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Posters

Sylwia Jarzynka et al., J Biotechnol Biomater 2018, Volume 8
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Antibiofilm activity of Human Milk Oligosaccharides against matured biofilms formed by different pathogen species

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Statement of the Problem: Due to its unique composition, human milk is an excellent source of nutrients and also many bioactive ingredients, which have as a potential healthy effect. Oligosaccharides (HMOs, Human Milk Oligosaccharides) is the main group with potential biocidal using, especially of medical procedures. Due to the concentration of the components of human milk, oligosaccharides, in addition to lactose and fats, are the third component. Due to their bioactivity, we hypothesised that HMOs exhibit an antimicrobial activity against a wide spectrum of human pathogenic bacteria. A systematic investigation of the antimicrobial spectrum of polled or individual HMOs has not been performed for bacterial biofilm structure.

Methodology & Theoretical Orientation: Clinical isolates and reference strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, MRSA, Burkholderia cepacia* and *Acinetobacter baumanii* were used in this study. Human milk was obtained from nine healthy donors from Human Milk Bank in Warsaw Poland. Milk samples were pooled, next in the skimming milk proteins were precipitated. Carbohydrates with oligosaccharides fraction were then lyophilized. In the quantitative analysis of the biofilm we done determination of the minimal biofilm inhibitory concentration (MBIC), determination of colony forming units in the planktonic phase and of the minimal biofilm eradicated concentration (MBEC). Live/dead staining of the biofilms and CLSM image acquisition were used.

Conclusion & Significance: In our preliminary work, we could show that the human milk saccharide fraction exhibit moderately activity against some planktonic bacteria species (e.g. clinical isolates of *P. aeruginosa* and *MRSA*) and inhibit biofilm formation of *P. aeruginosa*. HMOs showed a biofilm eradicating effect on most tested pathogens. Oligosaccharides may potentially constitute a new medicinal product of natural origin, used in the prophylaxis and treatment of respiratory tract infections in patients with cystic fibrosis and COPD.

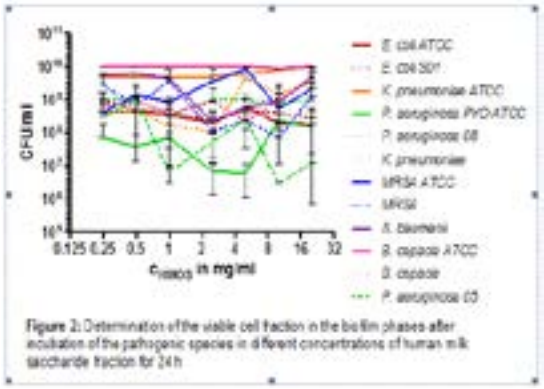

Figure 2: Determination of the viable cell fraction in the biofilm phases after incubation of the pathogenic species in different concentrations of human milk saccharide fraction for 24 h

Recent Publications:

1. Ackerman, D. L., et al. (2017). Human Milk Oligosaccharides Exhibit Antimicrobial and Antibiofilm Properties against Group B Streptococcus. ACS Infect Dis 3(8): 595-605.
2. Bode, L. (2015). The functional biology of human milk oligosaccharides. Early Hum Dev 91(11): 619-622.

3. Cosgrove, S. E., et al. (2002). Health and economic outcomes of the emergence of third-generation cephalosporin resistance in Enterobacter species. Arch Intern Med 162(2): 185-190.
4. Lewis, K. (2001). Riddle of biofilm resistance. Antimicrob Agents Chemother 45(4): 999-1007.
5. LiPuma, J. J. (2005). Update on the Burkholderia cepacia complex. Curr Opin Pulm Med 11(6): 528-533.

Biography

Sylwia Jarzynka, together with a team of scientists from the Medical Biology Department in cooperation with the Jena University Hospital, Center for Infectious Diseases and Infection Control are involved in the study of antimicrobial biotic and abiotic factors. Special achievements of the researchers concern the inhibition of bacterial biofilm with the use of equal biomaterials, which are potential carriers of antimicrobial agents.

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Notes:

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Synthesis and controllable time and feed ratio of organic-inorganic hybrids with near infrared absorption for solar cells

