Aksaray "Gate to Cappadocia"

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7TH INTERNATIONAL CONGRESS ON ADVANCES IN BIOSCIENCE AND BIOTECHNOLOGY

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İlker CAMKERTEN
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Dear Scientist,

The seventh International Congress on Advances in Bioscience & Biotechnology (icabb) was organized in Aksaray, TÜRKİYE. We are very happy for organizing this congress in such a beautiful city and country that we have strong historical ties.

We wanted to make this conference little bit special by bringing scientist together from different disciplines of biology and biotechnology area and also to open new research and cooperation fields for them. In this sense, we desired to bring the distinguished scientist together to get know each other and to develop and implement new joint projects.

The scientist joined the congress was from different country. Total over the hundered scientist were registered in the congress. The total number of submissions were 83 and after a careful evaluation 38 submissions were accepted by our scientific committee and 3 of them were accepted as poster presentation and, 35 of them were accepted as oral presentation and all those presentations was taken place in the conference booklet.

I would like to thank Prof. Dr. Hesham El Enshasy (Plenary/Orientation Lecture), Dr. Santosh Ramchuran (Plenary Lecture), Prof. Dr. Fikrettin Şahin (Plenary Lecture), Prof. Dr. Samina Mehnaz (Plenary Lecture) and Dr. Ernesto Hernandez (Plenary Lecture) for their valuable presentations.

We would like to send our special thanks to Mr. Musa Köse and Mr. İsmet Uzun, ZENITH Group workers for their special efforts. And finally, the most importantly I would like to thank to all the participants individually who came from far away to join this conference.

President

Prof. Dr. İlker Camkerten

Dear colleagues,

We are honor to welcome you this year in the 7th. International Congress on Advances in Bioscience and Biotechnology (ICABB). We are happy this year to have conference in Aksaray "The historic and wonderful city in the heart of Antolia, Türkiye. The conference this year is attended by many colleagues from all over the world to share their novel research with the scientific society. ICABB is also now considered as international hub for networking and building new partnership in research and to establish new cooperation agreements among researchers and between researchers and industries as well. I am happy to see that our ICABB family is growing from year to year in terms of number and diversity representing many countries around the world. The conference this year covers most of the biotechnology colors (Green: Agriculture and Environment; White: Industry; Red: Medical). In addition, some topics this year present very interesting integrated research between these three main fields of biotechnology.

I wish you all colleagues who are attending the conference physically in Aksaray or on-line a very nice conference and successful networking with other colleagues toward providing solutions to challenges we are facing nowadays to improve the quality of life on the earth.

With you all nice conference, successful network, and enjoying the beautiful culture of Aksaray.

Prof. Dr. rer. Nat. Hesham Ali El Enshasy

Chairman of ICABB

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Eurasian Biotechnology Association

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Prof. Dr. Hesham A. EL ENSHASY

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Dr. Avnish Pareek, Universiti Technology and applied Sciences-Sur Campus, Sultanate of Oman

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ORAL PRESENTATIONS

SESSION A-1

BIOTECHNOLOGY SOLUTIONS TO MINIMIZE THE IMPACTS OF CLIMATE CHANGE AND TO SOLVE PROBLEMS RELATED TO HUMAN HEALTH AND FOOD SECURITY

Plenary (& Orientation) Lecture

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Abstract:

Nowadays, biotechnology is considered as one of the key research field based on its wide range of applications and providing solutions and safe approach to overcome most of SDGs Challenges. Applications of biotechnology ranged from waste water treatment and bioremediation up to the production of high value therapeutic proteins, stem cell-based therapy and cloning technology. Climate change is one of the major threats we face nowadays and providing innovative solutions to mitigate the effect of climate change and to adapt to the new climate changes we face is critical for shaping of the human life on the earth. For example, food security is one of the main challenges we face with crucial need to have solutions to provide quality and healthy foods in the current irreversible and unpredictable changes in earth climate. However, climate changes not only affect the soil-water-air quality, water availability, which directly affect the plant yield, nutritional value and safety, but also can affect the overall all production and supply chain of food. This presentation provides a comprehensive global scenario of the current and future potential solutions for climate change mitigation and adaptation to minimize its effect of food availability and safety. It will also discuss the possible biotechnological solutions to minimize the impact of climate change on human health.

THE IMPACT OF DISRUPTIVE BIO-BASED INNOVATIONS GLOBALLY

Plenary Lecture

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Abstract:

Disruptive bio-based innovations are having a significant impact globally in moving away from the use of petrochemical feedstocks. However, there are many challenges in establishing a sustainable bioeconomy that will contribute to a country's socio-economic development through the creation and growth of new bio-based industries. The CSIR's Chemical Cluster believes that challenges in establishing a sustainable bioeconomy for the country can be enhanced by focusing on Small-to-Medium-Enterprises (SME) development and support as the main vehicle for economic growth. There is a strong focus on re-industrialization and the drive towards circular economy approaches using bio-based innovations. We believe that this will concomitantly lead to import replacement and increasing the competitiveness of local industries through the development of innovative knowledge products, processes, and relevant skills in support of new and established industries in the biomanufacturing sector. The CSIR has established several industry-facing centers that foster innovation, technology development support and entrepreneurship. These include the Biomanufacturing Industry Development Center (BIDC), the Industrial Biocatalysis Hub (IBH), and the Biorefinery Industry Development Facility (BIDF) which use an open innovation model that provides access to infrastructure and resources to enable technological advances that create and sustain bio-based industries and promote job creation. This session highlights the capabilities and offerings in biomanufacturing at the CSIR and showcases key industrial biotechnology innovations that have successfully led to commercialization. Also, key insights into how we have adopted rapid bioprocess development, optimization, and scale-up strategies to enable the "concept-to-commercial" model to be successful in a short-time frame to allow bio-based technologies and products for local and international uptake.

Keywords: Disruptive Technologies, Bio-Based, Innovations, Bio-Economy

[*]*CSIR Biomanufacturing Technologies*

APPLICATIONS OF BORON IN REGENERATIVE MEDICINE

Plenary Lecture

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Abstract:

Burn, chronic (non-healing) wounds and dermatitis are the major challenge of current dermatological applications. The destruction of skin integrity or tissue by biological, physical or chemical causes is the most common and destructive forms of wounds. Acute wounds usually heal within 3-6 weeks without the need for professional treatment modalities. However, chronic wounds are mainly associated with infection and prolonged inflammation, healing impairment and continuous tissue degradation. Radiation dermatitis is a common side effect of radiotherapy, which is one of the most frequently used treatments for cancers. Some people will experience mild redness and itchiness, while others may suffer painful, broken skin that is prone to infection. Any deformation that can occur in skin integrity can leave the human body vulnerable to many pathological conditions such as infection, excessive fluid loss and electrolyte imbalance. Although a vast amount of products have been introduced in the market, claiming to provide a better optimization of local and systemic conditions of patients, they do not meet the expectations of clinicians and patients. Therefore, developing new, safe, self-applicable, effective, and cheap wound care products with broad-range antimicrobial activity has always been an attractive area for scientists.
Therefore, a novel antimicrobial carbopol-based hydrogel formulated with boron and pluronic block copolymers was developed by Dr. Fikrettin Sahin, and evaluated for its healing activity using in vitro cell culture techniques. In addition, the preclinical and clinical studies were conducted to determine the effect of a novel hydrogel formulation containing NaB on the acute and chronic wounds healing. The results revealed that while both boron compounds significantly increased MSCs differentiation, and proliferation, migration, vital growth factor, and gene expression levels of dermal cells along with displaying remarkable antimicrobial effects against bacteria, yeast, and fungi, NaB displayed greater antimicrobial properties as well as gene and growth factor expression inductive effects. Preclinical and clinical studies proved that NaB-containing gel formulation enhanced healing rate of chronic wounds, burn and completely prevent the radiation-induced dermatitis in breast cancer tested. Therefore, experimental data showed that NaB, and its pluronics combination, could be used in dermatological clinics and be a future solution for chronic wounds and dermatitis.

Keywords: Boron-Based Gel, Wound Healing, Antimicrobial, Burn, Radiation Dermatitis.
**Yeditepe University and TUBA*

PSEUDOMONAS AURANTIACA – A BACTERIUM 'EXTRAORDINAIRE'

Plenary Lecture

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Abstract:

Pseudomonas aurantiaca - A rare species of Pseudomonas, till now few strains have been reported globally including Pakistan. Most of these have their genome sequenced due to their significant importance in agriculture. It is a gram-negative rod shape bacterium, well known for its ability to promote plant growth and kill plant pathogens, mainly due to production of large number of primary and secondary metabolites. In addition, it has ability to produce extracellular enzymes such as pectinase, protease, lipase and cellulase; and solubilizes potassium and zinc in soil to make it available to the plants. Our group has isolated large number of plant growth promoting bacteria including P. aurantiaca. Nine strains of this species have been isolated from sugarcane, cotton, paragrass, and cactus. These strains have been used as biofertilizer and promoted the growth of wheat, rice, corn, sugarcane, cucumber, bell pepper, and tomato under controlled environment and field conditions. These strains inhibited the growth of fungal pathogens including Fusarium spp. and Colletotricum falcatum, of economically important plants, rice, wheat, sugarcane, corn, etc., on plate assay. Several metabolites have been extracted and reported from these strains. Among these are phenazine derivatives, bacteriocins (antibacterial pepetides), Cyclic lipopeptides, Quorum sensing signals (Acyl homoserine lactones), Pyoverdin, Pyocin, pyoluterin, rhizoxin analogue, auxins, HCN, and pyrrolnitrin. A new compound "Lahorenoic Acid" (alkyl-substituted aromatic acid) has been reported by our group. Production of these metabolites vary depending on strain and growth media. Genomes of five strains PBST2, ARS38, RP4, FS2 and G7 are sequenced. Bacterial cultures and their metabolites have shown positive results for antimicrobial activity against human bacterial pathogens, antifungal and anticancer activity. These bacteria have great potential to be used as biofertilizer, biopesticide, and play role in cure of human diseases.

Keywords: Pseudomonas, Biofungicide, Biofertilizer, Microbial Metabolites

SUSTAINABLE SYSTEMS FROM PRICKLY PEAR CACTUS NOPALES

Plenary Lecture

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Abstract: The challenges from climate change require well-coordinated global efforts to impulse a circular economy for achieving Sustainable Development Goals. System approaches like those bringing together agriculture and biorefineries are needed to propose environmentally friendly and energy efficient solutions. Biomasses from the first to fourth generations look insufficient to meet current demands on products, energy, power and heat. Brown biotechnology could offer opportunities to exploit plants from arid regions. For instance, prickly pear cactus nopales. These strong invasive cacti are known for requiring comparatively less water, energy, care, carbon footprint and aggrotech than other biomass feedstocks. It seems possible to propose a novel sustainable biorefinery strategy using sustainable nopales. Well-designed systems can integrate experiments and assessments of realistic scenarios based on life cycle assessment, energy balances and efficiency. This enables a cleaner design for ethanol production from nopales in tune with the circular economy and sustainable development. We studied four realistic scenarios of systems blending agriculture-biorefinery, considering two fertilisers, two pretreatments and two operational modes. The scenarios were evaluated in terms of environmental effects via LCA and efficiency of storing energy in the ethanol molecule. Traditional acid hydrolysis and neutralisation do not lead to cleaner and energy efficient ethanol producing systems. Ionic liquids could offer a positive opportunity if fit-for-purpose chemical engineering designs are deployed. The best scenario considers organic fertilisers, ionic liquids and recycling and reuse of materials. It leads to a cleaner and energy efficient agriculture-biorefinery system for ethanol production. Also, it had the lowest impacts on environmental potentials such as acidification, eutrophication, global warming and more. This design also used the lowest amount of energy per unit of energy stored as ethanol fuel. Besides, it showed the best energy efficiency to capture net energy as ethanol fuel by three-fold compared to the worst scenario. Systems bringing together agriculture and biorefineries with fifth generation biomasses like nopales and novel biomass pretreatment can deliver beter solutions to help people, the environment and the climate.

PROCESS OPTIMIZATION OF 'HALAL' GELATIN PRODUCTION FROM OMANI FISH SPECIES

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Abstract:

Gelatin is a partial degraded collagen protein hydroxylate. It has specific rheological propertiy that is used extensively in industry. Most of the commercial gelatin produced is porcine and bovine in origin. This commercially gelatin has religious, socio-culture, and sanitary issues and world-over it is not accepted by many communities and creeds. Given this, an alternative source of gelatin is imperative from some halal source. Fish is considered halal, and can be utilized for halal gelatin production. During fish processing 40-60% /unit weight of fish waste is generated. However, the classical alkali or acid treatment for gelatin production does not work well with fish collagen because of its specific imino acid composition. Therefore, the enzymatic process for gelatin production from fish industry waste is used. So far such a process is not optimized for the warm water fish species waste. In our preliminary studies it was observed that this enzymatic gelatin production has some challenges. Firstly, since the gelatin has a specific visco-elastic rheological property, a strict treatment time and optimum temperature must be maintained for enzymatic treatment. Secondly, during product purification, molecules of desired size needs to be effectively removed from rest of the protein hydroxylates; and finally, the ready to harvest gelatin molecules must be protected from the indigenous proteolytic enzymes of the fish waste as well as from treatment enzyme. Given these challenges, the present study was conducted using a previously reported local thermophillic B. licheniformis thermo-alkaline protease. Process optimization was done on two most common warm water Omani catch fish species using experimental design and it was found that at 55-56°C, 2ml crude enzyme/100gms of fish waste, for 4 hours of treatment time at pH 9.5, 47% of working gelatin was produced as per GMIA manual of standards. The predicted results were found close to the actual results.

Keywords: Gelatin, Collagen, Halal Product, Process Optimization, Bioprocessing, Protease, Warm-Water Fish Species

*Preliminary part of this study was supported by The Research Council, Ministry of Higher Education, under the FURAP program.

BLIND DOCKING AGAINST HIV-1 PROTEASE USING AUTODOCK 4.2.6

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Abstract:

Currently, the HIV/AIDS virus is a major impediment to global health and development. Due to various negative effects, the development of a reliable vaccination is a now pipe dream. In addition, HIV-1 protease is accountable for rectifying the gag and gag-pol polyproteins during virion maturation. However, antiretroviral therapy (ART), increases the life expectancy of HIV-positive patients which is now being given to 14.9 million people globally. Tragically, there are still no HIV-1 therapeutics that work effectively. In this study, blind docking was performed in virtual box 6.1 using AutoDock 4.2.6 to determine effective phytochemicals that can target HIV protease (PDB ID: 2R5Q). Twenty phytochemicals were selected and a clinical trial drug (darunavir) was selected as a control. When the grid box was generated, the x, y and z values were 14.814, -15.206, and -54.457 and spacing were set to 0.54. Also, the genetic algorithm population was set to 150. Additionally, blind docking redocking value of 1.658Å was performed to validate the procedure. The ligands were evaluated according to the binding energy (BE) and inhibition constant (Ki), and the best potential phytochemicals were determined: carandinol (BE: -10.55, Ki: 18.54), withaferin A (BE: -9.96, Ki: 49.66), lupatic acid (BE: -9.77, Ki:68.9), maslinic acid (BE: -9.25, Ki:166) and sambunigrin (BE: -9.07, Ki: 68.73) kcal/mol, nM respectively. The best-docked poses and amino acid interactions were visualized using BIOVIA DS. The common amino acid interactions were observed in LEU24, LEU97 and PRO1. Moreover, common conventional hydrogen bonds were perceived in ASN98 and ILE3. In addition, ADMETlab2.0 was used to analyze ADMET properties. Withaferin A was the optimum ligand because it acknowledged the five Lipinski rules, exhibited significant Kis and consistent BEs, and also had an optimum logP. Additionally, findings anticipate the use of withaferin A for anti-viral purposes due to its good oral bioavailability.

Keywords: Keywords: Antiretroviral Therapy, Binding Energy, Inhibition Constant, Phytochemicals

**This research depicts the new therapeutics target for the HIV disease condition*

EXPLORING ELEMENTAL BALANCES AND STATISTICAL OPTIMIZATION TO EXPLOIT PGPR AND NITROGENASE PRODUCTION POTENTIAL OF GLUCONACETOBACTER DIAZOTROPHICUS UNDER BIOPROCESS SETTINGS

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Abstract:

Design of biopocesses to optimize microbial growth and production of industrial enzymes for food, feed as well as agriculture are of great interest both scientifically and economically. Gluconacetobacter diazotrophicus is rod-like shaped Gram-negative bacterium, with plant growth stimulating activity and being tolerant to acetic acid. Interestingly, it exibits nitrogenase activity, a key enzyme of the biological fixation pathway, thereby being potential of organic fertilizer, promising reduction in chemical fertilizer use. Despite its potential, very little is known on G.diazotrophicus under on bioprocess settings: only ad hoc media compositions are defined, elemental balances for fermentation is seldomly characterized, no optimization studies. However, being an important cost parameter as well as key to study (part of) the metabolism, finding a suitable/optimum medium composition and characterization of metabolism under production conditions is of great interest.

Statistical (as opposed to mechanistic) optimization is a technique used to explore relationships among explanatory variables as well as optimize systems where little is known on the mechanistic details of the system. The approach is nearly-universally applicable, typically uses an empirical model built using generated data to explore and optimize the system within a predefined domain. In particular Response surface methodology, uses a sequence of designed experiments (Central Composite or Box Behnken design) to obtain an optimal response using a second-degree polynomial model as an approximation.

