSN[2], Gunasekara TDCP[2]

[1]Department of Allied Health Sciences, [2]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [3]Department of Nidana Chikitsa, Institute of Indigenous Medicine, University of Colombo, [4]Industrial Technology Institute, Sri Lanka

Objectives: To formulate and evaluate an herbal aqueous cream using the aqueous extract of dried pericarp of *Garcinia zeylanica* and determine the antimicrobial activity *in-vitro*.

Methods: Dried pericarps of *Garcinia zeylanica* (60g) were boiled down from 1440 ml to 240 ml to obtain neat concentration following Ayurveda practice. Qualitative phytochemical analysis was done using standard methods. Three herbal aqueous creams were formulated containing 30%, 40% and 50% emulsifying ointment base respectively, according to British Pharmacopoeia 2013. The final extract concentration in all formulations corresponded to neat concentration. The color, odor, phase separation, pH and stability, bacterial loads and antimicrobial activity were determined at 0, 1 and 2 months at room temperature. Antimicrobial activity was tested against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), clinical isolates; *Klebsiella pneumoniae* and *Acinetobacterbaumannii* using the well diffusion method. Zones of inhibition (ZOI) of triplicate experiments were recorded and average ZOI calculated.

Results: The qualitative phytochemical analysis of aqueous extract confirmed the presence of phytochemicals including flavanoids, carbohydrates, tannins and saponins. The formulation containing 40% ointment base had appropriate physical properties and was selected for further study. The physical and microbiological evaluation indicated good stability and antimicrobial activity of the formulation. Average ZOI obtained were *Staphylococcus aureus* (11.67mm), *Escherichia coli* (12mm), *Klebsiella pneumoniae* (10.33mm), *Acinetobacter baumannii* (11.67mm) and *Pseudomonas aeruginosa* (11.33mm) after two months at room temperature. No bacterial contamination of the formulated ointment was detected.

Conclusions: *Garcinia zeylanica* aqueous cream shows good stability and antimicrobial activity against tested pathogens during the time period tested and should be evaluated further.

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Optimization of a real time PCR for quantification of *Leptospira* bacterial loads in patients using SYBR green based real time PCR

Gamage GGTN[1], Bandara AWMK[1], Marasinghe MGCP[2], Weerasekera MM[1], Fernando SSN[1], Gunasekara TDCP[1]

[1]Department of Microbiology, [2]Department of Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura

Objective: This study aimed to optimize a quantitative SYBR green based *Sec Y* real time PCR assay for *Leptospira* and establishment of a standard curve for detection of leptospiral genome copy numbers in patient samples.

Methods: To generate the standard curve, DNA was extracted from cultures of *Leptospira interrogans* serovar Manilae using QIAamp DNA blood mini kit (QIAGEN, Germany) and quantified by NanoDrop. *Leptospira* copy number was calculated based on published genomic size to construct a standard curve with tenfold serial dilutions of genomic DNA of *Leptospira interrogans*. Optimization of real time SYBR green *Sec Y* PCR was

carried out for the following parameters: annealing temperature, $MgCl_2$ concentration, primer and template DNA concentration. Analytical sensitivity and specificity of the *Sec Y* conventional PCR and real time PCR was evaluated. Further sensitivity of *Sec Y* and flaB conventional PCR was also evaluated.

Results: Using conventional PCR the *Sec Y* primers amplified all pathogenic *Leptospira* strains tested. The sensitivity of the flaB conventional PCR was 1000 copy numbers and *Sec Y* conventional PCR was 100 copy numbers while the real time PCR detected up to 30 genomic copy numbers of leptospiral DNA extracted from *Leptospira* culture isolate. The R^2 value of standard curve was 99.3%.

Conclusion: *Sec Y*Real time PCR technique was found to be highly sensitive and thus can be efficiently used as a diagnostic tool for diagnosis of leptospirosis and to determine the leptospiral loads in patients.

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High sensitive C reactive protein in lumbar disc herniated subjects with positive and negative disc microbes

Withanage ND[1], Pathirage S[2], Peiris H[3], Perera S[4], Athiththan LV[3]

[1]Department of Allied Health Sciences, Faculty of Medical Sciences, University of Sri Jayewardenepura, [2]Medical Research Institute, Colombo 08, [3]Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, [4]The Central Hospital, Colombo 8

Objectives: To identify the association of hs-CRP in lumbar disc herniated (LuDH) patients with positive and negative disc microbes.

Methods: Venous blood sample was obtained from 96 subjects who were undergoing lumbar discectomy and 200 µL of serum aliquot was taken for hs-CRP and analyzed using KONE 20 XT autoanalyzer. Surgically removed disc was taken for aerobic and anaerobic studies whereas muscle biopsies were used as controls. Gram stain, coagulase and catalase test were performed for the isolates and RapID ANA II ID kit (remel,USA) was used for the identification of anaerobes..

Results: Among the 18 (19%) subjects who were positive for disc microorganisms; 12 were positive for aerobes and 6 for anaerobes. There were 30/96 with elevated level of hs-CRP (>3 mg/L) and 34/96 with slightly elevated hs-CRP (1-3mg/L). All the anaerobic positive patients had either elevated or slightly elevated values for hs-CRP, but only 5/12 aerobic positive patients had either elevated or slightly elevated values. Though there was no significant relationship between elevated hs-CRP and presence of microorganisms, 66.7 % of the LuDH patients had either elevated or slightly elevated values. Mean hs-CRP values were high in both negative (4.14±7.1 mg/L) and positive microbial disc cultures (5.13±11.9mg/L).

Conclusions: Mean hs-CRP were higher in both microorganism positive and negative patients. No significant relationship was observed between elevated hs-CRP and presence of microorganisms though the mean value was high in microbe positive subjects. The majority (66.7%) of the LuDH patients had either elevated or slightly elevated values for hs-CRP.