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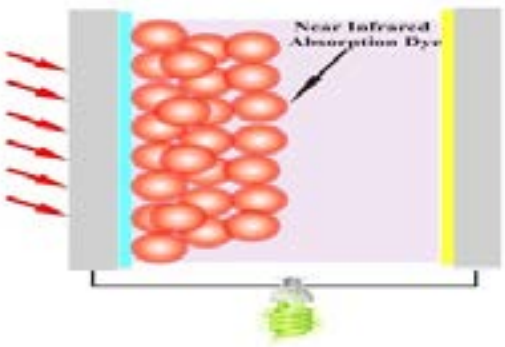
Nowadays organic dye materials are receiving more interest in solar cell due to improved solution process ability, scalable synthesis, tunable chemical and physical properties via molecular design and low cost. However, the compatibility and aggregation of squaraine dye limited broad application. In this study, we designed a system contend of octavinyl-polyhedral oligomeric silsesquioxane OV-POSS which used to solve these problems. These is the first time designed a novel hybrids large broad absorption visible to near infrared transient absorption spectroscopy, prepared by OV-POSS with 6-Bromoquanaldine and squaric acid (semisquaraine (SSQ) squaraine (SQ)) H2 their light properties a broad spectral coverage in a big region from 400 to 800 nm. Our systems were characterized by Fourier Transform Infrared Spectroscopy FTIR, 1HNMR, UV-Vis-Spectra, Water contact angle images and FE-SEM properties, and when a combination our system dyes with N719 as co-sensitization in photovoltaic performance using Ti foil-based solar cell DSSC. It showed highest properties a power conversion efficiency of 7.73%, with a short-circuit current density (Jsc) of 18.48 mA/cm^2 , an open-circuit voltage (Voc) of 0.73 V and a fill factor of 46.22% under the AM 1.5G illumination with an intensity of 100 mW/cm^2 from a solar simulator hence exhibited good performance in solar cell application.



Recent Publications:

1. Wang S, et al. (2015) Controllable preparation and properties of active functional hybrid materials with different chromophores. RSC Advances 5(2):1070-1078.
2. Puyad A L, et al. (2013) A comparative study of semi-squaraine and squaraine dyes using computational techniques: tuning the charge transfer/biradicaloid character by substitution. Journal of Molecular Modeling 19(1):275-287.
3. Yan Z, et al. (2012) Near-infrared absorbing squaraine dyes for solar cells: Relationship between architecture and performance. The Journal of Physical Chemistry C 116(16):8894-8900.

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Nisha Zahid et al., J Biotechnol Biomater 2018, Volume 8
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Atorvastatin, a double weapon in osteoporosis treatment: An experimental and clinical study

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Objective: The aim of this study was to evaluate the effect of atorvastatin on the bone formation and resorption markers in ovariectomized rats (experimental study), and to study its effect on the bone mineral density (BMD) in postmenopausal osteoporotic women (clinical study).

Materials & Methods: The study involved experimental and clinical aspects. In the experimental aspect, 42 female Wistar rats were divided into five groups: group I - sham-operated, group II - 1 mL of carboxymethyl cellulose [CMC] was administered orally, group III - 20 mg/kg orally of atorvastatin was administered, group IV - Untreated ovariectomized [OVX] rats and served as a model of osteoporosis [OP] and group V - 20 mg/kg orally of atorvastatin was administered to ovariectomized rats. After four weeks, serum acid phosphatase, alkaline phosphatase, osteocalcin, total calcium and inorganic phosphorus were assessed. Then, 3 µm thickness lumbar and femur sections were examined to assess cortical thickness, trabecular area, numbers of osteoblasts and osteoclasts. In the clinical aspect, 85 post-menopausal osteoporotic females with recently detected hyperlipidemia participated in the study. Atorvastatin 40 mg/day, calcium carbonate 500 mg/day and vitamin D 800 IU were given to all patients for a period of 18 months. BMD was measured at the start and at the end of the study by dual-energy X-ray absorptiometry (DEXA).

Results: In the experiment aspect, the biomarkers of bone remodeling were notably elevated in the OVX group. Whereas atorvastatin produced a significant decrease in the level of these bone metabolic markers and significantly ameliorates osteoporotic changes induced by ovariectomy. In the clinical aspect, after 18 months the DEXA showed improvement in the T-score, statistically significant only in the femoral neck area.

Conclusion: Atorvastatin was able to decrease the rate of bone metabolism and increase osteogenic activity. It has dual mode of action both anabolic and antiresorptive effect on bone. This lipophilic statin member may act as a double weapon drug.

Biography

Nisha Zahid has completed her Bachelor degree in Dental Surgery and currently, she is pursuing her MPhil in Pharmacology from a reputed institute Ziauddin University of Karachi, Pakistan. She has done with her course work and presently, she is doing her research which is on statins.

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Evaluation of medicine distribution, regulatory privatisation, social welfare services and liberalisation

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The strategy of price liberalisation and privatisation had been implemented in Sudan over the last decade and had a positive result on government deficit. The investment law approved recently has good statements and rules on the above strategy particularly to pharmacy regulations. Under the pressure of the new privatisation policy, the government introduced radical changes in the pharmacy regulations to improve the effectiveness of the public pharmacy i.e., resources should be switched towards areas of need, reducing inequalities and promoting better health conditions. Medicines are financed either through cost sharing or full private. The role of the private services is significant. A review and reform of financing medicines in Sudan is given in this article. Also, it highlights the current drug supply system in the public sector, which is currently responsible for the Central Medical Supplies Public Corporation (CMS). In Sudan, the researchers did not identify any rigorous evaluations or quantitative studies about the impact of drug regulations on the quality of medicines and how to protect public health against counterfeit or low quality medicines, although it is practically possible. However, the regulations must be continually evaluated to ensure the public health and is protected against by marketing high quality medicines rather than commercial interests and the drug companies are held accountable for their conducts.