The talk will provide a brief overview on bioprocess options for G.diazotrophicus, efforts on finding optimum medium composition for its growth using response surface methodology based on Box-Behnken experimental design, and translating the optimum conditions into benchtop scale bioreactor and full characterization in its C, electron, and ATP-balances. The results will be discussed vis-à-vis enzyme production and PGPR potential.

Keywords: Gluconacetobacter Dizatrophicus, Medium Optimization, Nitrogenase, Response Surface Methodology, Elelemental Balances

**This study is supported by TÜBİTAK, with project Nr 221M301*

EXTREMOZYMES CHARACTERIZATION: A CASE STUDY ON THE THERMOPHILIC BACTERIAL ALPHA-AMYLASE TFAMY48

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Abstract:

Extreme ecosystems such as thermal springs are known to harbor thermophilic microorganisms that produce thermostable enzymes, which are of interest to various industries that operate under extreme physicochemical conditions. Algeria, which is rich in thermal springs, has become a focus of research on thermophiles and their enzymes.

A study conducted on thermophilic bacteria isolated from a thermal spring in Algeria identified Tepidimonas fonticaldi as a producer of a highly active (30 U/mL) amylase, named TfAmy48. The enzyme was purified and characterized to determine its enzymatic properties. It was found that the optimal activity of TfAmy48 occurs at pH 8 and a temperature of 70°C, with the presence of calcium shifting the optimum to 80°C. The monomeric enzyme has a molecular weight of 48 kDa and is sensitive to classical inhibitors such as heavy metals, but not to chelating agents in detergents such as EDTA and EGTA. The enzyme's characteristics suggest its potential as an additive in detergents.

In comparison to a commercial enzyme, Termamyl® 300L, TfAmy48 was found to have superior relative and residual activity in the presence of different detergents and their constituents. The gene encoding TfAmy48 was cloned into E. coli BL21 using the expression vector pTrc99A, enabling the overexpression of the enzyme up to an activity of 300 U/mL. Homology modeling was used to elucidate the enzyme's structure.

In conclusion, the study characterized the thermostable amylase enzyme TfAmy48 produced by Tepidimonas fonticaldi from a thermal spring in Algeria. The enzyme's properties suggest its potential application in the detergent industry.

Keywords: Thermophilic ; Tepidimonas Fonticaldi ; A-Amylase ; Detergent Formulations. *

OPTIMIZATION OF PLASMA OZONE PRETREATMENT FOR LIGNIN EXTRACTION FROM BANANA PEELS FOR POTENTIAL COSMETIC APPLICATION

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Abstract:

Lignin derived from banana peels has huge application in cosmeceuticals industries. Lignin can act as natural UV protection due to its UVA and UVB absorbing properties and is believed to become a super alternative for the commercial UV protection products widely used in the market. Lignin from banana peels can be extracted by undergoing pretreatment. In this study, ozone pretreatment conditions were optimized and applied towards banana peel. To confirm the success of the pretreatment, compositional analyses were conducted before and after the ozone pretreatment. Subsequently, the pretreated banana peels underwent three different extractions (basic organosolv, soda and formic acid extraction) and the efficiency of the extraction methods were compared. The results revealed that formic acid extraction shows the least amount of lignin left in the banana peel (4.4 ± 0.25%) compared to basic organosolv and soda extraction methods with 8.91 ± 1.07% and 5.95 ± 1.15% remaining lignin, respectively. Meanwhile, the lignin-containing hydrolysate after the plasma ozone pretreatment was also collected. All type of lignin collected from different strategies were formulated into cosmetic creams. Structural characterizations using Fourier transform infrared (FT-IR) spectroscopy and Scanning Electron Microscope (SEM) were performed for all samples. The ozone pretreatment demonstrated an efficient method for pretreatment of banana peels, as it was capable of reducing lignin up to 39.14%. Sun Protection Factor (SPF) of lignin-based cream from banana peels showed significant values ranging from 1 to 13 according to the types and concentrations of the lignin used. Highest SPF values (13.219 ± 1.36) was obtained from the cream incorporated with the lignin from the hydrolysate. Lignin-based cream of formic acid lignin, soda lignin and solution lignin portrayed good UVB absorption, while basic lignin shows good UVA protection and thus proves its significant potential in cosmetics applications.

Keywords: Lignin, Plasma Ozone, Pretreatment, Banana Peels, UV, Cosmetic

**This work was financially supported by the Universiti Teknologi Malaysia (UTM) under the UTM Fundamental Grant, UTM-FR (20H88)*

INFLAMMATORY BOWEL DISEASE IN A HORSE: CLINICAL PRESENTATION AND CECUM MICROBIAL PROFILE

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Abstract:

In the clinical examination of the 11 year old horse brought to the hospital with complaints of chronic diarrhea, pain and weight loss, it was observed that the heart rate increased, the respiratory rate and body temperature were normal. Anemia, dehydration and neutropenia were detected in the hemogram findings, while a decrease in total protein and albumin values and an increase in liver enzymes were detected in the blood biochemistry findings. The horse died on day 4 after being treated for suspected inflammatory bowel disease (IBD). In order to clarify the etiology of the disease and to investigate the taxonomic bacterial composition, DNA extraction was performed for metagenomic analysis by taking samples from three different parts of the cecum. For bacterial profiling, primers that amplify the V3-V4 region of the 16S rRNA gene were used. Amplicon readings from the Illumina MiSeq System were analyzed using quantitative insights into microbial ecology 2 QIIME2 (2022.11) software. Campylobacter rectus (36.1%) and Roseburia inulinivorans (18.3%) were found to be higher in the areas of the cecum showing hemorrhagic lesions compared to the less inflamed parts. As a result, when the examination findings, hemogram and biochemistry changes and bacterial profile were evaluated together, it was determined that the case was compatible with IBD and the taxonomic bacterial composition of the disease was revealed.

Keywords: IBD, Bacterial, Clinical, Cecum

BIOAVAILABILITY ENHANCEMENT OF COENZYME Q10 USING CYCLODEXTRIN

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Abstract:

The increasing prevalence of heart disease, diabetes, cancer, and immune diseases because of unhealthy lifestyles has increased the demand for healthy food and supplements across the country, thereby raising the demand for Coenzyme Q10 (CoQ10) products among health-conscious consumers. CoQ10 is a critical component of the electron transport chain, which is responsible for producing adenosine triphosphate (ATP) and is frequently used as a supplementary treatment for some diseases. As human ages, the body production of CoQ10 reduces thus impairing ATP synthesis resulting in decreasing energy production and potential cellular damage. The hydrophobic property of CoQ10 powder limits its bioavailability as a supplement, which was reported less than 10%. Various strategies have been implemented in developing stable and soluble CoQ10 nutraceutical supplements with high bioavailability and efficacy. Emulsification of CoQ10 improves its bioavailability and enables its use in a wide range of products, such as skincare, supplements, and functional foods. The stability and effectiveness of the emulsified CoQ10 are depending on several factors, such as the type and concentration of emulsifier used, the pH of the formulation, and the manufacturing process. The use of cyclodextrins enhances the emulsification process by improving the solubility and stability of the molecule. The hydrophobic cavity of the cyclodextrin molecule encapsulates the CoQ10 molecule, forming an inclusion complex. This complex increases the solubility and stability of CoQ10 in the aqueous phase, preventing aggregation and degradation of the molecule. Overall, the use of cyclodextrins as emulsifiers for CoQ10 improves the bioavailability and stability of the molecule, making it more useful for various applications, such as in the pharmaceutical and cosmetic industries.

Keywords: Emulsification, Cyclodextrin Complex, Coq10, Bioavailability

**This study is supported by Kamarizs Medicare Sdn. Bhd.*

OPTIMIZING AND PURIFYING LACCASE FROM THE NEWLY ISOLATED WHITE ROT FUNGUS, CERRENA SP. WICC F39.

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Abstract:

White rot fungi have been studied extensively on a global scale due to their potential use in biotechnology. Of the ligninolytic exoenzymes produced, laccase has been one of the most studied and is associated with a variety of green oxidation processes. However, difficulties with producing sufficient quantities of these enzymes in economically viable processes have held back the study of ligninolytic enzymes. In this study, local soils were screened for white rot fungi producing ligninolytic enzymes and eight isolates were identified as potentially useful from 119 candidates. Cerrena sp. WICC F39 was selected for its high laccase activity and optimization work was conducted using ligninocellulosic wastes in submerged culture. The optimization work was conducted using both OFAT and statistical methods with OFAT producing 110% more laccase activity than the statistical method. Characterization of laccase from Cerrena spp. WICC F39 showed a molecular mass of 62 kDa, a fold of purification of 5834.68 with 158.6% recovery and an ABTS substrate Km and Vmax value of 0.107 mM and 77101.00 S-1 mM-1 respectively. The optimum pH, optimum temperature, pH stability and thermal stability of laccases were 2.5, 60°C, 4–6, 20-80°C, respectively. Sodium azide was found a true inhibitor for laccases from Cerrena spp. WICC F39. In accordance with the results showed in this study, such high-level secretion of laccase and other ligninolytic enzymes make Cerrena spp. WICC F39 as a potential candidate for enhanced bioremediation.

Keywords: Cerrena, Rice Straw, Submerged Fermentation, Purification

SACCHAROMYCES BOULARDII BIOTECHNOLOGY AND PROBIOTIC FUNCTIONAL FOOD DEVELOPMENT

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Abstract:

Over the past half century, modernization, population growth, has been associated with changes to energy-dense, appetizing but nutrient-poor foods, contributing to an increased risk of developing several diseases. In this context, we have tried to develop three types of functional foods (pasta, cereals and chips) based on the probiotic strain Saccharomyces boulardii for preventive purposes against digestive diseases and diarrheal symptoms with potential health benefits. In order to carry out this study, a questionnaire survey was distributed online and in the field on the consumption of probiotics and functional foods in Algeria, which allowed us to formulate functional foods that are appropriate to the expectations of the consumer. A series of tests were applied on functional foods (AF) developed namely the strain viability test, analysis of microbiological, physico-chemical, organoleptic, nutritional and «liking» quality. For the viability test, remarkable results were obtained, indeed the probiotic strain was able to survive in the three food matrices. The other tests ensured that our FAs are of satisfactory quality and comply with Algerian legislation. These formulations were patented. The AF market is continuously growing worldwide, which leads us to believe that the research and development of this field in Algeria is crucial and requires a lot of attention and future investigation.

Keywords: Functional Food, Probiotic, Saccharomyces Boulardii, Formulation, Pasta, Chips.

ANTIVIRAL EFFECT OF ESSENTIAL OILS ON TOBACCO MOSAIC VIRUS IN PLANT TISSUE CULTURE MODEL

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Abstract:

Tobacco Mosaic Virus (TMV) is one of the first plant viruses to be discovered. It is described as a positive single-stranded RNA viruses with a length of approximately 300 nm rod-shape morphology. TMV can infect the roots, stems, and leaves of live tobacco plants. Unlike other viruses, it can remain in the death plant tissue for a long time and maintain its virulence effect because of its morphological characteristics. TMV can infect 150 different plant species, including tomato, pepper, eggplant, tobacco, spinach, petunia and marigold. It follows the path of mechanical transmission and can spread with the hands, clothes, or tools of the workers. Thus, it can show contamination even from factory to factory. TMV affects the development process of chloroplasts in infected plants, creates mosaic-patterned yellow-dark green necrotic spots on the leaves of the plant and affects fruit development. Since there are no currently available options or products for management of the plant viruses including TMV, it can cause serious damage in agriculture and negatively affect the food sector in the long term. The annually agricultural lost cost attributable to plant viruses are estimated more than $30 billion in worldwide. For this reason, there is a need to develop antiviral substances for plant viruses, which will protect plant viral contamination, reduce the virulence effect or inhibit the viral replication in infected plants. In order to develop such products, it is thought that it should be done in controlled areas such as plant tissue culture in order to protect the health of other plants. Therefore, in this project it was aimed to isolate TMV from dried tobacco samples, proliferate the virus in plant tissue culture and perform antiviral experiments. Briefly, mature plant callus parts were infected with isolated TMV and passaged on agar. Infection period was observed during 7 days on agar culture. Accordingly, the infected group was defined as pale and brown, while the control group appeared lively and green. Then, the callus was fragmented and TMV isolation was done by ultracentrifugation. TMV were detected by Transmission Electron Microscopy (TEM). In our ongoing project, the antiviral activity of lavender and tea tree essential oils will be tested against TMV in callus agar and/or suspension culture. Antiviral activity analysis will be done by RT-PCR method. According to preliminary results it was shown that both lavender and tea tree essential oil applications were effectively inhibited TMV in both callus culture.

Keywords: TMV, Antiviral, Lavandula, Lavender Oil, Tea Tree Oil, Callus Culture, Plant Viruses*

LONG-TERM NUTRITION WITH MEAT AND BAKERY MEALS, REFLECTION ON THE HEMATOLOGICAL PROFILE: AN EXPERIMENTAL STUDY ON RODENTS

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Abstract:

Background/Aim: In the modern world, it is concerning low-quality food or so-called „fast food“, of the barbecue type and at the same time oversaturated with carbohydrate and lipid components, which inevitably leads to obesity and other, often malignant diseases, in humans and some pets. On the other hand, obese people try to correct an inadequate diet, consuming only one or two types of food, neglecting the intake of unbalanced and insufficient amounts of essential nutrients, in which is leading the so-called meat diet, that is widespread and as such represents an attempt to correct obesity. In the research, we used the animal model of the laboratory rat, which is omnivorous in terms of nutrition, which represents a certain similarity with humans and pigs, so the rat is the animal of choice in our study. The aim of the study is to determine the possible adverse effects of long-term consumption of meat products and bakery products, following the hematological status of the examined animals. Material and methods: Twenty-four rats were randomly divided into three groups, eight in each group. The first group (A) was fed with bakery products. The second group (B) was fed with meat products. The third group (C) was fed with conventional, briquetted food for rodents, and it represented the control group. The animals consumed the specified food for seven weeks (49 days). The analysis of hematological parameters was determined using the cell counter „Idex Laser Cyte“ flow hemocytometer and the usual parameters were determined. Results: The Bonferroni test of individual differences between the two experimental and control groups show that in group (A) the MCV was significantly lower compared to group (B) (MD=-4.45, $p<0.001$) and the control group (MD=-3.08, $p=0.014$), MCH compared to group (B) (MD=-2.08, $p<0.001$) and the control group (MD=-0.9, $p=0.013$), MCHC compared to the meat group (MD=-1.26, $p=0.052$). Group (A) had higher results for WBC compared to the group (B) (MD=2.97, $p=0.031$) and for PLT (MD=418.0, $p=0.004$). Group (B) had significantly higher results for the parameter MCH compared to the control group (MD=1.18, $p=0.001$) and for the variable MCHC (MD=1.38, $p=0.031$), while it had lower results for WBC in compared to the control group (MD=-3.10, $p=0.002$) and PLT (MD=-301.8, $p=0.042$). Conclusion: We concluded that a long-term diet exclusively with group (A) bakery products, as well as group (B) meat products, adversely affected a number of hematological parameters. In support of this statement, certain poikilocytotic forms of erythrocytes were determined after microscopic analysis of the peripheral blood of the examined animals.
Keywords: Bakery Products, Meat Products, Hematological Parameters

FORMULATION DEVELOPMENT OF A WOUND HEALING CREAM FROM EXTRACTS OF OPUNTIA SPP.

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Abstract:

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. Various studies have been done to assess the wound healing potential of Cactaceae family. Wound healing agents support the natural healing process, reduce trauma and likelihood of secondary infections and hasten wound closure.

This study aimed at formulating and preparing herbal cream from Opuntia stricta; establishing the quality, wound healing efficacy and toxicity profile of the prepared herbal Cream.

Ethanolic extracts of the aerial parts of the study plant were prepared and screened for presence of alkaloids, flavonoids, terpene, steroids, and anthraquinones.

Formulation of Herbal Skin Cream for wound healing was successfully developed that met the relevant pharmaceutical characteristics. The prepared formulations are then evaluated for parameters like physical properties, pH, viscosity, Spreadability and stability of the formulated cream. The prepared formulations showed good Spreadability, no evidence of phase separation and good consistency during the study period. Stability parameters like visual appearance, nature, viscosity and pH of the formulations showed that there was no significant variation during the study period. The prepared formulations showed proper pH range that is approximately pH 6; it confirms the compatibility of the formulations with skin secretions. The creams were found to be stable during stability to ICH guidelines (40±2 °C/ 75±5 % RH) for 3 months.

In-vitro Diffusion studied conducted on all the 8 formulations has shown good diffusion when compared to other formulations.

Now it can be possible to develop creams containing herbal extracts and can be used as a barrier to protect skin.