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Assessment of inflammatory markers IL-6 and its relation with clinical status in stroke patients

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Introduction & Aim: Acute stroke is the most common neurological disease. Stroke is the second leading cause of mortality worldwide and is a major cause of long-term disability. Also, in occurring of inflammatory cascade, the stroke will be activated and play the main role in disease separated from them. This study was carried out to investigate the association of serum TNF-a, and IL-6 levels with clinical outcome in acute stroke.

Methods: The study involved 90 patients. 45 control and 45 patients with the first-time stroke aged 71.2±10.8 years of both sexes entered the study consecutively. Modified Rankin Scale (mRS) for stroke severity was evaluated on two months. Serum IL-6 and TNF-a level were measured by enzyme-linked immunosorbent assay (ELISA) on day 1. The association between serum TNF-alpha and Il-6 levels in stroke patients with control values and stroke outcome was evaluated by T-test (SPSS software 22). Moreover, statistical significance was defined as $P<0.05$.

Results: 90 patients with 45 stroke (14 female and 31 male) and 50 control subjects (34 male and 16 female) were included in the study. Mean serum TNF-α and IL-6 level in the control group and mean serum TNF-α level in the stroke patients group was (26.57 Pg/ml, 45.30 Pg/ml. 112.55 pg/ml, 140.02). The levels of TNF-alpha and IL-6 in serum were no significantly correlated with the volume of dysphagia ($r=.099$; $P<0.05$ and $r=.170$; $P<0.05$). However, the difference of IL- 6 levels among groups was not significant. In contrast, there was no significant association between inflammatory markers with the severity of dysphagia, MRs, and serum albumin.

Conclusion: The results of this study demonstrate that increased inflammatory markers increase the severity of dysphagia and worsening clinical status of patients. Therefore inflammatory markers can be used as reliable prognostic factors for predicting the prognosis of patients with stroke.

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Formulation and characterization of dacarbazine loaded stearic acid nanoparticle cream and its cytotoxic potential

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In current years, rapid increase in the number of skin cancer patients has led to socio-economic burden worldwide. Dacarbazine (DZ) is a single drug approved by US-FDA for the treatment of a type of skin cancer melanoma. DZ have short half-life in systemic circulation as well as poor water solubility, slow response rate with severe toxicity which limits its market potential. In view of the above background present study is designed for formulation, characterization and pharmacological evaluation of dacarbazine laden nanocream (DZC) for the treatment of melanoma. Dacarbazine loaded stearic acid nanoparticles (DZP) prepared by diffusion method. DZC prepared by oil-in-water emulsion technique by using DZP. Dacarbazine nanoparticle and its cream were characterized for shape size of particle, drug loading capacity, nanoencapsulation efficacy, zeta potential, transmission electron microscopy (TEM), pH value, spreadability and viscosity, *in vitro* drug releasing capacity and its cytotoxic effect by using MTT assay. The particle size of DZNP and DZNC was 16.3±8.1 nm and 16.9±7.8 nm respectively. pH value and spreadability of nanoparticle cream were found to be 6.7±0.14 g cm/sec and 55.23±3.13 g cm/sec respectively. Nanoencapsulation efficiency and drug loading capacity were 67.4±3.5% and 6.73 mg/10 mg respectively. From above results, it can be concluded that DZC can be effectively utilized for the treatment of melanoma.

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Nanobiotechnology for treatment of neurological disorders