Keywords: Wound Healing; Antimicrobial Activity, Antioxidant Activity; Formulation; Opuntia Stricta

**THE HIGHER SCHOOL OF BIOLOGICAL SCIENCES OF ORAN. ALGERIA*

EFFECTIVE AND EFFICIENT PROLIFERATION OF BHK-21CELLS USING SERUM-FREE-MEDIUM IN FED-BATCH CULTURE SYSTEM FOR FMD VIRUS PRODUCTION

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Abstract

Foot-and-mouth disease (FMD) is a highly contagious and devastating a viral disease of cloven-hoofed animals and is considered a severe threat to the livestock industry worldwide. Today, BHK 21 cells adapted to suspension culture systems are widely used in large-scale inactivated FMD vaccine production. The serum is a broad supplement in animal cell culture media. Although it has many advantages, it has many drawbacks and a substantial cost. Current biotechnological approaches to cell culture avoid using serum; therefore, this study aims to grow BHK-21 suspension cells in serum-free media (SFM) in stirred bioreactors. In our study, BHK-21 cells were maintained in suspension culture up to 20 passages in a 2L stirred bioreactor. After ten passages of suspension cell culture, SFM was used without serum, while control groups were maintained with 6M medium, including 10% serum. FMDV culture was prepared between the 10th and 20th passages level of the cells. During the process, the growth kinetics of the cells culture, antigenicity and infectivity of the FMD virus were assessed comparatively. The determined cell count and percentage of viability of the cultures in both media complied with each other. Also, virus antigenicity and infectivity values of the virus harvests were similar for the test and control groups (SFM and 6M). This study showed that large-scale suspension BHK-21 cells used in industrial-scale production of the FMD vaccine could be grown in SFM without serum without compromising quality and quantity.

Keywords: Serum-Free Medium, BHK-21 Cells, Foot-And-Mouth Disease, Vaccine, Pilot-Scale Production

[*] *This study is supported by the General Directorate of Agricultural Research and Policies (TAGEM) and the ŞAP Institute*

THE EFFECT OF FULLERENE C_{60} NANOPARTICLE ON COX-2, HO-1, P53 AND CASPASE-3 PROTEIN SIGNALING PATHWAYS AGAINST LIVER TISSUE INJURY

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Abstract:

In this study, the treatment effect of fullerene C_{60} nanoparticle against liver tissue damage caused by 7,12-dimethylbenz [a] anthracene (DMBA) in Wistar albino female rats was investigated. The animal experiments part of this study was conducted in the Firat University Experimental Animal Research Center (FUDAM) with the permission of the Firat University Animal Experiments Ethics Committee dated 27.01.2021 and numbered 2021/02. In this study, 60 Wistar albino female rats (n=60, 8 weeks old) were used. These rats were divided into 4 groups and each group included 15 rats. Groups are as follows: (1) Control Group: Group fed with standard diet; (2) Fullerene C_{60} Group: The group given Fullerene C_{60} (1.7 mg/kg bw, oral gavage); (3) DMBA Group: The group given DMBA (45 mg/kg bw, oral gavage); (4) Fullerene C_{60} + DMBA Group: The group given Fullerene C_{60} (1.7 mg/kg bw, oral gavage) and DMBA (45 mg/kg bw, oral gavage). The rats were decapitated after 16 weeks and their liver tissues were examined. Expression levels of COX-2, HO-1, p53 and caspase-3 proteins in liver tissue were determined by western blotting technique. Compared to the DMBA-treated group, COX-2 protein expression level was decreased in the fullerene C_{60} administered groups, while the HO-1, p53 and caspase-3 protein expression levels were significantly increased. According to the results, it was determined that fullerene C_{60} has a preventive and therapeutic effect on liver tissue damage.

Keywords: Caspase-3, COX-2, HO-1, p53

**This work was supported by Scientific Research Projects Coordination Unit of Firat University. Project number: FF. 20.07.*

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EFFECT OF DIFFERENT EXOSOME ISOLATION METHODS ON EXOSOME CHARACTERIZATION AND EFFICIENCY

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Abstract:

Objectives: Exosomes, one of the extracellular vesicles, provide intercellular communication, are considered extracellular organelles. The isolation of exosomes, which are frequently used in drug studies, especially in cell-free cellular therapy and nano-size, are made with different methods, and each method has advantages and disadvantages. It was aimed to investigate the effects of exosome characterization, proliferative effect and by using two of the most widely used isolation methods, ultracentrifugation, and commercial kit.

Research Methods: L929 mfibroblast cell line was used in the study. Cells were grown in DMEM without FBS for exosome. The medium transferred into two different tubes. One of the tubes was used for exosome isolation by ultracentrifugation (15000xg); the other was used for isolation with a commercial kit, the miRCURY Exosome Kit. Exosome were characterized by SEM, NTA and surface markers and compared according to isolation methods. The effect of exosomes on proliferation was compared on A549 and Wl38 cell lines.

Results and Conclusion: The shape of exosomes isolated by ultracentrifugation with SEM is smaller and more constricted, their number is higher. The shape of the exosomes isolated with the kit is more compatible with the literature, and they are seen in the form of round membranes and in small numbers. While there was no difference between the two groups in NTA results, less CD63 was detected in exosomes obtained in ultracentrifugation. The antiproliferative effect was the same in both groups; It was determined that exosomes obtained from ultracentrifuge isolation were more effective, especially in the study performed on the Wl38 cell line (P=0.001). As a result, isolation performed with a mass yielded more successful results in characterization; On the other hand, isolation by ultracentrifuge gave more successful results in terms of demonstrating the function of exosomes.

Keywords: Exosome, Isolation Methods, Proliferation, Cell Culture

PRELIMINARY PHYLOGENETIC FINDINGS OF THE NEAR EASTERN FIRE SALAMANDER (SALAMANDRA INFRAIMMACULATA, MARTENS, 1885) IN ANATOLIA

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Abstract:

The Near Eastern Fire Salamander (Salamandra infraimmaculata), a globally categorized as near threatened species in the IUCN Red List, is distributed in southern and eastern parts of Türkiye, north of Iraq, northwest of Iran and along the Levant. There are three known subspecies of fire salamanders in Anatolia, which are, S. i. infraimmaculata, S. i. orientalis and S. i. semenovi. All knowledge about subspecies boundaries is only based on morphological and morphometric studies so far. Except for a few samples, there is no genetic characterization using mtDNA or nuclear genes. To reveal the phylogenetic relationships among those three subspecies, we sequenced the 638 bp long mitochondrial cytochrome oxidase I gene (cox1) in 148 individuals collected from 41 localities throughout their range in Türkiye. Phylogenetic analyses indicated that these three subspecies formed well-supported monophyletic groups. The nominotypical subspecies S. i. infraimmaculata split off first from the ancestral population and is now found only in the south of Hatay province. Subspecies S. i. orientalis is the next diverged group and is found in Adana, Osmaniye, Kahramanmaraş, Adıyaman and the north of Hatay provinces. The last monophyletic group, S. i. semenovi, is distributed in eastern Anatolia ranging from Erzincan south to Batman and Şanlıurfa. Interestingly, a previously unknown but related population is found in Mersin province. The relationship within this last subspecies is unresolved as more samples are needed to conclude whether the Mersin populations are a separate taxon or not. We argue that better understanding the intraspecific variation and determining the precise distributional boundaries for this threatened species are critical to developing effective conservation strategies.

Keywords: S.I. Infraimmaculata, S.I. Orientalis, S.I. Semenovi, Cox1 Gene

Acknowledgement: **This study was supported by TUBITAK 121Z819.*

DNA ISOLATION AND GENDER DETERMINATION FROM ONE SHED FEATHER OF RAMPHASTOS TUCANUS BIRD

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Abstract:

Gender determination by PCR method is important in birds whose gender cannot be determined morphologically,. DNA sources to be isolated for gender determination in birds may consist of samples such as blood, fresh feathers, feces, tissue, and saliva. It is important to obtain DNA non-invasively in birds. This case study was planned on a caged and waiting feather sample belonging to a Ramphastos Tucanus bird brought to Kayseri Zoo. In this study DNA isolation from shed and waiting feather and gender determination with universally declared primer pair are aimed. The obtained feathers were delivered to the laboratory under sterile conditions. DNA isolation was isolated by standard phenol-chloroform isolation method. The OD260/280 ratio of the DNA obtained 1.84 and the amount was determined as 73 ng/µl. As a result of the analysis performed with the gender-specific primer, a single band representing the male gender was observed.

Keywords: Bird, Shed Feather, Gender Determination, Pcr

CONSTRUCTION OF NEW GENERATION PROBIOTICS: TECHNICAL DEMANDS

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Abstract:

Problem. The search and implementation of naturally derived probiotic components for creating synbiotics products that possess both technological and physiological functionality is promising for the 3P medicine application. Today, the production of pharmabiotics has gained new significance through the use of “next-generation” probiotic strains. Therefore, there is a need to develop new, more affordable and reliable technological procedures for screening, cultivation and store of any unique strains or its composition. Research methods. The work is based on the analysis and systematization of our experimental and theoretical scientific data. Results. Special attention should be paid to the safety of strains, their functional characteristics, produced metabolites, adhesive properties, allowing successful colonization of the host's epithelial cells along with no exhibiting inhibitory effect to the commensal representatives, and ability to specifically modulate local and systemic immune responses. Since conventional probiotic preparations contain live cultures, this requires strict demands to enable their viability, appropriate transportation, as well as mandatory compliance with the shelf life, which is usually longer for lyophilized preparations compared to liquid or gel forms (up to three months). In addition to optimizing the composition of the nutrient medium, great attention is given to the microorganism cultivation, particularly to the nature (source) and concentration of carbon in the medium and its influence on biomass accumulation. Analysis of trends in the processing industry in Western Europe and America shows that cryogenic technologies occupy a priority position in obtaining high-quality food products and additives with increased content of biologically active compounds.

Conclusions. The lyophilization is the commonly accepted method, since microbial strains can be stored significantly longer than frozen. Lyophilized preparations require fewer types of filler in the final product, ensuring their standardization and relative stability during long-term storage. The manufacture of NG probiotical strains mixed with prebiotical components requires revision of technical procedure.

Keywords: Pharmabiotics, 3P Medicine, Probiotic Strains, Technological Procedures*

ALKALIPHILIC BACILLUS LEHENSIS G1: FROM BASIC SCIENCES TO BIOTECHNOLOGY APPLICATION

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Abstract:

Extremophilic microorganism have been a great interest to scientific and industrial communities not only for fundamental scientific knowledge discovery but most importantly for its potential in biotechnological application. Bacillus lehensis GI is an alkalophilic bacterium that was isolated in Malaysia. The bacterium high pH survivability and capability to express hydrolytic enzymes including cyclodextrin glucanotransferase, maltogenic amylase, levanase and many more has led to further investigation on its pH adaptation characteristics as well as the functionality and structural studies of its biocatalysts. Antiporter gene or protein involved in pH adaptation has been identified and cloned into E. coli for functional study. Another interesting feature of this Bacillus lehensis G1 is its ability to secrete enzymes extracellularly. Proteomic approach was used to identify several potential signal peptides and secretive efficiency study was carried out using E. coli as the expression host to investigate their extracellular expression and secretion efficacy. The study on extremophilic microorganism such as alkalophile Bacillus lehensis G1 has led us to the importance of understanding biological and fundamental scientific knowledge for future application in biotechnology industries.

Keywords: Extremophile, Signal Peptides, Ph Adaptation, Hydrolytic Enzymes

[*]Plenary talk (video, Invited by Chairman)

BIOCONVERSION OF BIOMASS: FROM WASTE TO WEALTH

(Keynote)

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Abstract:

Industrial production of many products heavily relies on fossil resources. In the meantime, biomass from lignocellulosic has received significant attention as an alternative material due to its renewable nature and abundant availability. Various bioconversion technologies have been used to produce or extract value-added products from biomass. However, the bioconversion efficiency process depends on the types of biomass used as a raw material, which are different in terms of their compositions and the processes involved. In most cases, the bioconversion of lignocellulosic biomass usually involves three stages which are pretreatment, hydrolysis and fermentation processes. The processes are necessary for breaking down polysaccharides into monosaccharides and eventually for efficient conversion into targeted products. Several bioconversion studies into value-added products were conducted using different lignocellulosic biomass sources obtained in Malaysia. Conversion of biomass oil palm frond into xylooligosaccharide and pineapple leaves fibre into total reducing sugar involved pretreatment and enzymatic hydrolysis. In contrast, the fermentation process involved converting tropical fern into pectinase enzyme and local lignocellulosic biomass into mycelium-based biofoam. These studies also focus on optimizing bioprocessing parameters involved to improve the production yield. Therefore, bioconversion of lignocellulosic biomass showcases different biomass properties, product variations, pretreatment and hydrolysis methods, as well as fermentation conditions, which are valuable input for current and future renewable sources of industrial raw materials.

Keywords: Bioconversion, Lignocellulosic, Biomass, Pretreatment, Hydrolysis

[*]This study is supported by research grant under Universiti Teknologi Malaysia

UTILIZATION OF RHIZOBACTERIA TO INCREASE ANTIOXIDANT AND PHYTOCHEMICAL CONTENT OF LOCAL GINSENG (TALINUM PANICULATUM GAERTN) LEAVES

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Abstract:

The development of organic herbal products from the local community is expected to support tourism and increase regional economic income. One of the local herbal products that has the potential to be developed is local ginseng tea because it has properties to increase immunity and stamina. To provide added value from this product, it is necessary to plant organically, because organic products are in great demand in the world because they are free of harmful chemicals. One method that can be used to improve the quality of this local ginseng tea such as phytochemical content, antioxidants, and chlorophyll is to use rhizobacterial biostimulants. The methods used are the calorie metric method for phytochemical analysis, DPPH method for antioxidant analysis, IAA analysis method to determine the content of IAA hormone in rhizobacteria, nitrogen-fixing test using Jensen media to determine the ability of rhizobacteria to fix nitrogen, and bacterial ability test to dissolve phosphate using pikovskaya media The results obtained from preliminary tests that 50 types of rhizobacteria were taken from plant roots. From the IAA test, 17 positive IAA hormones were obtained, these 17 rhizobacteria were tested nitrogen fixing and testing the ability of rhizobacteria to dissolve phosphate. 4 rhizobacteria were taken with the best IAA hormone content, fixing nitrogen and dissolving phosphate, then applied to ginseng plants in the field at a concentration of 2% each. As a result, the four rhizobacteria can increase the content of antioxidants, phytochemicals, and chlorophyll in ginseng plants when compared to controls. The highest antioxidant content with an IC50% value (31.03 ppm) in the very strong antioxidant category was found in tingho 6 isolate, the highest total flavonoid content (87.03mg QE/100 mL) was found in tingho 7 isolate, the highest total phenol content (791.92 mg GAE/100g) was found in tingho 7 isolate, the highest chlorophyll content was found in tingho 9 isolate (1422.26 ppm)

Keywords: Phytochemicals, Ginseng, Medecine, Local

**Back2Nature Regenerative Farm Kuala Pilah Malaysia*

OPTIMIZATION OF HIGHER PRODUCTION, CHARACTERIZATION, ANTIMICROBIAL AND ANTICANCER ACTIVITY OF BIOACTIVE METABOLITES ISOLATED FROM PSEUDOMONAS AURANTIACA PB-ST2

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Abstract:

Metabolites and plant growth promoting abilities of Pseudomonas chlororaphis subsp. aurantiaca, PB-St2, have been extensively studied. This study focuses on isolation and optimization of higher yield of bioactive metabolites, from PB-St2, and their potential as antimicrobial and anticancer agents. Initially, maximum production of metabolites was optimized at different temperatures and incubation periods. PB-St2 was subjected to bulk extraction (5 L) and metabolites were detected by thin layer chromatography (TLC), and purified through gravitational column chromatography and HPLC; and characterized by LC-MS. Collected fractions were screened for antimicrobial activity against fungal phytopathogens (Fusarium equiseti, Fusarium incarnatum, Alternaria alternata, Colletotrichum falcatum) and bacterial human pathogens (Bacillus cereus, Pseudomonas aeruginosa, Salmonella enterica, Klebsiella oxytoca) and anticancer activity (HepG-2 , SF767). Bacterial culture grown at 32°C for 72 h yielded 5 g crude extract. Through gravitational chromatography, two pure compounds, Mupirocin and Phenazine Carboxylic Acid (PCA); and a complex of three compounds (PC3) were isolated from crude extract. PC3 was further purified by HPLC and three compounds, pyoluteorin, PCA and 2-hydroxyphenazine (2-OH-phz) were identified. Complex of compounds PC3, and PCA exhibited highest inhibitory activity against B. cereus and A. alternata. Maximum antifungal activity of 2-OH-phz was observed against A. alternata followed by C. falcatum and F. incarnatum. PC3 also achieved the highest IC50 against HepG-2 and SF767 cell lines followed by PCA and 2-OH-phz.

Keywords: P. Aurantiaca; Bioactive Metabolites; Chromatography; Antimicrobial; Anticancer

Higher Education Commission (HEC) of Pakistan

MAXIMIZING PULLULAN PRODUCTION: UNLOCKING THE POTENTIAL OF AUREOBASIDIUM MELANOGENUM DSM2402 THROUGH BIOPROCESS OPTIMIZATION

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Abstract:

Pullulan is a water-soluble homopolymer composed of maltotriose subunits. It is a bio-degradable biopolymer essentially a linear glucan containing α-1,4 and α-1,6 linkages in the ratio of 2:1. The unique structural and physical properties of pullulan provide its structural flexibility, easy derivability, and superior solubility. It has potential applications in the pharmaceutical, food industries and as biodegradable plastic because of its advantageous chemical and physical properties. Some of its outstanding properties including low viscosity, non-toxicity, slow digestibility, high plasticity, and excellent film-forming capabilities. Pullulan is produced by Aureobasidium pullulans which is also called black yeast. Although pullulan shows great potential in industries, the high production cost and low productivity are the major drawbacks. Thus, the main objective of the present work is to focus on bioprocessing optimization for high pullulan production by Aureobasidium melanogenum DSM 2404. The experiment started with selecting the best medium production and was followed by medium optimization using one- factor-at-a-time method and statistical method in the shake flask. Using the optimal medium compositions obtained, the effect of pH-controlled and uncontrolled pH conditions were compared in a bioreactor. The cultivation medium that produced the highest pullulan production from statistical optimization was composed of (in g L-1): sucrose 57.47; BSFL powder, 5.03; K2HPO4, 20.0; MgSO4.7H2O, 0.6; NaCl, 1.0. On the other hand, the optimal medium composition using one-factor-at-a-time method composed of (in g L-1): sucrose 40.0; BSFL powder, 6.0; K2HPO4, 18.0; MgSO4.7H2O, 0.6; NaCl, 1.0. The maximal pullulan production obtained from the statistically optimized medium was 33.11 g L-1, which was 158.17% higher than the unoptimized medium (12.83 g L-1), and 18.12% higher than the medium optimized using one-factor-at-a-time (28.03 g L-1). Further cultivation in a 16-L bioreactor using controlled and uncontrolled pH shows that higher pullulan production was obtained from uncontrolled pH condition (37.53 g L-1) compared to controlled pH condition (26.87 g L-1). Cultivation of culture in a 16-L bioreactor under uncontrolled pH condition using statistically optimized medium composition shows an increment of 192.69% pullulan production when compared to unoptimized medium composition in the shake flask.