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There are approximately 400 known neural disorders, some being due to a disruption or failure of the blood brain barrier (BBB) such as, e.g., meningitis, epilepsy, multiple sclerosis, prion and prion-like diseases (Parkinson's, Alzheimer's), HIV, encephalitis, and systemic inflammation (sterile or infectious). As a consequence of the growing aging population, many such neurodegenerative diseases, cancer, and infections of the brain will become more prevalent. Unfortunately, the developmental process for new drugs has not kept pace with progress in molecular neuroscience because most of the new drugs discovered are unable to cross the BBB. This clinical failure may be largely attributed to a lack of appropriate drug delivery systems. Of interest here are those disorders requiring treatment by delivery of nanobiotechnology (NBT)-based drugs through the BBB-one of the most promising applications in clinical neuroscience. Nanoparticles, utilized as drug delivery agents, could potentially carry out multiple tasks in a predefined sequence. They can be effective careers in delivery of conventional drugs, recombinant proteins, vaccines, etc. The following nanoparticles such as: liposomes, peptides, radiolabeled polyethylene glycol coated hexadecylcyanoacrylate nanospheres, polyalkylcyanoacrylate or poly-lactic-co-glycolic acid (PLGA) nanoparticles with polysorbate 80 or poloxamer, and magneto-electric nanoparticles (MENs) are available. Localized and controlled delivery of drugs at their desired sites of action is preferred because it reduces toxicity and increases treatment efficiency. Author will discuss the various strategies that have been explored to increase drug delivery into the brain and their attending difficulties, with particular emphasis on NBT-based drug delivery systems. However, although the use of nanotechnology is expected to reduce the need for invasive procedures for delivery of therapeutics, some devices such as implanted catheters and reservoirs will still be needed. Further, there is some concern about the safety of nanoparticle entry in the brain which needs to be resolved before human use.

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The impact of antiepileptic drugs on vitamin levels in epileptic patients

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Background: The impact of antiepileptics on serum vitamin levels is controversial and uncertain. With no clear conclusion on the impact of antiepileptics on serum levels of vitamins, there is a need for further clinical studies in order to ascertain the impact of old and newer antiepileptic drugs on serum levels of vitamins in epileptic patients, thus accomplishing a suitable usage of vitamins supplementation.

Objective: The intention of the present research is to confirm the hypothesis of whether vitamin levels are altered with antiepileptic drugs or not. The study also aims to reveal which vitamin levels are particularly more altered, are vitamin levels affected by gender and the type and number of antiepileptics used.

Methods: The present research was piloted in collaboration with the Department of Neurology at Qilu Hospital of Shandong University. A total of 63 serum samples of epileptic patients receiving antiepileptics as monotherapy or polytherapy were requested for analysis of nine vitamin serum levels. Total nine vitamins (B_1, B_2, B_6, B_9, B_{12}, A, C, D and E) in epileptic patients receiving antiepileptic drugs were analyzed. The serum results of all vitamins were compiled and evaluated with SPSS.

Results: It was alarmingly found that serum levels of vitamin D were particularly very low in almost all (90%) epileptic patients in this study. Notably, serum levels of vitamin C and vitamin B_1 were also below reference range in 72% and 46% epileptic patients, respectively. The remaining vitamins were almost in reference range for most of the patients. In our study, mean and frequency of vitamin D, C and B_1 levels do not vary too much among different gender groups. The patients receiving newer antiepileptic drugs displayed a slightly increased serum vitamin D levels in comparison to the patients receiving older antiepileptic drugs. We found low vitamin D, C and B_1 serum levels in patients who were on monotherapy as in comparison with patients on polytherapy.

Conclusion: The most significant and surprising finding of this study revealed that serum vitamin D levels in particular were very low in almost all patients and in some patients' vitamin B_1 serum levels were also below the reference range. More importantly, it is first time reported here that vitamin C serum levels were also below reference range in the majority of these Chinese epileptic patients. It is recommended that all these vitamins should be regularly monitored in addition to therapeutic drug monitoring of antiepileptic drugs. Additional clinical trials are required for further evaluation. It is also recommended that epileptic patients with low serum levels of these vitamins may be prescribed vitamins supplementations with antiepileptic drugs in order to control their seizures more effectively and efficiently.

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Verification of the efficacy of targeting peptides linked liposomal nanoparticles for therapy of hepatocellular and nasopharyngeal carcinomas and breast cancer

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The efficacy of systemic cytotoxic chemotherapy has been widely assessed in patients with advanced hepatocellular carcinoma (HCC). For example, doxorubicin is the most commonly studied chemotherapeutic agent for HCC. However, it has been shown to have a response rate of only 10-20% in clinical trial. In addition, its potential benefit has been reduced by the related adverse effect. So far, the multikinase inhibitor sorafenib is considered to provide survival benefit over supportive care. However, the long term prognosis of those cancer patients still remains poor. Therefore, in the present experiment, we proposed to use the so-called peptide targeting chemotherapy to overcome the adverse event in the conventional targeted chemotherapy. In order to perform this experiment, we have constructed some peptides which can bind specifically to the cancer cells and cancer vascular endothelia by using a phage displayed 12-mer random peptide library. We obtained three different peptides and one control peptide. Each contains 12 amino acids: a. L-peptide: RLLDTNRPLLPY (anti-different cancer cell membrane); b. control peptide: RLLDTNRGGGGG; c. SP-94-peptide: SFSHHTPILP (anti-NPC tumor cell and hepatoma cell membranes) and d. PC5-52-peptide: SVSVGMKPSPRP (anti-tumor endothelia). The L-peptide (L-P), SP-peptide (SP-P), PC5-52-peptide and the control peptide (C-P) were linked to liposomal iron oxide nanoparticles; and also to liposomal doxorubicin (L-D). Using peptide linked liposomal iron oxide, we can localize the peptide targeted tumor cells and tumor endothelia, and then we used those peptides linked liposomal doxorubicin to treat SCID mice bearing different cancer xenografts. Our results showed that when L-P-L-D containing 2mg/kg of SCID mouse body weight was used to treat xenografts bearing SCID mice, the tumor could be well controlled, and no specific adverse event was seen. However, when the control peptide was used to replace the specific peptide, the xenograft size was also decreased, but the visceral organs revealed marked apoptotic change. In conclusion, the specific peptides linked liposomal doxorubicin nanoparticles can be used for treatment of SCID mice bearing cancer xenografts with minimal adverse event, especially in the SCID mice γ species (NGS), which shows remarkable tumor suppression.