Keywords: Optimization, Aureobasidium Melanogenum DSM 2404, Pullulan, Production*

M FORMULATION FROM HIBISCUS SABDARIFFA LINN. EXTRACT

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Abstract:

Hibiscus sabdariffa Linn. (HSL) or otherwise commonly known as 'Roselle' or 'Karkade', has been widely known for its traditional use as food and herbal medicine. It is a plant that grows in tropical regions such as Malaysia. It has been widely studied for its antimicrobial and antioxidant properties due to the presence of anthocyanins for wound healing application, but formulating it into a stabilized topical delivery cream is still lacking. This study aims to investigate the wound-healing activity of HSL and formulate it into a nanocream for topical delivery. The cytotoxicity of the ethanolic HSL extract was assessed via MTT assay on HSF 1184 cells and results have shown that the IC50 value of the HSL extract was 1000µg/mL, indicating that it is weakly cytotoxic to HSF 1184 cells. The HSL extract was further verified for its wound-healing activity on HSF 1184 cells via Scratch assay and at a concentration of 250µg/mL showed the highest cell migration activity of HSF 1184 compared to the control (without treatment). Then, the HSL extract was formulated into a water-oil based nanocream. Based on characterization, it had a mean droplet size of 477.03nm, polydispersity index of 0.542, conductivity value of 0.11 mS/cm, pH of 5.53, and no phase separation was observed in both centrifugation tests and freeze-thaw cycles. The characteristics of the HSL nanocream indicate that it is a stable W/O formulation that is suitable to be applied on skin. In a nutshell, HSL extract demonstrated a great potential as natural wound healing treatment as it is weakly cytotoxic, and shows potential for accelerating cell migration, and can be incorporated into a stable nanocream for topical delivery.

Keywords: Hibiscus Sabdariffa Linn., Cytotoxicity, Wound Healing, Nanocream, Formulation

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BIOACTIVE COMPOUNDS FROM PEPEROMIA OBTUSIFOLIA

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Abstract:

Peperomia obtusifolia (L.) A. Dietr., native to Middle America, is an ornamental plant also traditionally used for its mild antimicrobial properties. Chemical investigation on the leaves of P. obtusifolia resulted in the isolation of two previously undescribed compounds, named peperomic ester (1) and peperoside (2), together with five known compounds, viz. N-[2-(3,4-dihydroxyphenyl)ethyl]-3,4-dihydroxybenzamide (3), becatamide (4), peperobtusin A (5), peperomin B (6), and arabinothalictoside (7). The structures of these compounds were elucidated by 1D and 2D NMR techniques and HREIMS analyses. Compounds 1–7 were evaluated for their antifungal (against Botrytis cinerea, Septoria tritici and Phytophthora infestans), antibacterial (against Bacillus subtilis and Aliivibrio fischeri), and antiproliferative (against PC-3 and HT-29 human cancer cell lines) activities. The known peperobtusin A (5) was the most active compound against the PC-3 cancer cell line with IC50 values of 25.6 µM and 36.0 µM in MTT and CV assays, respectively. This compound also induced 90% inhibition of bacterial growth of the Gram-positive B. subtilis at a concentration of 100 µM. In addition, compound 3 showed anti-oomycotic activity against P. infestans with an inhibition value of 56% by using a concentration of 125 µM.

Keywords: Piperaceae, Peperomia Obtusifolia, Isolation, Cytotoxicity, Anticancer, Antibacterial

TRIPLE COMBINATION RHIZOME EXTRACT IN ENHANCING SYNERGISTIC ANTIOXIDANT ACTIVITY AGAINST FREE RADICALS

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Abstract:

Rhizomes of medicinal plants which are known to contain secondary metabolites of phenols and flavonoids. The purpose of this study was to determine the antioxidant activity of the combination of extracts of the rhizome. The combination used for comparison in the extract mixture was extract A (1:1:1), extract B (1:2:1), extract C (2:1:2), and ascorbic acid was used as a positive control. This research uses descriptive and experimental methods. The research design used was a completely randomized design with two factorials, the first factor was a combination of extracts consisting of 3 variations A (1:1:1), extract B (1:2:1), and extract C (2:1:2) . The second factor is concentration consisting of 5 variations (10, 50, 100, 150, 200 ppm). Data were analysed using a two-way ANOVA test and further analysed by Duncan's test. This study showed that the combination of extracts A, B, and C contained secondary metabolites of phenols and flavonoids. The types of flavonoid compounds contained in the combination of extracts A, B, and C are flavanones and dihydro flavanols. The results showed that the combination of extract B with a concentration of 200 ppm in the DPPH method was the most optimal variation in reducing free radicals (78.21%). Based on the ABTS method, the combination of extract A with a concentration of 200 ppm was the best free radical scavenger (91.11%). The results of the analysis of antioxidant activity based on the FRAP method showed that the combination of extract A with a concentration of 200 ppm was able to reduce free radicals (52.76%). The analysis of the DPPH, ABTS, and FRAP methods shows that the combination of extracts as a source of antioxidants has the scavenging ability so that the combination of extracts can be used as an alternative antioxidant.

Keywords: Rhizomes, Antioxidant, Synergistic, Free Radicals

**This study is supported by BLU funding of FMIPA UNJ Jakarta*

OPTIMIZATION OF PERSICARIA ODORATA OIL USING SUBCRITICAL WATER EXTRACTION

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Abstract:

Persicaria odorata (P. odorata), commonly known as Vietnamese coriander or kesum, is a herbal plant widely used in Malaysian traditional cuisines and known for its unique flavor and medicinal properties. As the demand for natural extracts from botanical sources continues to grow, the development of sustainable and environmentally friendly extraction methods become crucial. Subcritical water extraction (SWE) has emerged as a green extraction process that only used water as solvent that offers numerous advantages over conventional solvent extraction methods. The study was aimed to identify the optimal conditions to obtain high oil yields from P. odorata by using SWE. An experimental design utilizing the Box-Behnken design was employed to systematically investigate the effects of three important parameters: temperature, extraction time, and volume of water. The temperature range explored in this study was 130-180 °C, the extraction time was varied from 10 to 20 minutes, and the volume of water was ranged from 15 to 20 mL. The obtained experimental results were analyzed using response surface methodology to evaluate the individual and interactive effects of the parameters on the oil extraction efficiency. Significant findings from the optimization process revealed that temperature exerted the most dominant influence on the extraction process. The optimum operating conditions obtained for SWE extraction of global oil yield were at 164°C, 16 minutes and 17 mL with predicted yield of 17.68 % per dry weight of sample. The findings highlight the potential of SWE as a sustainable and efficient method for extracting oil from herbal plants, contributing to the promotion of sustainable resource utilization and reducing the environmental footprint associated with extraction processes.

Keywords: Persicaria Odorata, Response Surface Methodology, Subcritical Water Extraction

DEVELOPMENT OF HIGH CELL MASS PRODUCTION PLATFORM FOR LIMOSILACTOBACILLUS REUTERI USING MIXED SUBSTRATES CULTIVATION SYSTEM

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Abstract:

The rising concerns about environmental and economic trends have prompted an expanding interest in green science technology. There is a growing interest in value-added chemicals such as 1,3-propanediol (1,3-PDO) using biological approaches and sustainable feedstock such as glycerol. Limosilactobacillus reuteri (L. reuteri), an exclusive obligate heterofermentative lactic acid bacterium, employs both Embden, Meyerhof and Parnas (EMP) and phosphoketolase (PKP) pathways for cell growth on a carbon source such as glucose as the substrate and hence produces lactate, acetate and ethanol. In addition, L. reuteri is also a potential bio-factory for the production of 1,3-PDO. This study aims to develop a new cultivation strategy for optimised biomass production on a semi-industrial scale. In this study, the effect of various operational strategies was studied for biomass production of the L. reuteri DSM 20016 strain. The cultivations were performed at a shake flask level. The compositions of the best cultivation medium were optimised to increase the biomass. The concentration of the cultivation medium was optimised using one-factor-at-a-time (OFAT) and Response Surface Methodology (RSM). The main medium components studied included glucose, lactose and yeast extract. In comparison to the un-optimised cultivation medium, the optimisation process using OFAT and RSM increased biomass significantly by 29.26% and 109.66%, respectively. RSM-optimised cultivation medium produced a biomass of 62.21% higher than OFAT-optimised cultivated medium. The optimised medium was further cultivated in batch mode using a 5 L bioreactor. In batch fermentation, biomass produced 194.10% and 162.85%, under uncontrolled and controlled pH respectively, was noticeably higher compared to the shake flask level. The findings of this study improved the process of L. reuteri production by using a carbon sources mixture and a single nitrogen source as the feedstock in a shake flask level. Furthermore, L. reuteri production also improved on a semi-industrial scale under batch mode uncontrolled pH.

Keywords: Bio-Fermentation, Biomass Production, Probiotics

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CASE STUDY ON THE SCALE-UP OF HERBAL EXTRACT PRODUCTION

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Abstract:

The demand for herbal medicine are expected to increase substantially in the upcoming years. Effective scaling-up plays a crucial role in ensuring laboratory findings could be brought to the market. One of the scaling-up study was on Eurycoma longifolia root, (commonly called tongkat ali, pasak bumi, or longjack). In this study a dimensional analysis was used for the solid-liquid extraction process as cost-effective and scalable method used for herbal extraction industry. The dimensionless number ShSc-1 was determined to be suitable model. Under the optimal extraction conditions, a laboratory-scale extraction yielded an extract yield of 8.76% with a ShSc-1 number of 0.0312. With a scale-up factor of 7.65, the pilot-scale extraction achieved an extract yield of 8.65%, ShSc-1 number of 0.0376 with an error of 1.37% and. This study's findings offer valuable insights for successfully transitioning from laboratory-scale to pilot-scale extractions, which paved the way for effective production of herbal extract.

Keywords: Herbal Extract, Scale-Up, Dimensional Analysis, Dimensionless Number

IMMOBILIZATION OF CYCLODEXTRIN GLUCANOTRANSFERASE ON RICE HUSK BIOCHAR FOR CYCLODEXTRIN PRODUCTION

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ABSTRACT

Cyclodextrin glucanotransferase (CGTase) is a highly catalytic efficient enzyme that is used to produce cyclodextrin (CD) which has a high demand in cosmetic, pharmaceuticals, food and agricultural industries. The instability of CGTase is a nagging issue because the activity is easily affected by harsh conditions, especially extreme pH, and temperature, thereby reducing product formation. Adsorption enzyme immobilization technology is a versatile method that has been used to improve enzyme stability, efficiency, and reusability. However, the method often suffers from enzyme leaching problem that limits CD production. In this study, CGTase was immobilized onto activated rice husk biochar (ARHB) using an adsorption and covalent bonding method for cyclodextrin (CD) production. Optimization of the CGTase immobilization onto the ARHB was investigated using one factor at a time and Response Surface Methodology (RSM) by measuring the effects of immobilization contact time, immobilization temperature, pH immobilization, agitation rate, ARHB to CGTase ratio, enzyme concentration, and crosslinker glutaraldehyde (GA) concentration on the immobilization efficiency. A kinetic model and adsorption isotherm that describe enzyme adsorption immobilization on the RHB and ARHB was deduced. The recovery efficiency and reusability test were conducted to ensure the effectiveness of the CGTase-ARHB immobilized to produce CD. Thus, immobilization of CGTase on ARHB improved CGTase stability and reduced enzyme leaching hence increase the CD production, hence suggesting that ARHB proved to be an effective support for the immobilization process of enzymes.

Keywords: Cyclodextrin Glucanotransferase, Rice Husk Biochar, Activated Carbon, Enzyme Immobilization.

**Myrgs*

INDUSTRY TREND: REGENERATIVE FARMING

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Abstract:

The Back2Nature Regenerative Farm's with Soil Microbes

Back2Nature Regenerative Farm is based in Kuala Pilah, Malaysia. Its focus is to harness the powers of soil microbiome for quality food cultivation. The objective is to demonstrate the technical and economic feasibility of growing food without resorting to toxic agrochemicals against plant diseases and synthetic fertilizers to promote plant growth. We applied selected local soil microbes on the cultivation of chili, papaya, black ginger, star fruits, eggplant, rosella, passion fruits, and herbal plants with surprising results.

Our experiment on the dry, hard and tired clayish soil on our farm with soil bioremediation has yielded positive results over a two-year period. This lead us to conclude that selected high performance mycorrhizae fungi and solubilizing bacteria, when working in concert as a consortium, are capable of restoring natural fertility to soil. Nutrient recycling involving the use of appropriately formulated organic composts with high performance soil microbes are expected to feature more prominently in regenerative farming in the future. We have produced a range of bio-products on the farm to support our experiment.

Current food and agriculture practices are heavily influenced by the adoption of 1960s "Green Revolution" of large-scale industrial chemical farming approach. The focus of conventional agricultural practices on raising output yield and lowest unit cost for mass produced foods. Such practices have inadvertently led to an explosion of chronic diseases worldwide, including obesity, type 2 diabetic, autism, various types of cancers, neurodegeneration, social isolation, environmental degradation and loss of biodiversity. It is urgent to develop a more sustainable approach to food cultivation by harnessing the powers of soil microbiome.

Over the past 3 years, we have made a number of breakthrough discoveries on identifying and isolating specific microbes which can raise the growth performance of plants, suppress plant diseases and remediate toxic substances in the soil ecosystem. We are in the process of introducing such bio-products to the user community to raise awareness about regenerative farming. Our soil is indeed rich with diverse biological resources waiting to be tapped for higher quality of life.

Keywords: Regenerative Farming Microbes

BACK2NATURE

RADICALS SCAVENGING AND ANTI-INFECTIVES OF INDIGENOUS SIMPOR LEAF EXTRACT (DILLENIA SUFFRUTICOSA MARTELII GRIFF) OF INDONESIA

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Abstract:

Many studies have focussed on the use of the plant as a promising source of radical scavenging, and anti-infective agents. Plants have been used for centuries as one of the main components of traditional medicine in different cultures. Indigenous plant sources in many countries and regions may promote different potentials and provide a wide range of health benefits. Increased threat of microbial resistance against the currently used antibiotics, creates increased demand for the potential application of plant extracts (as the historical biofactory of antimicrobial metabolites) for medical applications. This study evaluates the radical scavenging and anti-infective properties of indigenous Simpor leaf extract from Indonesia. A factorial Completely Randomized Design (CRD) with two factors (graded maceration and concentration was assigned in radical scavenging analysis and one factor (concentration) for anti-infectives against Bacillus cereus and Escherichia coli. Radicals of ABTS and FRAP were used at 734 nm and 593 nm absorbance. Data were analyzed with ANOVA (sig. 0.05). Results suggest that Indigenous Simpor extract has good scavenging activity against ABTS and FRAP at 60 ppm (85.05% and 56.04%). Moreover, the concentration of 0,25 g/ml extract showed anti-infective properties with an inhibition zone of 10,73 ± 0,85 mm on B. cereus. While no positive result was seen on E. coli. It can be concluded that the Indigenous Simpor of Indonesia possesses radical scavenging and anti-infective potentials on gram positive bacteria.

Keywords: Radicals, Anti-Infective, Simpor, Indigenous

**This study is supported by BLU funding of FMIPA UNJ Jakarta*

SCREENING OF THE BACTERIOCINOGENIC POTENTIALS OF SOME BACILLUS STRAINS ISOLATED FROM MIANG FOR POTENTIAL PROBIOTICS APPLICATION IN FISH FARMING.

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Abstract:

With the high need for bio-antimicrobials that effectively serve as an alternative to conventional antibiotics and beneficial feed additive in fish farming, this communication elucidated the bacteriocinogenic potentials of six Bacillus strains (K2.1, K6.1, K7.1, K15.4, K22.6, K29.2) isolated from Miang. Streptococcus agalactiae and Aeromonas hydrophila were this study's pathogenic strains of interest. From our antimicrobial agar well diffusion assay, strains K15.4 and K29.2 have broad antimicrobial activity against these indicator strains. With an inhibition zone(mm) of 9 and 20.4, respectively, against Streptococcus agalactiae and 22 and 34 against Aeromonas hydrophila. However, strain K7.1 inhibited only Streptococcus agalactiae with an inhibition zone(mm) of 10; strains K2.1, K6.1 and K22.6 showed no antimicrobial activity against all the indicator strains. The 16S rRNA identification of these Bacillus strains revealed (K2.1, K6.1, K7.1, K15.4 and K22.6) are Bacillus tequilensis and (K29.2) is Bacillus siamensis. Their phylogenetic analysis revealed their close genetic relatedness to Bacillus subtilis, thus reassuring their probiotic safety. As potential probiotic strains, their antibiotics resistance was assayed against some selected antibiotics (10µg/ml of erythromycin 30mg/ml, chloramphenicol 35mg/ml, streptomycin 20mg/ml, kanamycin 25mg/ml and ampicillin of 25mg/ml) via agar well diffusion. Our result revealed that only Bacillus tequilensis strains K6.1 and K15.4 were slightly resistant to streptomycin. Furthermore, their protease activity, evaluated via a nutrient agar + 10g/l skim milk plate, was positive with a suitable IP (proteolytic index). This additional property has buttressed these strains as multifunctional probiotics. Therefore, subsequent in vivo and in vitro studies are required to ascertain these properties' efficacy properly.

Keywords: Antimicrobials, Aquaculture, Antibiotic-Resistant Microbes, Miang, Probiotics And Protease Enzymes.

PREDICTING THE IN SILICO AND IN VITRO PLANT-GROWTH PROMOTING POTENTIAL OF PLANT-ASSOCIATED BACTERIA ISOLATED FROM MIANG TEA LEAVES (CAMELLIA SINENSIS VAR. ASSAMICA)

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Abstract:

A total of 70 endophytic bacteria were isolated from Miang tea leaves out of which two strains including Pseudarthrobacter enclensis NIO-1008T and Curtobacterium citreum DSM 20528T were selected due to their ability to produce high levels of indole acetic acid (IAA). The gene sequences of these bacterial species were retrieved from NCBI and screened against an online repository of 5,565 plant-associated bacteria (https://plabase.cs.uni-tuebingen.de/pb/plaba_db.php) where a match was found to Pseudarthrobacter phenanthrenivorans J015, Pseudarthrobacter chlorophenolicus Mor30.16, Curtobacterium citreum DSM 20528 and Curtobacterium citreum NS330 respectively with >98% sequence identities to the original query sequences (NCBI BLAST). The algorithm predicted plant growth promoting traits such as colonizing plant system (34%), stress control (20%), competitive exclusion (14 - 16%), biofertilization (10 - 13%), phytohormone production (10%), bioremediation (9%) and plant immune response stimulation (1%). Furthermore, 5,986 (Curtobacterium citreum) & 3738 (Pseudarthrobacter enclensis) protein sequences were retrieved from UniProtKB (www.uniprot.org). The curated sequences were supplied as a FASTA or. fas format input file to PLaBAse database (https://plabase.cs.uni tuebingen.de/pb/form.php?var=PIFAR-Pred) for analysis and annotation of plant bacterial only interaction factors (proteins) “PIFAR” using blasp+hmmer algorithm. Possible factors predicted for the genomes included: Cellulose_synt, Pilin, dps, katB, katE, katG, pip, galU, gpsX, gumH, ethylene, salycilic_hydroxylase, galU, rfb303 amongst others. Using the blast as well as blast+hmmer modes, the plants growth promoting traits were also annotated and 5220 possible genes were predicted as able to facilitate plant-growth promotions by the bacteria. Preliminary in vitro experiments using Pseudarthrobacter enclensis NIO-1008T and Curtobacterium citreum DSM 20528T treated sunflower and tomato seeds showed a significant increase in %germination and other plant growth parameters compared to untreated controls. Future research could work towards molecular optimization of these bacterial strains for better applications in biofertilization.

Keywords: Camellia Sinensis; Endophyte; Growth Promotion; Protein Sequence; Biofertilizer

COMPARATIVE ANALYSIS OF METABOLIC AND MORPHOLOGICAL CHARACTERISTICS OF DOF1 TRANSGENIC WHEAT UNDER NITROGEN STRESS

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Abstract:

Over the last century, the increased crop production has largely been attributed to rampant input of nitrogen fertilizers, which either gets lost through leaching or volatilization, hence resulting in environmental pollution and several human health hazards. In order to cater this widespread concern, the need of the hour is to engineer crops that require less fertilizer input and use the applied nitrogen in an efficient manner. In this regard, a number of transcription factors have been reported to improve the nitrogen use efficiency (NUE) in plants. Triticum aestivum Dof1 (TaDof1) is a transcription factor that modulates the activity of multiple genes, specifically that are involved in carbon and nitrogen metabolism under nitrogen-limiting conditions. Previously, transgenic wheat plants overexpressing TaDof1 were developed, and assessed with respect to their expression profiles along with biochemical and morphological traits (Hasnain et al., 2020). The main premise of the current investigation was to compare the role of TaDof1 in T2 generation of six different transgenic wheat lines (F1, G1, G2, G3, G4, G5) in terms of their metabolic, biochemical, and morphological traits under normal and nitrogen deficient conditions. The screening of positive plants was done through BASTA and conventional PCR. The expression level of TaDof1 in transgenic lines was evaluated through obtaining total RNA from each group. Then, the expression level of four genes (Glutamine synthetase, nitrite reductase, phosphoenolpyruvate carboxylase and pyruvate kinase) associated with TaDof1 in carbon and nitrogen metabolism were quantified through RT-PCR and real time PCR. Moreover, a number of biochemical and morphological assays were also performed.

Keywords: Triticum Aestivum L.; Tadof1 Transcription Factor; Nitrogen Use Efficiency; Quantitative RT-PCR

KAM School of Life Sciences (FCCU)

BENEFICIAL MICROORGANISMS IN CONVENTIONAL MICRO-BASED BIOFERTILIZERS

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Abstract:

Modern agriculture must be more productive, more sustainable and environmental friendly. There are macronutrients such as Nitrogen (N), Phospohorus (P), Potassium (K), and Sulfur (S) which is greatly contributes to the vital crop production. Apart from that, beneficial microorganisms may also be instrumental to crop improvement and fertilizers efficiency. Some of the beneficial microorganisms consist of biological N2 fixation, P solubilisation and phytohormone production. Microbial-based bioformulations that increase plant performance are greatly needed and in particular bioformulations that exhibit complementary and synergistic effects with mineral fertilization. In this study, four fertilizers are analysed for five analyses which are Total Lactobacillus count, Yeast Count, Actinomycetes bacteria, Nitrifying Bacteria and Nitrogen Fixing Bacteria. It was observed that the insect-based organic fertilizer detected no Lactobacillus, Actinomycetes and Nitrogen Fixing Bacteria. Meanwhile, fish emulsion fertilizer verified that most of the beneficial microbes are not strong compares to other fertilizers. EM microorganisms have proffered that some of the beneficial microbes are substantial among other fertilizers. Continuous designing, developing and testing on the microbial-based formulation fertilizers to be used in efficient integrated plant nutrient management system has gained worldwide interest recently to enhance crop productivity and soil fertility. This presentation highlights the recent developments in the quality control approaches for biological feterilizers for the qualitative and quantitative detection of functional microbes.

Keywords: Microbial-Based Biofertilizer, Beneficial Microorganisms, Biological N2 Fixation

PHYTOCHEMICAL, ANTIOXIDANT, AND ANTIBACTERIAL PROPERTIES OF COLOURED LIP BALM ENRICHED WITH CITRUS ESSENTIAL OIL: TANGERINE (CITRUS RETICULA L.), LEMON (CITRUS LEMON L.), BERGAMOT ORANGE (CITRUS BERGAMIA).

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Abstract:

Citruses are well-known for their application in cosmeceutical product. The cosmeceutical product, such as coloured lip balm, gained attention among various age range. Application of citrus to coloured lip balm enhanced the product's value. The phytochemical, antioxidant, and antibacterial properties of coloured lip balm enriched with tangerine (Citrus reticula L.), lemon (Citrus lemon L.), bergamot orange (Citrus bergamia) were examined. The result showed that lemon exhibited the highest total phenolic content, total flavonoid content, and the strongest DPPH scavenging activities compared to tangerine and bergamot. However, the study showed that coloured lip balm enriched with tangerine, lemon, and bergamot essential oil did not show any antibacterial properties against two tested foodborne pathogens namely Staphylococcus aureus and Escherichia coli. This study indicated that coloured lip balm enriched with citrus essential oil has potential as a natural antioxidant agent.

Keywords: Phenolic, Flavonoid, Ultrasound, Foodborne Pathogens, Citrus

**We would like to thank Institute of Bioproduct Development, Universiti Teknologi Malaysia for support throughout the study.*

MOLECULAR DYNAMICS STUDIES OF TAXOL BOUND TO TUBULIN DIMER-GDP INTERFACE

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Abstract:

Microtubules are one of the major components of the eukaryotic cell cytoskeleton. Heterodimers composed of GTP-bound α- and β-tubulin molecules polymerize into microtubule protofilaments and associate laterally to form hollow microtubules. Taxol is a chemotherapy drug used to treat ovarian, lung, Kaposi's sarcoma, esophageal, cervical, breast, and pancreatic cancers. Taxol stabilizes microtubules, disrupts mitosis, and ultimately affects cell division. However, the mechanism of taxol binding to the αβ-tubulin dimer interface with GTP is still unclear. To elucidate this mechanism, three complexes (tubulin dimer-GDP, taxol-bound tubulin dimer-GDP, and tubulin dimer-GTP) were constructed and subjected to microsecond molecular dynamics simulations coupled with MMGBSA binding energy calculations. The MM-GBSA calculations revealed that taxol increased the overall binding affinity between α-tubulin and β-tubulin proteins. Furthermore, taxol reduced the conformational changes of the dimer as reflected in the root-mean-square displacement (RMSD) profile. Also, the root-mean-square fluctuation (RMSF) profile showed that residual variation was greatly reduced, especially at the interface. This supports the role of taxol in stabilizing the protein interface and preventing the dissociation of α-tubulin and β-tubulin. These results provide further insight into the interaction of taxol and heterodimers and may aid future drug design targeting this protein.

Keywords: Taxol, αβ-tubulin dimer interface, stability, MD simulations, MMGBSA

Blind docking against HIV-1 protease using AutoDock 4.2.6

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ABSTRACT

Currently, the HIV/AIDS virus is a major impediment to global health and development. Due to various negative effects, the development of a reliable vaccination is a now pipe dream. In addition, HIV-1 protease is accountable for rectifying the gag and gag-pol polyproteins during virion maturation. However, antiretroviral therapy (ART), increases the life expectancy of HIV-positive patients which is now being given to 14.9 million people globally. Tragically, there are still no HIV-1 therapeutics that work effectively. In this study, blind docking was performed in virtual box 6.1 using AutoDock 4.2.6 to determine effective phytochemicals that can target HIV protease (PDB ID: 2R5Q). Twenty phytochemicals were selected and a clinical trial drug (darunavir) was selected as a control. When the grid box was generated, the x, y and z values were 14.814, -15.206, and -54.457 and spacing were set to 0.54. Also, the genetic algorithm population was set to 150. Additionally, blind docking redocking value of 0.000Å was performed to validate the procedure. The ligands were evaluated according to the binding energy (BE) and inhibition constant (Ki), and the best potential phytochemicals were determined: carandinol (BE: -10.55, Ki: 18.54), withaferin A (BE: -9.96, Ki: 49.66), lupatic acid (BE: -9.77, Ki:68.9), maslinic acid (BE: -9.25, Ki:166) and sambunigrin (BE: -9.07, Ki: 68.73) kcal/mol, nM respectively. The best-docked poses and amino acid interactions were visualized using BIOVIA DS. The common amino acid interactions were observed in LEU24, LEU97 and PRO1. Moreover, common conventional hydrogen bonds were perceived in ASN98 and ILE3. In addition, ADMETlab2.0 was used to analyze ADMET properties. Withaferin A was the optimum ligand because it acknowledged the five Lipinski rules, exhibited significant Kis and consistent BEs, and also had an optimum logP. Additionally, findings anticipate the use of withaferin A for anti-viral purposes due to its good oral bioavailability.

Keywords: Antiretroviral therapy, Binding energy, Inhibition constant, Phytochemicals

INTRODUCTION

HIV -1

The retrovirus HIV causes AIDS in humans. HIV infects CD4+ T-cells, which are important helper T-cells in the human immune system. HIV is a single-stranded, positive-sense RNA virus that is encapsulated. HIV-1 and HIV-2 are the two forms of HIV that have been identified (Phillips *et al*., 2018). In 1999, scientists discovered a chimp SIV virus that was identical to HIV. According to researchers, in 1920 SIV was initially transmitted to humans in the Congo. More than 70 million people have been infected with HIV, and about 35 million

have died of AIDS at the beginning of 2021. Africa continues to be the most badly affected in every 25 adults living with HIV more than two-thirds of all HIV-positive people worldw*ide* (WHO, 2021*)* (Fig 1). However, HIV still represents a significant global epidemic. The lifespan of HIV-positive individuals has risen due to ART (Fig 3). Therefore, ART is vital for survival to reduce morbidity caused by drug toxicity and the developing resistance. Therefore, the development of an efficient vaccination is still a distant goal.

There are five phases in the HIV cycle. When a CD4 cell is identified, the virus invades it by adhering to receptors on its outer membrane, fusing with the cell, and releasing viral RNA and enzymes. The virus uses an enzyme called reverse transcriptase to reverse-transcribe its single-stranded viral RNA into double-stranded DNA. During integration, the virus interrupts the CD4 cell by integrating its produced viral DNA into the cell's nucleus using the enzyme integrase. Following that replication, the CD4 cell continues the process of producing new virus copies, leading to mutations in the new virions. During budding and maturation, new HIV virions penetrate the CD4 cell's outer membrane. Proportionately, it can devastate the entire immune system (Fig 4*)* (Maartens, Celum and Lewin, 2014). Furthermore, HIV infection can be transmitted in various ways, although it is most typically done by infected blood or blood components (Laila *et al*., 2019).

Protein-ligand docking

Protein-ligand docking is a computational approach that is objective in accurately predict non-covalent interactions receptor-ligand binding (Jenwitheesuk and Samudrala, 2003). There are two main techniques: BD and SSD. The technique known as BD involves docking a ligand to the entire protein surface without being aware of the target pocket. Docking a ligand to the active site of a protein knowing the target pocket is known as SSD (Huang and Zou, 2010). Furthermore, two modes of docking exploration are frequently used: evaluation of docking scores to predict which ligands are likely to bind favourably, and analysis of binding poses to establish which interactions are significant in forming the protein-ligand bond (Lippert and Rarey, 2009).

Protein receptor selection

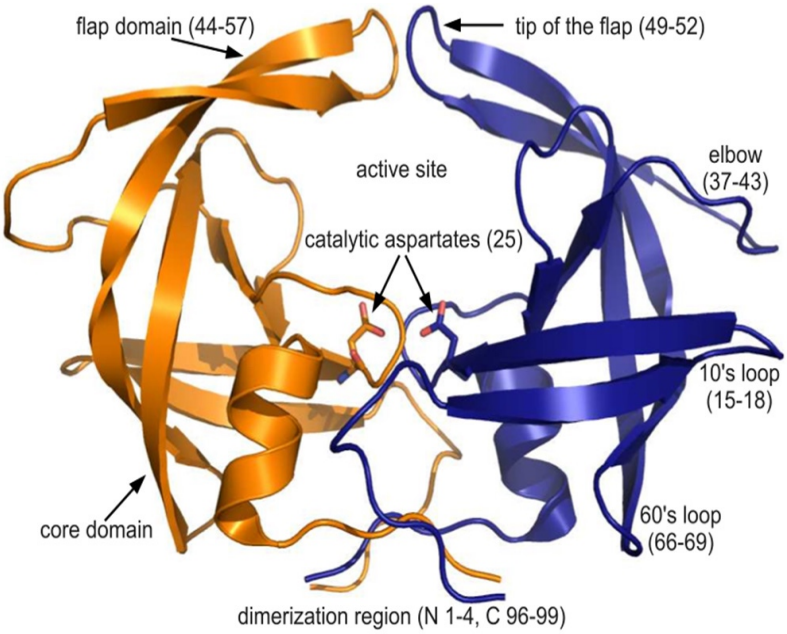
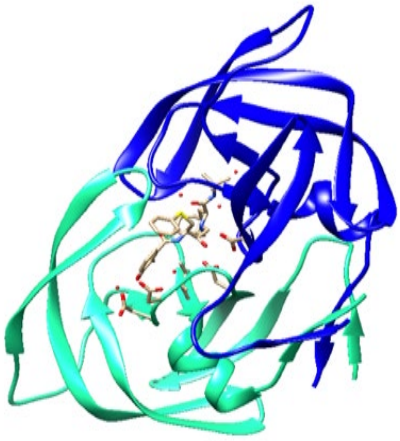


Figure 1. HIV protease receptor (Venkatakrishnan et al., 2012)

HIV-1 protease also has a homodimeric C-2 symmetric structure that aids in stabilising the two monomers for dimerisation. The dimer interface is formed by four antiparallel strands. Dimerisation interaction is necessary for substrate binding and the formation of the active site (Fig 1). In protease, each monomer contains an extended beta-sheet region known as the flap (a glycine-rich loop) that functions as part of the substrate-binding site and controls the recognition and the entry of substrates/inhibitor to the active site (Jenwitheesuk and Samudrala, 2005). Inhibiting HIV-1 activity or changing the HIV protease active site residues can terminate viruses from maturing and packing properly. HIV protease has become one of the primary targets for the design and development of many novel HIV-1 therapies. Mutations in the protease generated drug resistance, which resulting in reduced efficacy of HAART (Highly active antiretroviral treatment). 2R5Q subtype C is a crucial target for HIV-1 regulation and it presents nelfinavir (Table1). Nelfinavir was the first protease inhibitor to be optimised using Monte Carlo simulation, and it was also the first to be used by HIV patients. (Bardsley-Elliot and Plosker, 2000).