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Ethnopharmacological survey of medicinal plant used for the treatment of liver damage, nephrolithiasis and cardiovascular disease in the south region of Morocco, Marrakech

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This study was carried out to identify the widely used medicinal plant in folk medicine for treating liver damage, nephrolithiasis and cardiovascular disease by the population of Marrakech province, in South of Morocco. This investigation was undertaken during more than 4 months started in 2015. Data were collected from (54%) housewife, (63%) traditional healers using ethnobotanical questionnaire. A total of 67% reported that *Rubia tinctorum* L. is the only plant used for the treatment and management of the above-mentioned diseases. Data collected was separated in two parts. The first part concerned interviewee information's (age, sex, and level of education) and the second part was designed for plant uses (vernacular names, uses, parts used, and mode of preparation). Use value (UV), fidelity level and family UV (FUV) were calculated. The dried root (57%) of *Rubia tinctorum* L. was prepared in the form of raw condition (78%). Oral administration of the plant was most (90%) common route of administration. Finally (96%) were reported that the plant has no side effects. The results of the study show that *Rubia tinctorum* L. is used for the treatment and management of both common and specialized human diseases. Further studies are needed to better understand the underlying mechanism behind it therapeutic actions.

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The activity of dental membranes on the reduction of bacterial growth in the course of peri-implantitis

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Medical University of Warsaw, Poland

Statement of the Problem: Peri-implantitis treatment is an important clinical challenge with a significant estimation for getting a local infection under control, preserving soft and bone tissue in the area around the implant and in result maintaining the aesthetics and stability of the implants. Microorganisms, including drug resistant *Staphylococcus aureus*, play a crucial part in the etiology of peri-implantitis. Depending on the treatment strategy, only 34 to 79% cases are treatable. Important methods of treatments are nonsurgical and surgical procedures, which are often unsatisfying for the physician, as well as the patient, therefore the constant search for alternative, effective methods matters. The important treatment process in peri-implantitis poses a use of membrane for oral soft tissue and bone regeneration guided regeneration.

Aim: The aim of this study was to establish the usefulness in laboratory conditions of a bioactive collagen membranes and titanium-reinforced membranes for reduction of microorganism growth in the active stages of peri-implantitis. The proposed solution is cost effective and painless in application and in addition could be a promising alternative as a vehicle for transferring antibiotics and other antibacterial substances directly to the tissues.

Methodology & Theoretical Orientation: Antibacterial activity of five different membranes was observed for the chosen bacterial strain *S. aureus*. In a spectrophotometric assay the incubation of bacteria with membranes in Luria broth on micro titrated plates on 24 hours at 37°C was being monitored.

Results: The results show that the growth of *S. aureus* was lower for: T-Gen®0-collagen membrane, Dyna®-extracellular membrane with glycoproteins and glycosaminoglycans, Cytoplast®-titanium reinforced membrane, and Mucograft®-collagen membrane.

Conclusion & Significance: This work supports that the bioactive membranes can be an effective antibacterial *in vitro*. The new challenge is the use of these membranes as precise drug carriers, in the treatment strategy used for peri-implantitis.

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Synthesis, antibacterial activity and molecular docking studies of some new imine derivatives

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The aim of this research work was to synthesize some new imine derivatives (Schiff bases) by using the reaction between different heterocyclic amines and aromatic aldehydes. We have synthesized nine compounds (imine derivatives), five of them are not known yet. These products are synthetized under reflection condition. The structure of the synthesized products was determined based on IR and 1H NMR spectra. Compounds were screened for their antibacterial activity against gram positive bacteria *Staphylococcus aureus* (clinical isolate), *Staphylococcus aureus* (food isolate), *Listeria monocytogenes*, and against gram negative bacteria *Escherichia coli*. Compounds were found to be potent antimicrobial agent against gram positive bacteria. All the compounds were subjected to molecular docking studies for the inhibition of the enzyme L-glutamine D-fructose-6-phosphate amidotransferase [GlcN-6- P] (EC 2.6.1.16). The *in silico* molecular docking study results showed that from nine synthesized compounds, seven of them shows minimum binding energy and have good affinity toward the active pocket, thus, they may be considered as good inhibitor of GlcN-6-P synthase. From this research work we can conclude that this synthesized compounds show antibacterial activities. The synthesized compounds can be used further by modifying their structure with the aim to increase their biological activity.