Table 1. Description of the receptor

Receptor	Active chain	Organism	Natural ligand	Mutations
HIV – 1 Protease **PDB ID:2R5Q** 	A B C* D	Human immune deficiency virus	Nelfinavir	V32I I47V V82I

Ligand selection

These FDA-approved drugs are utilized as standard drugs in protein-ligand docking due to their safety and controllability (Thind and Kowey, 2020). Although, natural compounds such as flavonoid, alkaloid, and phenolic compound have been suggested as potential anti-HIV therapeutics (Salehi *et al*., 2018). Therefore, experimental research into innovative HIV therapeutics should be done using ethnomedicines and other natural sources.

Software selection

Protein-ligand docking will be performed using AutoDock suite 4.2.6 comprised of AD4 and AutoDock vina. In order to reduce the flexibility of the protein, AD4 combines the Lamarckian algorithm and the empirical force field (Forli *et al*., 2016) and to ease the binding confirmation and free energy associations of the docked complex. In contrast, the ligand data file is converted into the required formats(*pdbqt*) using Open Babel GUI. USCF Chimera is a high-performance software with a fundamental expansion that facilitates docking visualising, and analysing user data (Butt *et al.,* 2020). BIOVIA DS and LIGPLOT+ are used to visualize the high-quality 2D interactions diagram representing conventional hydrogen and

hydrophobic interactions. Also, PyMOL is used for the 3D visualization of docked complex poses. However, the precision of the results is impacted by the speed of the CPU cores (Ritchie and Venkatraman, 2010).

Validation

Redocking and Ramachandran plots are used for validation of the docking procedure. In redocking, a cluster tolerance of 2.0 Å in positional RMSD is used to cluster the docking result. The procedure of protein-ligand docking is valid when a natural ligand co-crystal structure is superimposed with a redocked complex whether the RMSD value is less than 2Å (Zubair *et al*., 2020). In addition, the Ramachandran plot is utilised to analyze the docking procedure by obtaining percentages of the most favorable region. Receptor destruction can be estimated by determining the percentages.

MATERIALS

The hardware included a Lenovo laptop with Windows 10 and configured with an Intel(R) Core 2, CPU N3060 @ 1.60GHz 64-bit processor, 2GB RAM, and virtual box 6.1.3.4 with a 64-bit processor and 8GB RAM. AutoDock 4.2.6, Open Babel GUI 2.4.1, and BIOVIA Discovery Studio Visualizer 21.1.1.0.0 were the software used. In addition, Python 3.10.0 and MGL Tools 1.5.6 were installed to support the execution of AutoDock 4.2.6. The NCBI PubChem database, the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB), PROTOX II and ADMETlab2.0 were the web tools used. The three-dimensional structure of the HIV-PR protein (PDB ID: 2R5Q) was received from the PDB while the ligands were acquired from NCBI PubChem.

METHODOLOGY

The pdb file of the receptor was retrieved. The water molecules and chains (A, D) were deleted using Autodock 4.2.6. The heteroatom nelfinavir was eliminated from chain B. Following the addition of the polar hydrogen bonds and the merging of the non-polar and Kollman charges, the missing atoms were identified and repaired. A pdbqt file was used to store this content. The ligands were acquired in. sdf format and Open Babel GUI was used to convert them to a pdbqt file. Gasteiger charges were added and the number of rotatable bonds for each ligand was computed when the file was opened in Autodock 4.2.6. Torsions were set

and the ligands were saved in a .pdbqt file. A grid box was created to enclose a specific region of the A chain. The grid dimensions were adjusted as follows; x = 122, y = 122 and z = 112, while the grid center values were x = -14.814, y = -15.206 and z = -54.457 and its spacing was 0.54. The docking search parameter chosen was the Lamarckian genetic algorithm where the genetic algorithm runs were set to 10 with a population size of 150. Subsequently, Autogrid was executed, which produced grid maps for individual atoms of the ligand to be docked. A docking log (DLG) file was produced, containing the binding energies (BE) and inhibitory constant (Ki) values for all the poses generated per ligand. Docking poses were obtained from the BIOVIA discovery 2021 opening docked complex using BIOVIA DS and chimaera 1.16. Selected Options "Publication 1" and "Interactive 1" were used to optimise docking poses. Opening the *docked complex* allowed LIGPLOT+ to display interactions. It was possible to obtain the 2D interaction plot with HPI and HI. The visualisation of conventional HIs and HPIs (Pi-sigma, Pi-sulfur, alkyl, Pi-alkyl, and Pi-amide) was performed using BIOVIA 2021. The technique was validated by re-docking. The PROCHECK from SAVESv 6.0 was used to validate the re-docked complex by uploading the PDB file. However, the most favourable region was investigated by obtaining percentage differences between dockings before and after. PyMOL 2.5 was utilised to validate the ligand. BD was applied to the nelfinavir using the same docking process that was used for the previous ligands. Using PyMOL, the redocked file of the co-crystallized 2R5Q and nelfinavir structure was acquired, and the superimposed structure was viewed along with the associated RMSD value. Then, the redocked complex and the receptor's co-crystal structure were examined using LIGPLOT to find the common amino acid interactions. The PubChem database was used to obtain the phytochemicals' canonical SMILEs. The absorption, distribution, metabolism, and excretion (ADMET) parameters were predicted using these in ADMETlab2.0. Their potential drug receptivity was determined using Lipinski's rule of five. Utilising the PROTOX -II webtool for toxicity analysis, additional testing was performed on the compounds that were included in the short list. When evaluating the compounds, the following criteria were used: Toxicity class (Oral toxicity), predicted LD50, hepatotoxicity, carcinogenicity, and immunotoxicity

RESULTS

Docking Parameters

The phytochemicals Crandinol, Withaferin A, Lupatic acid and Sambunigrin have a higher affinity than the drugs used for the treatment currently, their binding energies are -10.55, -9.96, -9.77, and -9.77, respectively. The binding energies of the drugs darunavir, nelfinavir, doravirne, and indinavir are -8.55, -8.37, -7.59, and -7.21, respectively. Carandinol was the best potential HIV-1 inhibitor, with the lowest BA and inhibition constant respectively (*Table 2*).

Table 2. Binding Energy of HIV protease with top 14 phytochemicals as ligands.

		2R5Q		
		FDA- approved drugs		
	Ligand	*BA (kcal/mol)*	*Ki (nM)*	*Ligand efficiency (LE)*
1.	*Darunavir*	*-8.55*	*538.42*	*-0.23*
2.	*Nelfinavir*	*-8.37*	*734.72*	*-0.21*
3.	*Doravirine*	*-7.59*	*2740*	*-0.26*
4.	*Indinavir*	*-7.21*	*5220*	*-0.16*
5.	*Nevirapine*	*-6.80*	*10400*	*-0.34*
6.	*Tipranavir*	*-6.98*	*7590*	*-0.17*
7.	*Fosamprenavir*	*-6.19*	*28860*	*-0.31*
		Phytochemicals		
1.	*Carandinol*	*-10.55*	*18.54*	*-0.33*
2.	*Withaferin A*	*-9.96*	*68.73*	*-0.29*
3.	*Lupatic acid*	*-9.77*	*68.9*	*-0.30*
4.	*Sambunigrin*	*-9.77*	*68.73*	*-0.29*
5.	*Maslinic acid*	*-9.25*	*166.05*	*-0.3*
6.	*Andrographolide*	*-9.07*	*223.34*	*-0.27*
7.	*Lupeol*	*-9.00*	*253.06*	*-0.41*

Docking Poses

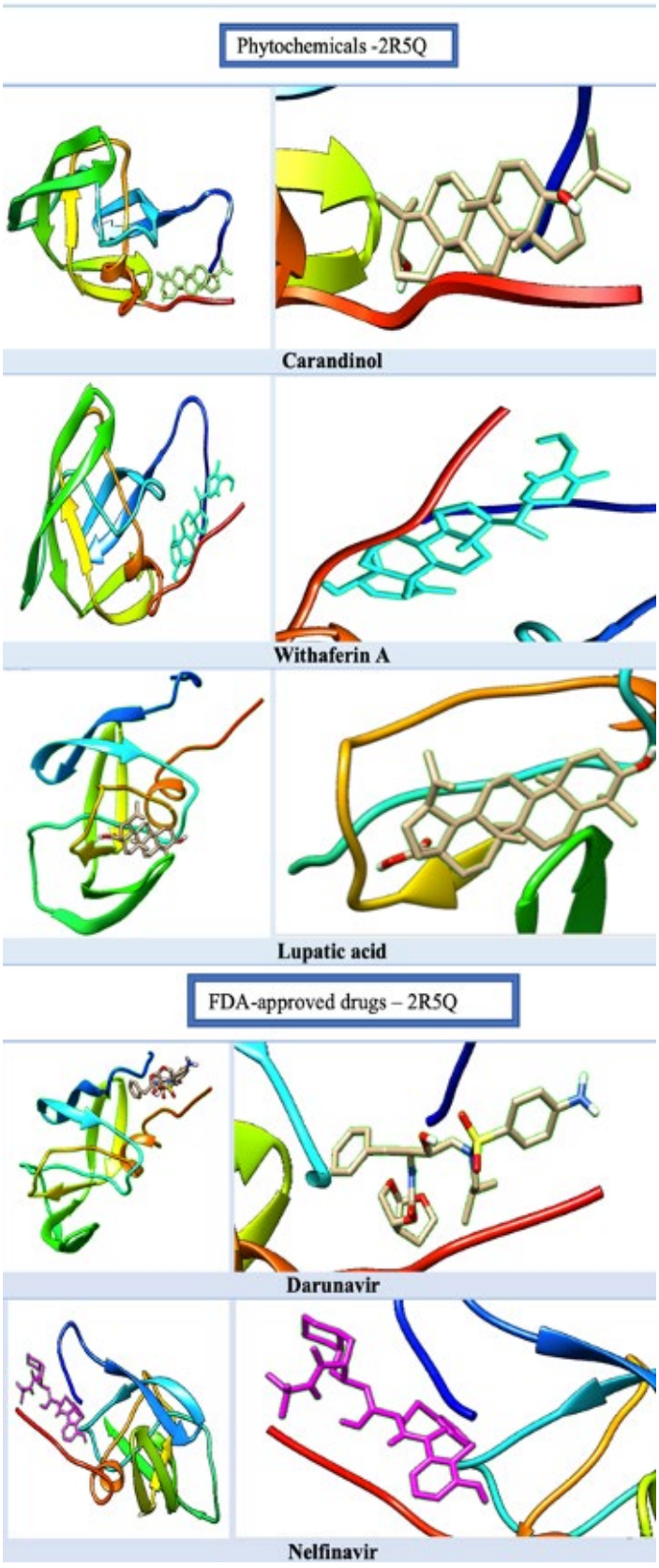


Figure 2. Docking poses of HIV protease with top 5 ligands.

Docking Interactions

The common amino acids with respect to HPIs were LEU24 and LEU5. Further, the observed common amino acid involved in hydrogen bonding was ASN98 in Carandinol, Darunavir and Nelfinavir.

Table 3. Interactions of 2R5Q

Phytochemical	Interactions		
	HI	**No**	**HPI**
1. Carandinol	ASN98 LEU93	2	CYS67, ILE3, ILE66, LEU24, PRO1, CYS95
2. Withaferin.A	GLU66 GLY17	2	PRO39, LYS70, ILE63, ILE62
3. Lupatic acid	GLY27 ASP29 PRO79	3	PRO81, ILE47, ILE54, ALA28
4. Sambunigrin	ASP30	1	PRO81, ALA28, ILE47, ILE54
FDA approved drug			
	HI	**No**	**HPI**
1. Darunavir	PRO1 THR96 ASN98	3	LEU24
2. Nelfinavir	ASN98 PRO1	2	THR26, LEU97, LEU24, LEU5

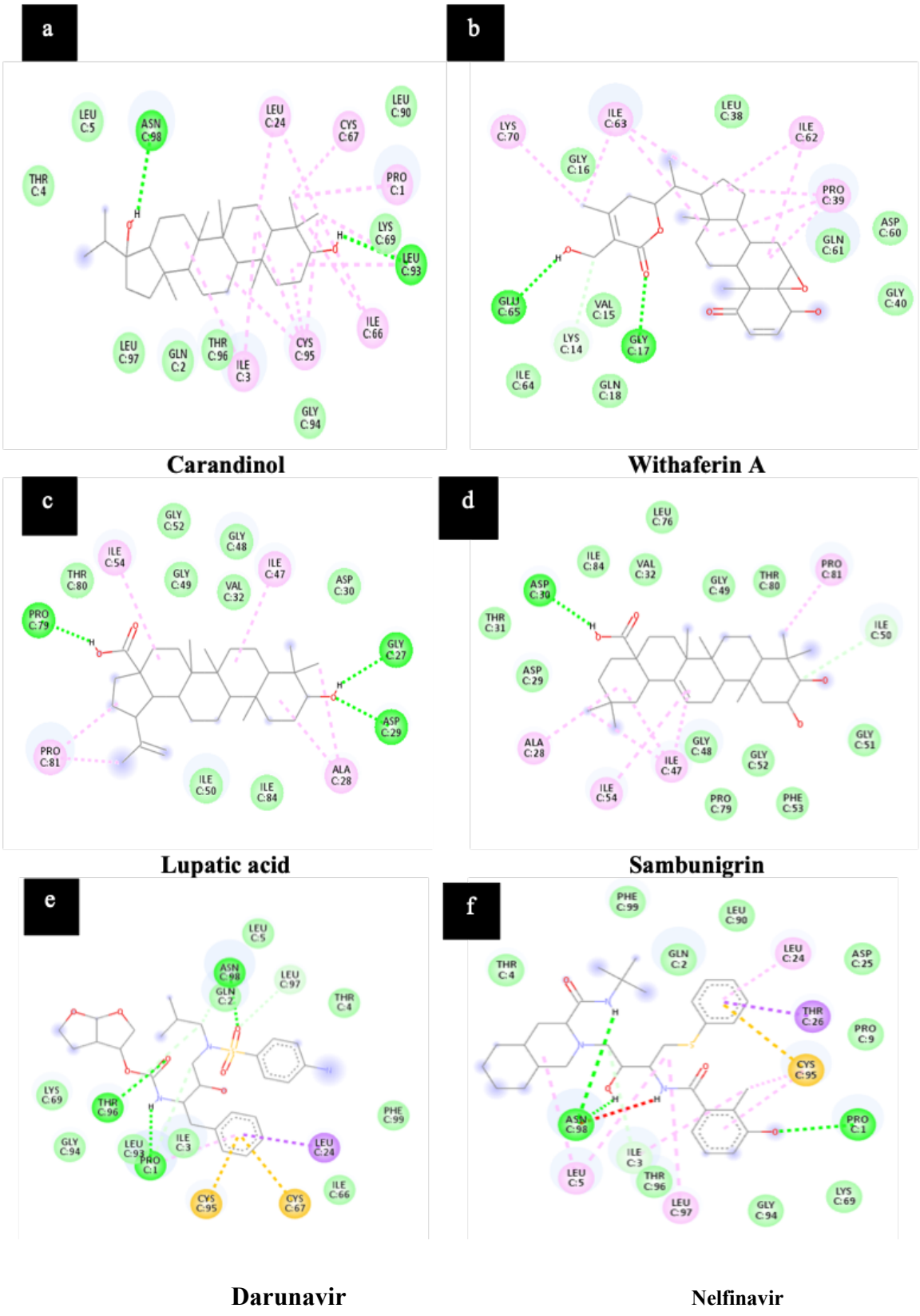


Figure 3. BIOVIA interactions of HIV protease with top 6 ligands. (a-d: phytochemicals; e-f: FDA approved drugs)

ADMET property analysis of the ligands

Table 4. Comparison of the ADMET properties of the best ligands

Phytochemical	MW	nHA	logS	logP	Lipinski rule	$CaCO_2$ Permeability	BBB penetration	AM09ES	CYP 142	hERG
Carandinol	444.40	2	-4.886	6.341	√	- 4.987	√	X	0.1	X
Withaferin A	470.27	6	-4.965	3.398	√	- 4.981	√	X	0.1	X
Maslinic acid	472.36	4	-3.766	5.667	√	-5.331	√	X	0.1	X
Lupatic acid	456.36	3	-4.434	6.005	√	-5.248	√	X	0.1	X
Lupeol	426.39	1	-6.199	7.291	√	-5.020	√	X	0.1	X
Gallic acid	170.02	5	-1.220	0.645	√	-5.728	X	X	0.1	X
Carindone	512.310	6	-4.199	4.080	√	-5.095	√	X	0.1	X
Sambunigrin	295.110	7	-1.237	0.528	√	-5.306	√	√	0.1	X
Andrographolide	350.210	5	-2.879	1.580	√	- 4.793	√	√	0.1	X
Iso chlorogenic acid A	516.130	12	-2.262	1.599	√	-6.177	X	X	0.1 – 0.3	X
Artemisinin	262.120	4	-2.241	1.318	√	-4.695	√	X	0.1	X

*Physiochemical property - MW- Molecular Weight contains hydrogen atoms. Optimal Range (OR): 100-600; nHA- number of hydrogen bond acceptors (OR: 0 -12); logS –Log of the aqueous solubility (OR: -4 - 0.5 logmol/L); logP – lipophilicity (OR: 0 - 3) Medicinal chemistry - Lipinski rule is acceptable (OR: MW ≤ 500/ logP ≤ 5); Absorption- Caco2 (colon adenocarcinoma cell line) permeability - higher than -5.15log unit; Distribution - BBB – blood brain barrier penetration (√); Toxicity- AMES – probability of being toxic (√), hERG Blockers – the probability of being active (√); Metabolism - CYP 142 – probability of being inhibitor (OR:0.5 -0.1)

Bioavailability radar and toxicity prediction

The poor water dissolvability and high lipophilicity of several oral medications (Crandinol, Lupeol, and lupatic acid) limit the therapeutic impact. It is recommended that the drug's therapeutic efficacy be increased by enhancing bioavailability and minimising interpatient variability in plasma level concentrations. A medication with a poor aqueous solubility will also have a low saturation solubility, resulting in a limited bioavailability (Table 4). Lipophilicity is required for a drug to interact with the lipid membrane. A divergence from these criteria indicates that the ligands (Crandinol, Lupeol, and lupatic acid) are not orally bioavailable (Jha *et al*., 2022).