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Thyroid cancer incidence and clinicopathological differences in patients with end-stage renal failure

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Aim: In the present, study we aimed to determine the prevalence of thyroid cancer and the clinicopathological properties of papillary thyroid cancer (PTC) in a patient population undergoing dialysis for end-stage renal failure (ESRF).

Materials & Methods: We retrospectively reviewed all thyroid ultrasonography (USG) examinations performed between January 2007 and December 2015 to determine the incidence of nodular thyroid disease in ESRF and normal patient populations. For both patient groups, differences between patient and tumor characteristics were evaluated in patients diagnosed to have PTC.

Results: Among 29.381 patients, who underwent thyroid USG examination, 3.491 were included in the ESRF group (Group 1) and 25.890 in the control group (Group 2). Thyroid cancer was detected in 77 (2.2%) of 3.491 patients in Group 1 and 338 (1.3%) of 25.890 patients in Group 2. Thyroid cancer was significantly more prevalent in patients with ESRF ($p<0.001$).

Discussion: When only patients with papillary thyroid cancer were considered, no significant difference existed between the two groups with respect to the prevalence of PTC, although PTC cases in the ESRF group had a significantly higher rate of aggressive characteristics such as capsule invasion, multifocality, and lymph node metastasis. Whereas thyroid cancer is more common in patients with ESRF compared to normal controls, papillary thyroid cancer was not significantly more prevalent in the ESRF group.

Conclusion: PTC in the ESRF group having more aggressive properties than those in the control group suggests that PTC should be diagnosed earlier in their course, treated more aggressively, and followed more closely in ESRF.

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Use of 3'-untranslated region of messenger RNA from measles virus matrix protein as a stabilizing cis-element for increased protein production yields

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The market for the use of recombinant proteins for medical applications has been increasing in recent years. Expression vectors are one of the decisive factors in the cost-effectiveness of the production process of recombinant proteins. The genetic factors found at the 3'untranslated region of mRNA expressed by such vectors, play an important role in determining its stability and thus in the efficiency of the recombinant protein production process. We studied the effect of 3'UTR of matrix protein mRNA from measles virus on mRNA stability by a GFP-based reporter construct in three cell lines. Application of 3'UTR of matrix protein mRNA from measles virus increased the GFP-mRNA stability in a time and cell dependent manner. Analysis of the 3'UTR of matrix protein mRNA from measles virus for presence of known cis-acting motifs indicated the occurrence of two PABPC1 binding sites, known for its stability and translation enhancing effects. Our results verified the potentiality of 3'UTR region of matrix protein mRNA for improvement of recombinant protein production and vector design for mammalian cell hosts.

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Britannin induces apoptosis through targeting AMPK in human breast cancer

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Nowadays, there is an urgent need for novel drugs with improved efficiency against tumor cells. Of note, induction of cell death pathways with regard to molecular targets is considered as a promising treatment method in cancerous cells. AMP-activated protein kinase (AMPK) which is involved in suppression of cell growth and induction of apoptosis is emerged as an attractive target molecule for cancer treatment. Recently, naturally derived products such as britannin had drawn growing attention as an agent in cancer therapy. Britannin was extracted from Inula aucheriana by using chromatography methods. Human breast cancer cell line (MCF-7) and normal breast cancer cell line (MCF10A) were applied to investigation of anticancer properties of britannin. Cytotoxic effects of britannin were examined by MTT assay. Apoptosis induction was evaluated by flow cytometry and also, activity of caspase3 was assessed by colorimetric assay. Furthermore, intracellular changes in protein expression of Bax, Bcl2 and AMPK were analyzed by western blotting method. The viability of MCF-7 and MCF10A cells inhibited after 24h treatment by britannin with the IC50 values 15±2.3μM and 80±5.4μM, respectively. Apoptosis induction in MCF-7 cells was confirmed by annexin V-FITC/PI staining and caspase3 activation. In addition, britannin decreased the expression of Bcl2 (anti-apoptotic protein) and increased the expression of Bax (pro-apoptotic protein) in MCF-7 cells. Moreover, britannin increased phosphorylated active form of AMPK in MCF-7 cells. Taken together, the data shows that the cytotoxic effect of britannin on the cancerous cells is significantly higher than on normal cells. Furthermore, results demonstrate that britannin induces mitochondrial-apoptosis through activation of AMPK in breast cancer cells and may potentially serve as an adjuvant agent for the treatment of human breast cancer.