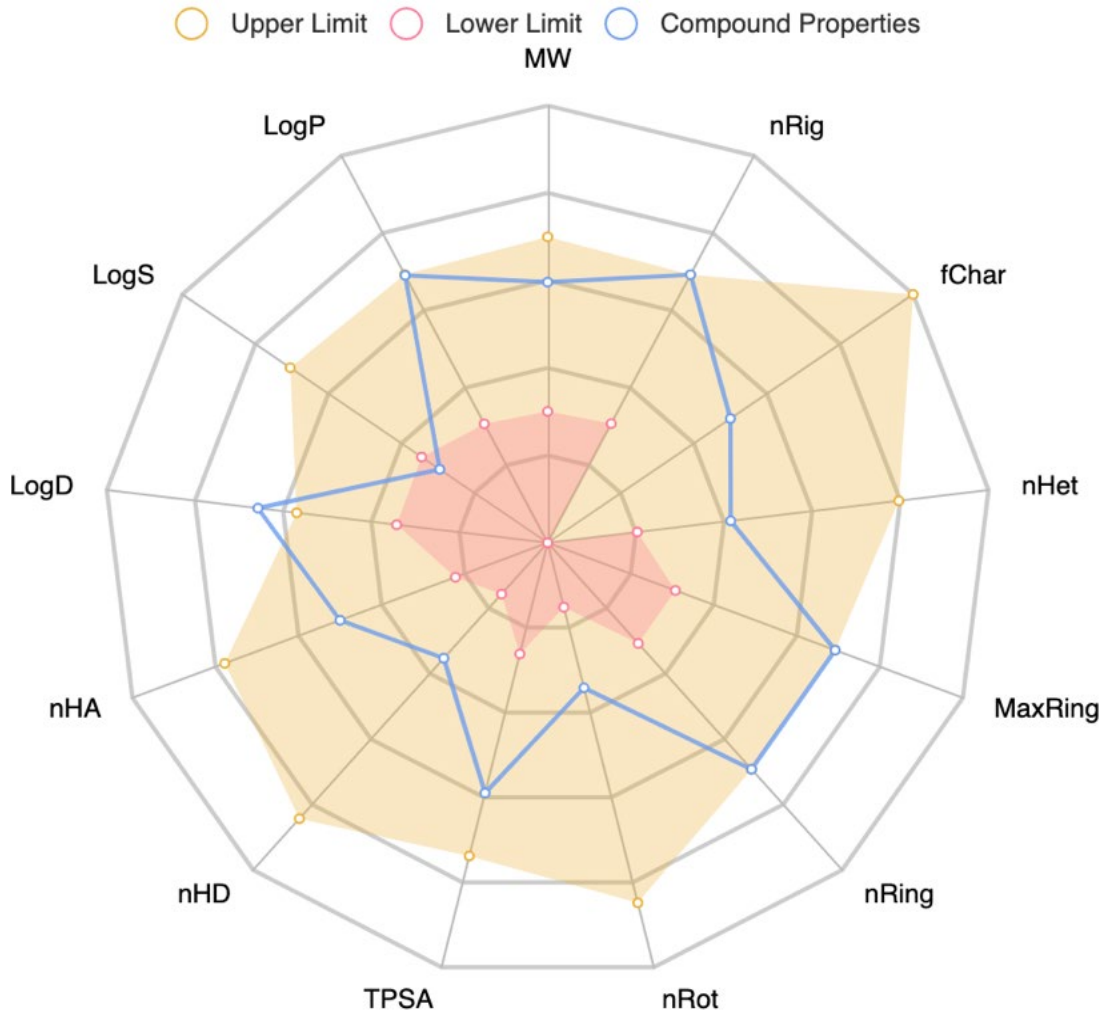


Figure 4. Bioavailability radar diagram of Withaferin A

Prediction of toxicity

Considering the bioavailability radars (Figure 4) of the best ligands and the toxicity profile, (Table 5) it can be deduced that Withaferin A is a potential candidate for drug development, and it can be used for the treatment of HIV infection. In comparison to other ligands, it shows the maximum number of hydrogen bonds with the receptor. However, Carandinol possesses hepatotoxicity and high lipophilicity which make it unsuitable for oral consumption.

Table 5. Toxicity prediction of the best ligands

Phytochemical	*Target*	*Prediction*	*LD50 mg/Kg*
Withaferin A	*Hepatotoxicity*	*Inactive (93%)*	*300*
	Carcinogenicity	*Inactive (55%)*	
	Immunotoxicity	***Active (99%)***	
Carandinol	*Hepatotoxicity*	*Inactive (72%)*	*500*
	Carcinogenicity	*Inactive (75%)*	
	Immunotoxicity	***Active (94%)***	

Validation

Redocking

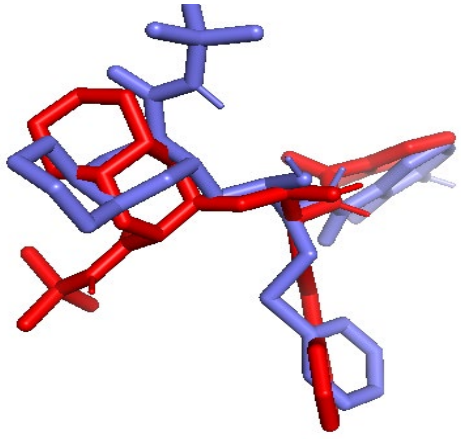


Figure 5. Superimposed structure and the RMSD of the redocked nelfinavir (purple) and its native (red) obtained from PyMOL (RMSD = 0.000 Å)

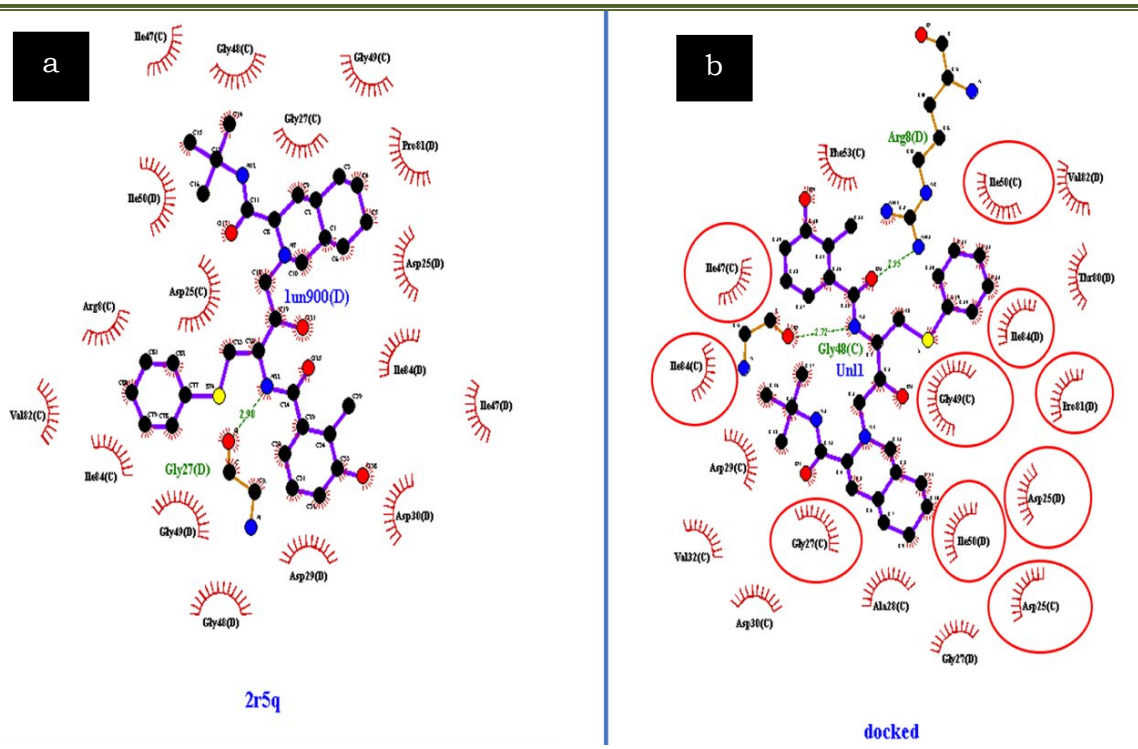


Figure 6. Interactions of LIGPLOT^{+} (a) visualization of the nelfinavir in the crystal structure of 2R5Q (b) visualization of the nelfinavir in the BD redocked complex with common amino acids

Ramachandran plot

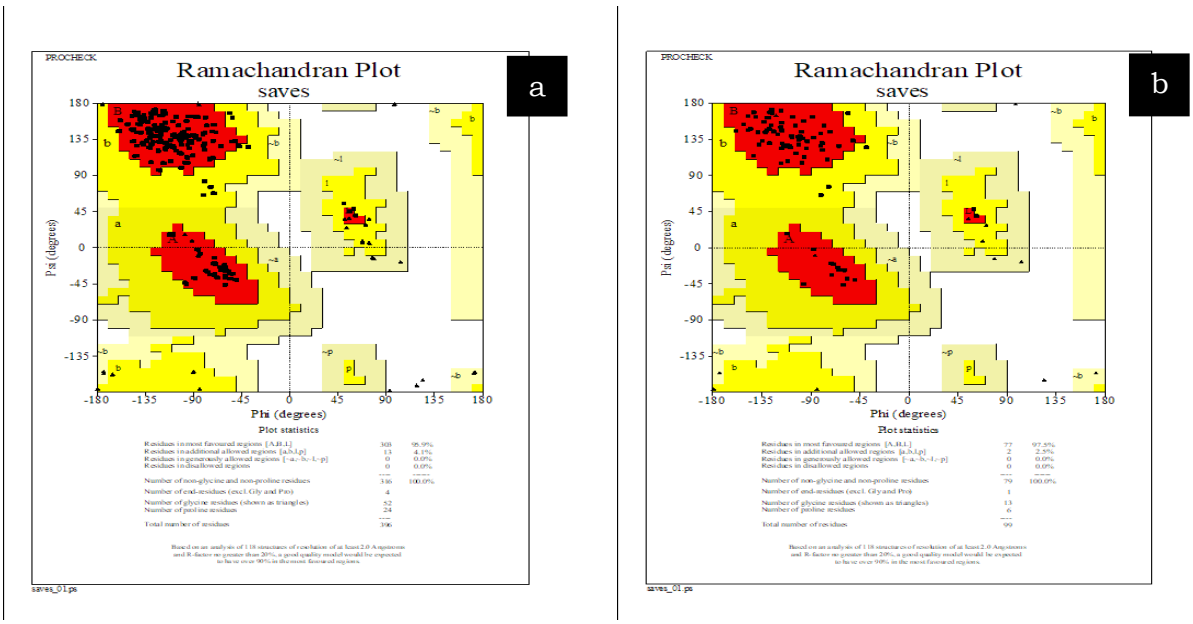


Figure 7. Ramachandran Plot analysis a) Ramachandran plot of 2R5Q (b) Redocked complex AD4 of 2R5Q

Most favorable region is displayed in red and amino acid residues are displayed in black squared

DISCUSSION

Protein-ligand docking is a procedure to find an optimum binding pocket between ligand and receptor. Through the formation of receptors, water molecules were eliminated to clear the binding pocket to the ligand. In addition, polar hydrogens and hetero atoms were added and removed to form desirable hydrogen bonds by merging non-polar. Because these hydrogen bonds are essential to stabilize the protein-ligand docking (Lemmon and Meiler, 2013). While non-polar atoms were merged, it prevents unnecessary hydrogen bond formation and helps to elevate the protein structure by distributing their charge to the nearby carbon. The AD4 type was assigned using AutoDock to identify the hydrogen bond acceptors/donors and aliphatic/aromatic carbon and hydrogen bonding state of hetero atoms in BD. Kollman charges were also added to both receptors (PDB ID: 2R5Q), therefore able to calculate the template value of each amino acid residue of the receptor (Forli *et al*., 2016). Furthermore, the vina is a docking program based on a simple scoring function and rapid gradient optimization conformational search, whereas AutoDock is based on an empirical free energy force field and rapid Lamarckian algorithm search approach (Forli *et al*., 2016). The scoring function is immensely beneficial for structure-based drug development due to its quick and precise scoring, ability to detect an appropriate docking pose, and strong binding between a small molecule and its native receptor (Zheng *et al*., 2022). Additionally, the estimation of pair-wise atomic incorporates assessments for a variety of secondary interactions, including hydrogen bonding, electrostatics, dispersion/repulsion, and desolvation. With AD4, users can utilize empirical charge calculations using Kollman and Gasteiger charges. However, Empirical approaches for electrostatic potential calculation have the benefit of speed, but they also have certain disadvantages, therefore empirical scoring function yield less accuracy of binding affinity and geometry than the semi-empirical method (PM6 method). Furthermore, the Gasteiger charge calculation is unable to control the electrons, which provides a significant defect for the docking calculation of metalloproteins (Morris and Corte, 2021).

Through the preparation of ligands, the TORSDOF was configured to indicate the number of rotatable bonds allocated during BD that are allowed to rotate freely, and the torsion tree was used to enable the ligand to have multiple confirmations while binding to the protein. The Gasteiger approach, which forms the basis of its orbital electronegativity partial equalisation, can be used to calculate partially charged proteins and ligands. But docking accuracy can be increased by polarizing the ligand using quantum mechanical methods like the AMBER score

(Bikadi and Hazai, 2009). Moreover, GA use the concept of natural genetics and biological evolution. The ligand has specified parameters (translation, orientation and confirmation) concerning the protein. In the GA, these are described as state variables, and each state variable corresponds to a gene. Whereas the ligand's state is being generated by the genotype, the phenotype originates from the atomic coordinates (Phillips *et al.,* 2018). In contrast, higher binding affinity (more negative score) and stability of a docked complex, which is obtained by the formation of lower potential energy are reflected in lower BA. Additionally, a high interaction number between HI and HPI was found to be associated with stability, BA, and exposure to bond breakage. The phytochemicals with greater binding energies could contain more hydroxyl groups, which form hydrogen bonds with the target protein, indicating a favourable interaction. Furthermore, alkyl and pi-alkyl linkages increase the hydrophobic interaction of ligands in the receptor's binding pocket. The pi-sigma bond introduces stabilising charges that allow the drug to intercalate into the binding regions of the receptor. The pication bond balances all negative atoms, such as chlorine atoms. Lower Ki is correlated with stronger drug potency and better binding energy; Ki is the lowest concentration required to suppress an enzyme by 50% or the percentage of a drug's binding efficiency (Meng *et al*., 2011) (Kataria and Khatkar, 2019). Furthermore, LE of lupatic acid and lupeol (LE ≥ - 0.3) contribute to removing size effects and optimising compounds based on their effective binding and pharmacokinetic properties (García-Sosa, 2011).. These bonds are crucial in the structural binding and free energy of the ligands with their respective targets.

Considering the best ligands, the binding affinity was in the range of – 10.55 to -9.77 (Table 3 and Figure 8), suggesting that Carandinol (-10.55) is a better candidate as compared to the other ligands.

Carandinol has a binding energy of – 10.55 kcal/mol. It has two types of bonds: the conventional hydrogen bond and the alkyl bond. ASN98 and LEU93 formed the conventional hydrogen bond. CYS67, ILE3, ILE66, LEU24, PRO1, CYS95 of the C chain formed an alkyl bond with the ligand. In this analysis and previous research, the amino acids ASP25, ASP29, ASP30, and GLY48 formed hydrogen bonds with the 2R5Q protease active site and HPIs with VAL32, ILE84, and ALA28, respectively (Umaarasu *et al.,* 2019).