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Ethanol content in traditionally fermented ayurvedic formulations has compromised good manufacturing practice regulations-compromised health

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A large part of the population of India prefers the traditional medicine for primary healthcare. However, the effective quality control of herbal medicine is still a big challenge. Numerous reports indicate noncompliance with good manufacturing practice (GMP) guidelines by the manufacturers which may lead to adverse drug reactions or toxic effects. Asava and arishta are the classical herbal dosage forms wherein fermentation occurs during production leading to the generation of ethanol. The presence of ethanol in these preparations may lead to their abuse for the pleasure and enjoyment. The self-generated ethanol is responsible for extraction of active constituents and acts as self-preservative. As the procedure for preparation for asava and arishta is same, the ethanol content is also expected to be the same irrespective of the manufacturer. The objective of the present study was to assess and compare the ethanol content of some traditionally fermented ayurvedic formulations available in the market. Method in this study include 20 formulations from 3 different manufacturers available as over-the-counter (OTC) product were obtained and their ethanol contents were determined using gas chromatograph (GC) fitted with flame ionization detector (FID). Statistically significant differences were noted in the ethanol content of various formulations. A simple, less time consuming, economic and validated gas chromatographic method for estimation of ethanol in fermented ayurvedic formulations was also developed successfully in present study. The data generated during study reflected poor compliance of GMP guidelines by the manufacturers and hence the quality is being grossly compromised posing a safety hazard.

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Modeling and optimization of fermentation conditions for glycolipopeptide production using response surface methodology and artificial intelligence approaches

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Statement of the Problem: Pseudomonas aeruginosa strain IKW1 produced a biosurfactant when grown in waste frying sunflower oil-basal medium. The active compound reduced surface tension of fermentation broth to 24.62 dynes/cm at a critical micelle concentration of 20.80 mg/L. It was identified by high performance liquid chromatography and Fourier transform-infrared spectrometry as a glycolipopeptide. It demonstrated considerable emulsification and foaming capabilities suggesting suitability for applications in pharmaceutical and detergent formulations. However, product yield was low, making large-scale production for recommended applications impracticable. Several researchers have reported yield improvement by strategic medium optimization approaches. Earlier, we adopted response surface methodology (RSM) for major nutrients optimization and recorded commendable yield increase. Later on, we employed Placket-Burman design (PBD) and RSM to screen and optimize trace nutrients and obtained significant yield improvement. However, research reports indicate that artificial neural network (ANN) is a better optimization approach.

Methodology & Theoretical Orientation: In this study, we optimized fermentation conditions like temperature, pH, agitation and duration using RSM, and compared results to those obtained with ANN linked with genetic algorithm (ANN-GA) and particle swarm optimization (ANN-PSO).

Findings: Our results showed that the biosurfactant response model, predicted by a quadratic function of RSM, was significant ($P<0.0001$; adjusted $R^2=0.9911$; RMSE=0.034), setting factor levels at temperature-32°C, pH-7.6, agitation speed-130 rpm and fermentation time-66 h. Maximum glycolipopeptide concentration was 107.19 g/L with a yield (Yp/x) of 4.24. Comparative results from ANN-GA ($R^2=0.9997$; RMSE=0.055) and ANN-PSO ($R^2=0.9914$, RMSE=0.047) showed that model and optimized factor settings were not significantly ($P>0.05$) different from those obtained with RSM.

Conclusion & Significance: This suggests that RSM, when meticulously executed, could be as good a modeling and optimization tool like neural network methods.

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Antidiabetic activity of aqueous extract of *Ruta montana L.* in streptozotocin-induced-diabetic rats

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The aim of the present study was to evaluate antidiabetic activity of aqueous extract of *Ruta montana L.* in streptozotocin induced diabetic rats. Diabetes mellitus was induced in rats by single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg body weight). After STZ induction, the hyperglycemic rats were treated with aqueous extract orally at the dose 250 mg/kg body weight daily for 15 days. Insulin (6 U.I/kg) was used as reference drug. The control group was administered with distilled water for the same duration. The fasting blood glucose levels were measured on every 5th day during the 15 day treatment. The results showed that oral administration of aqueous extract at the dose of 250 mg/kg body weight decreased blood sugar level in 5 to 15 days of treatment. The antidiabetic effect of aqueous extract was found to be comparable to that the effect exerted by the reference drug, insulin at the dose of 6 U.I/kg. blood sugar level in rats treated by aqueous extract was 2.41±0.05, 1.32±0.36, 1.10±0.12 and 0.98±0.04 mg/dl at the 1, 5, 10 and 15 days respectively and in rats treated by insulin was 2.25±1.30, 1.04±0.33, 1.19±0.45, 0.48±0.15 mg/dl at the 1, 5, 10 and 15 days respectively.

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DNA profiling from fetus and placenta preserved in formalin

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Cells in fetus and placenta like other biological materials contain the DNA. During the termination of pregnancy, fetus and placenta are preserved in formalin and normal saline in sexual assault cases. The preservation depends from case to case and availability of preservatives in hospitals. It is a common presumption that foetuses preserved in formalin are of no use for DNA profiling in forensic cases as formalin is an inhibitor. It is recommended by several researchers to preserve the foetus or placenta in normal saline instead of formalin. Study aimed to obtain the appropriate quantity of DNA from the samples preserved in formalin. 50 samples of fetus and placenta preserved in formalin were analyzed. Our results indicate that a proper manual removal of formalin may generate an appropriate quantity of DNA. DNA profiling from fetus and placenta can be preserved in formalin up to one year.

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Optimization of fermentation conditions for extracellular production of a therapeutic protein drug uricase, by *Aspergillus welwitschiae* using response surface methodology

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Uricase is commonly used in clinical analysis for the determination of urate in blood and other biological fluids. Microbial uricases as protein drug have been found effective in the treatment of hyperuricemia and gout, as well as in prophylaxis and treatment of tumor lysis hyperuricemia. A potential culture, *Aspergillus* sp. strain 1-4 displayed high extracellular uricase activity. This uricolytic fungal isolate was identified as *Aspergillus welwitschiae* strain 1-4 on the basis of phenotypic characteristics, together with ITS region sequence analysis. Sequencing product was deposited in the GenBank database under accession number MG323529. The Plackett-Burman experimental design with 20 runs was applied for screening of fifteen variables for their significances on uricase production by *Aspergillus welwitschiae*. Incubation time was the most significant variable affecting uricase production followed by yeast extract and inoculum size with significant P-values of 0.0002, 0.0083 and 0.0118; respectively. These variables were chosen for optimization studies using central composite design. It was found that, the maximum uricase production (59.01 U/mL) by *Aspergillus welwitschiae* is achieved at the following fermentation conditions g/L: sucrose 30, uric acid 3, peptone 2, yeast extract 2, $NaNO_3$ 2, K_2HPO_4 1, $MgSO_4.7H_2O$ 0.2, NaCl 0.2 and $FeSO_4.7H_2O$ 0.03", incubation time 7 days, temperature 35, pH 6, inoculum size 4 mL/50 mL medium, inoculum age 72 h and medium volume 50 mL/250 mL conical flask. An overall 2.5-fold increase in uricase production by *Aspergillus welwitschiae* was achieved in the medium after statistical optimization as compared with the unoptimized basal medium (23.58 U/mL) before applying Plackett-Burman.

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Sequential/parallel production of potential malaria vaccines – a direct way from single batch to quasi-continuous integrated production

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An intensification of pharmaceutical protein production processes can be achieved by the integration of unit operations and application of recurring sequences of all biochemical process steps. Within optimization procedures each individual step as well as the overall process has to be in the focus of scientific interest. This paper includes a description of the development of a fully automated production plant, starting with a two-step upstream followed by a four step downstream line, including cell clarification, broth cleaning with microfiltration, product concentration with ultrafiltration and purification with column chromatography. Recursive production strategies are developed where a cell breeding, the protein production and the whole downstream is operated in series but also in parallel, each main operation shifted by one day. The quality and reproducibility of the recursive protein expression is monitored on-line by golden batch and this is controlled by model predictive multivariate control (MPMC). As a demonstration process the production of potential malaria vaccines with Pichia pastoris is under investigation.

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A new nano technique tool in medical studies and diagnostic assays: A literature review

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Atomic force microscopy (AFM) is a three-dimensional topographic technique with a high atomic resolution to measure surface roughness. AFM is a kind of scanning probe microscope (SPM) and it's a near field technique which is based on the interaction between a sharp tip and the atoms of the sample surface. There are several methods and many ways to modify its tip to investigate surface properties including friction and adhesion force measurements, viscoelastic properties and determination of Young's modulus and imaging magnetic or electrostatic properties. AFM can analyze any kind of samples like polymers, adsorbed molecules, films or fibers, powders in air, controlled atmosphere or in liquid medium. In the past decade, AFM has been emerged as a powerful tool to obtain nano structural details and biomechanical properties of biological samples including biomolecules and cells. AFM application techniques and in particular its force measurement parts are not still familiar to most of the clinicians. This paper reviews the literature regarding the main principles of AFM and highlights the advantages of using AFM in biology, medicine and especially in dentistry. This literature review was performed through e-resources including Science Direct, PubMed, Blackwell Synergy, Embase Elsevier and Google Scholar for the references published in 1985 to 2010.

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