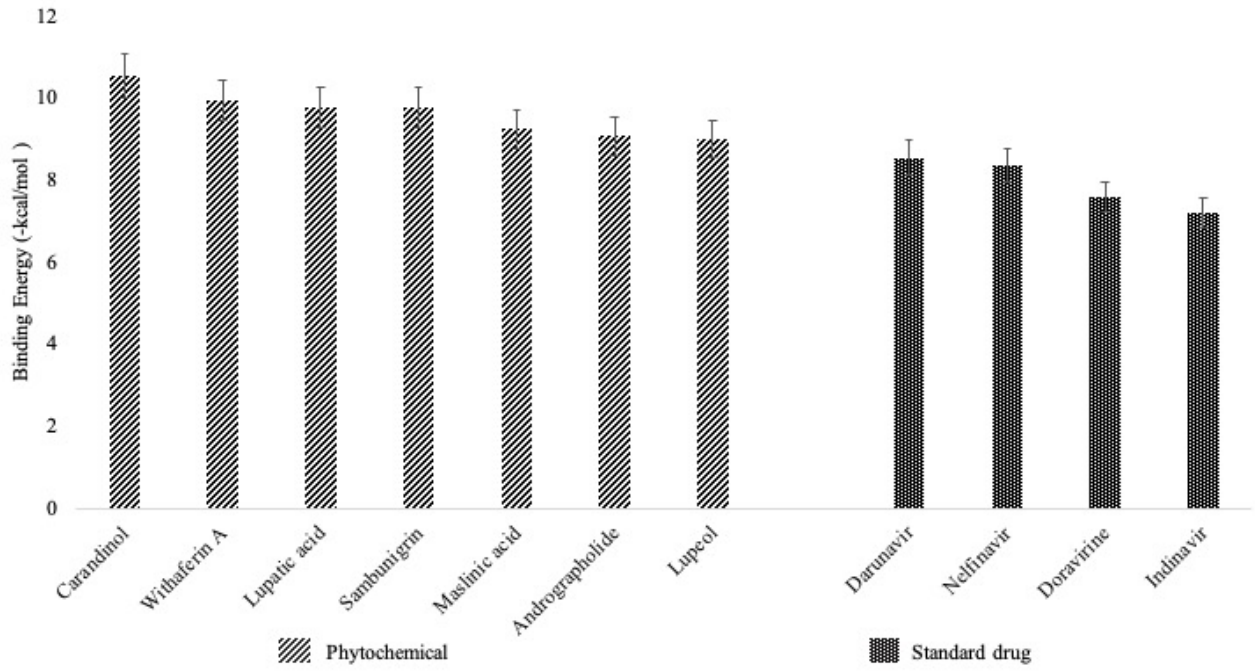


Figure 8. Comparative binding energy analysis between standard drug and best phytochemicals

Moreover, chemicals are more likely to have an affinity for several targets whether their logP is too high. The term "ligand-lipophilicity efficiency" (LLE) was implemented to facilitate affinity optimization concerning logP. Furthermore, molecular size and lipophilicity are crucial aspects to consider to achieve optimum ADMET characteristics (Schultes *et al*., 2010). Considering the bioavailability radars (Figure 11) of the best ligands and the toxicity profile (Table 1,7) it can be deduced that Withaferin A is a potential candidate for drug development, and it can be used for the treatment of HIV infection. In contrast, carandinol has smaller number of hydrogen bonds with the receptor and high lipophilicity which makes it unsuitable for oral consumption and it possesses carcinogenicity (Jha, 2022).

PyMOL was used to superimpose and validate the redocked AD4 pose, and to obtain good performance, the redocked complex's binding affinity needed to be lower and its RMSD value needed to be < 2.00Å (0. 00Å) García-Sosa, 2011). Therefore, the docking methodology was validated obtaining along with confirmation of ligand orientation with common amino acids (ILE47, GLY27, ASP25, ILE50, ILE 84, PRO81 AND GLY 49) between crystal structure and redocked complex. The results of the docking process revealed a substantial improvement, with the most favorable region showing a percentage of 97.5%, surpassing the

pre-docking state. This enhancement is likely due to slight conformational adjustments in the protein structure to accommodate the ligand. These adjustments influence the torsional angles of amino acid residues (phi and psi angles), ultimately leading to an overall improvement in the conformational quality. It is satisfying to observe that the receptor remains undamaged, as more than 88% of its residues are still within the most favored region. However, there are some limitations in AD4 such as the presence of lots of degrees of freedom and docking methods are not accessible to confirmational space, the protein targets often show significant conformational flexibility. However, AD4 and vina employ several simplifications that affect the results obtained. The main simplification is the use of rigid receptors because it can reduce the size of conformation space and scoring function. These challenges can be avoided by using receptor structure that has already been docked complexes, docking to a variety of distinct receptor structures, and using explicit receptor side chain flexibility during docking (Forli *et al.,* 2016).

CONCLUSION

In conclusion, a total of 27 phytochemicals were used for protein-ligand docking and among all the Withaferin A exhibited to be the most potent inhibitor against to 2R5Q with the lowest BE. Furthermore, darunavir was the best potential FDA-approved drug against to 2R5Q. In addition, ASP25, ASP29 and GLY48 novel interactions with HIs were identified in 2R5Q. This study exhibited that carandinol had the lowest binding energy and the second lowest was withaferin A. According to the analysis of ADMET properties, the best phytochemical for oral bioavailability was withaferin A. When visualising the interactions, undesirable bumps were observed for andrographolide which proved to be ineffective HIV-1 inhibitors. As shown by the findings, Withaferin A was the most effective phytochemicals because of their less-toxicity, accepted Lipinski rules and excellent GI absorption due to optimum lopP. Finally, all of the study's objectives were accomplished, and further work will be done in the future.

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Ethical approval

Name: W.Agrani Perera

Title: Protein-ligand docking studies for small molecules (ligands/phytochemicals/antiviral drugs) prediction and binding site identification against HIV protein receptor molecules.

Supervisor: Ms Heshani Mudalige

Millions of people are infected with HIV-1, which causes acquired immunodeficiency syndrome. The purpose of the project is to find optimal ligand binding sites against protein (CD4) receptors and this will be tested using bioinformatics computational approaches. Protein-ligand docking will be performed using AutoDock/AutoDock Vina. Protein receptors data files will be retrieved from the Protein Data bank. The ligands data files will be retrieved from PubChem/ ZINC databases. Then ligand data files were converted into required formats using PyMOL/ Open Babel. Grid box generation and AutoGrid, AutoDock execution runs will be performed and docking results will be generated. Based on the docking poses/ scores/ energy values and interactions, the docking results will be analyzed to identify the best binding sites. PyMOL/Chimera/BIOVIA drug discovery studio will be used to visualize the protein-ligand best-docked poses. LIGPLOT will be used to generate 2D/3D interactions of protein-ligand docked complexes. Redocking and Ramachandran plot analysis will be carried out to validate the study. Project overview form will be submitted to counteract computational safety measures. This study contains no animals, human tissue samples, clinical investigations, or personal data, it falls under the category of green ethics. The ethics approval for the study was received by the supervisor

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Effective and efficient proliferation of BHK-21cells using serum-free-medium in fed-batch culture system for FMD virus production

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ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious and devastating a viral disease of cloven-hoofed animals and is considered a severe threat to the livestock industry worldwide. Today, BHK 21 cells adapted to suspension culture systems are widely used in large-scale inactivated FMD vaccine production. The serum is a broad supplement in animal cell culture media. Although it has many advantages, it has many drawbacks and a substantial cost. Current biotechnological approaches to cell culture avoid using serum; therefore, this study aims to grow BHK-21 suspension cells in serum-free media (SFM) in stirred bioreactors. In our study, BHK-21 cells were maintained in suspension culture up to 20 passages in a 2L stirred bioreactor. After ten passages of suspension cell culture, SFM was used without serum, while control groups were maintained with 6M medium, including 10% serum. FMDV culture was prepared between the 10th and 20th passages level of the cells. During the process, the growth kinetics of the cells culture, antigenicity and infectivity of the FMD virus were assessed comparatively. The determined cell count and percentage of viability of the cultures in both media complied with each other. Also, virus antigenicity and infectivity values of the virus harvests were similar for the test and control groups (SFM and 6M). This study showed that large-scale suspension BHK-21 cells used in industrial-scale production of the FMD vaccine could be grown in SFM without serum without compromising quality and quantity.

Key words: Serum-free medium, BHK-21 cells, Foot-and-Mouth Disease, vaccine, pilot-scale production

INTRODUCTION

Foot-and-mouth disease is one of the most feared viral diseases for cloven-hoofed animals. FMD virus, which is the causative agent of the disease, is an aphtovirus belonging to the picornaviridae family and has seven serotypes (A, O, Asia, C, SAT1, SAT2, SAT3) with many subtypes. (Brown, 2003; Chakraborty et al., 2014). Many factors, such as the highly

contagious nature of the virus, the absence of cross-protection between serotypes and the existence of various susceptible animal species, complicate the combat of the disease (Domingo et al., 2002; Rodriguez et al., 2009).

Vaccination plays a significant role in the fight against with FMD, and for this purpose, about 2.35-2.5 billion doses of FMD vaccine are produced worldwide every year (Knight-Jones & Rushton, 2013; Belsham, 2020). Today, BHK-21 cells adapted to suspension culture systems are widely used to produce industrial-scale FMD vaccines (Li et al., 2019). BHK-21 cell line was established from 1-day-old Syrian hamsters kidney cells by Stoker and MacPherson in March 1961, and the original BHK-21 cells are fibroblastic on growth pattern as a monolayer culture (MacPherson & Stoker, 1962). They are susceptible to the FMD virus and can be grown in either monolayer or suspension culture systems (Capstick et al., 1962; Mowat & Chapman, 1962). During the cultivation proccess of BHK-21 cells in suspension, factors such as stirring, cell culture media and advanced passage levels an cause essential changes in FMD virus-specific cellular receptor expression, which may adversely affect both the infectivity and plaque character of FMDV (Yılmaz et al., 2020). Cell culture medium and its essential component serum, which constitutes 5-25% of it, are indispensable in animal cell culture systems (Gstraunthaler, 2003). In BHK-21 cell culture, approximately 10% of the medium is serum (Merten, 2002).

Serum plays a vital role in the culture medium by enzyme inhibition, toxin neutralization, providing hormones, vitamins and growth factors, and protecting cells against mechanical stress (Chelladurai et al., 2021). In addition, it may have some disadvantages due to the quality differences from batch to batch, toxins it may contain, high cost, potential to carry various contaminants, and difficulties in downstream processing. Moreover, the Transmissible Spongiform Encephalopathy risk led to using plant-origin materials in media (Cruz et al., 1999; Merten et al., 1999; Zhang et al., 2018). For all of these reasons, researchers have focused on working with serum-free media in the production of biological substances in recent years (Gürhan and Ozdural, 1990; Leist et al., 1990; Jan et al., 1994; Merten et al, 1999; Merten, 2002; Chelladurai et al., 2021). Bradshaw et al., 1983, obtained similar results with modified DMEM serum-free medium to 10% serum-containing medium for BHK-21 cells. Saha and Sen 1989, evaluated 11 different formulations containing peptones and casein hydrolysate without serum and had successful results with 10 of them. Rourou et al. (2014) have successfully produced rabies virus in Vero, BHK-21 and MRC-5 cells in a spinner flask

and a 2L stirred bioreactor using the animal component-free medium, with an in-house formulation. Recent studies obtained satisfactory results with SFM for virus culture in BHK and other cell types compared the effect of nine combinations of cell growth media, including Cellvento™ BHK-200 cell culture medium to FMDV propagation in erlenmayer flasks. Their results suggested that the best suitable medium is Cellvento™ BHK-200 cell culture medium (Kim et al., 2021). Park et al. (2021) produced O, and A strains of the FMD virus endemic to South Korea with Cellvento^{TM} BHK-200 cell culture medium in BHK-21 cells on a pilot scale stirred bioreactor.

In this study, it was aimed to establish the adaptation conditions of BHK-21 suspension cells to commercially available SFM in pilot-scale in stirred bioreactors and compare serum-free medium and conventional medium with 10% serum for the parameters such as the cell viability, growth kinetics of cells and antigenicity and infectivity titer of the harvested virus.

MATERIALS AND METHOD

Cell lines, viruses and medium: BHK cell lines were obtained from HUKUK (WDCM 756 Animal Cell Culture Collection, Ankara, Türkiye). A/TUR/11, A Nepal/84, Asia1/TUR/11, O1 Manisa FMD vaccine strains obtained from Virus Bank of FMD Institute, Ankara, Türkiye. Commercial SFM Cellvento™ BHK-200 (Merck KGaA, Darmstadt, Germany) was used for suspension cell production in 2L stirred bioreactor and virus culture in 125 ml spinner flasks. 6M cell culture medium described by Radlett et al., 1971 was prepared as an in-house formulation with 10% adult bovine serum (ABS) (Himedia, New Zealand) as a standard control medium. GMEM (Applichem, Germany) without serum was used for virus culture as standard control. For monolayer cell culture, GMEM medium was used with 10% ABS as a standard control medium,

Adaptation of BHK-21 cells to the serum free-medium and growth curve: BHK-21 cells were grown in roller bottles that have 850 cm^2 surface by semi-direct adaptation method to SFM using Cellvento™ BHK-200 medium with 1% FBS (Yılmaz et al., 2009; Van der Valk et al., 2010). The control group consisted of GMEM with 10% serum. Five serial subcultures have been realized every 48 hours. Cell counting was carried out by using the trypan blue dye exclusion method using a Bürker hemacytometer. The generation number has been calculated according to the formula (n= logCt-logCo/log2), and the growth curve was plotted depending on the generation number. The cells grown in monolayer culture in SFM with 1% FBS were

transferred to a 2L stirred bioreactor fixed pitch blade impeller (Biostad B plus, Sartorius, Germany). Suspension culture were made using the protocol, which is slightly modificated from studies of Telling and Elsworth (1965) and Girard et al. (1973). BHK-21 cells were maintained in suspension culture for up to 20 passages. In the suspended culture, the serum ratio was gradually decreased from 1% to 0%. After the 10th passage, it was continued with 100% SFM without serum. The cell cycle analyses and apoptosis rate were measured during 20 cell culture passages. The growth curve was plotted depending on the generation number, like in monolayer culture. Virus cultures were performed between 10th and 20th cell culture passage.

Cell viability, apoptosis stages and cell cycle phases: During all 20 suspension passages, cell viability and apoptosis levels were determined using the Annexin V kit (Muse Annexin V Dead cell kit, Luminex, USA) and cell cycle phases using a cell cycle analysis kit (Muse cell cycle kit, Luminex, USA) by the device (Muse cell analyzer, Merck Millipore, USA).

Virus assays: Cells were centrifugated for virus culture and resuspended by SFM and GMEM without serum. Suspension cells (cell count $2x10^6$ cells/ml) were infected at a multiplicity of infection (MOI) of 0.05 and incubated for 18-20 hours. Following virus culture, harvested viruses were tested for infectivity by plaque assay on BHK-21 An_{31} cells, capable of adherent proliferation, in 6-well plates. The virus titer was calculated according to the formula suggested by Bachrach et al. (1957)

PFU/ml=Average of plaques / Dilution x Volume of diluted virus added to the plate

146S antigenic particle amount was measured by sucrose density gradient (SDG-UV) assay (Doel et al., 1981). The virus experiments were repeated five times for each virus type.

In the statistical evaluation of the results of our study, the SPSS (Statistical Package for the Social Sciences) program was used, and their significance was determined by applying the "Repeated Measures ANOVA" to the obtained data and the "Post Hoc Tukey" test for further evaluations. The differences between the experimental groups, which emerged as a numerical value (p) as a result of all statistical applications, were accepted as significant at the $p<0.05$ significance level.

RESULTS

Adaptation of BHK-21 An_{30} cells to the serum-free medium and growth curve: Five serial monolayer subcultures have been performed with SFM and control group medium (GMEM) with 10% serum. Depending on generation time, the growth curve has been drawn by counting the cells after each subculture (Figure 1). The results showed that the cell growth was significantly higher in the SFM than control group medium. The process was expanded to bioreactor culture after adaptation to SFM in monolayer culture. The final viable cell number of each passages was recorded, like in monolayer culture, and growth kinetics were demonstrated. The cell proliferation was statistically higher in SFM ($t(59)=7,77$, $p<0.001$) (Figure 2).

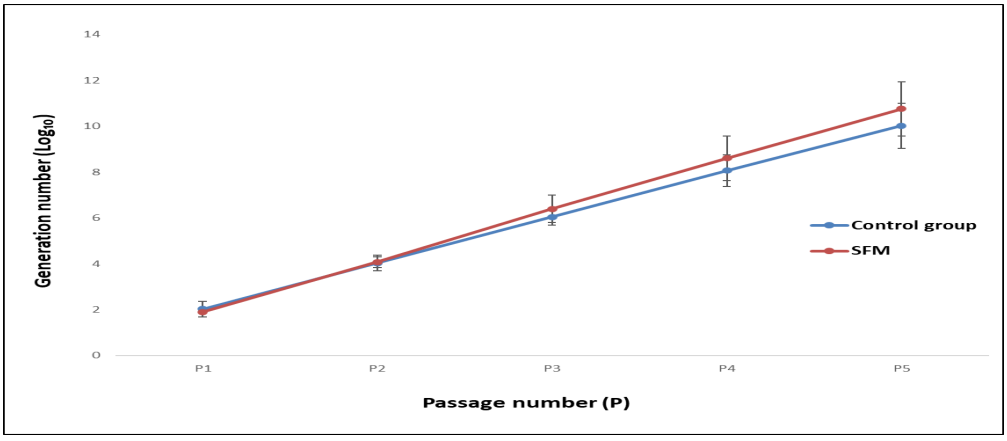

Figure.1. Adaptation of monolayer BHK-21 An_{30} cells to the serum-free medium and growth curve based on number of generations. Error bars represent standard deviations (n=3).

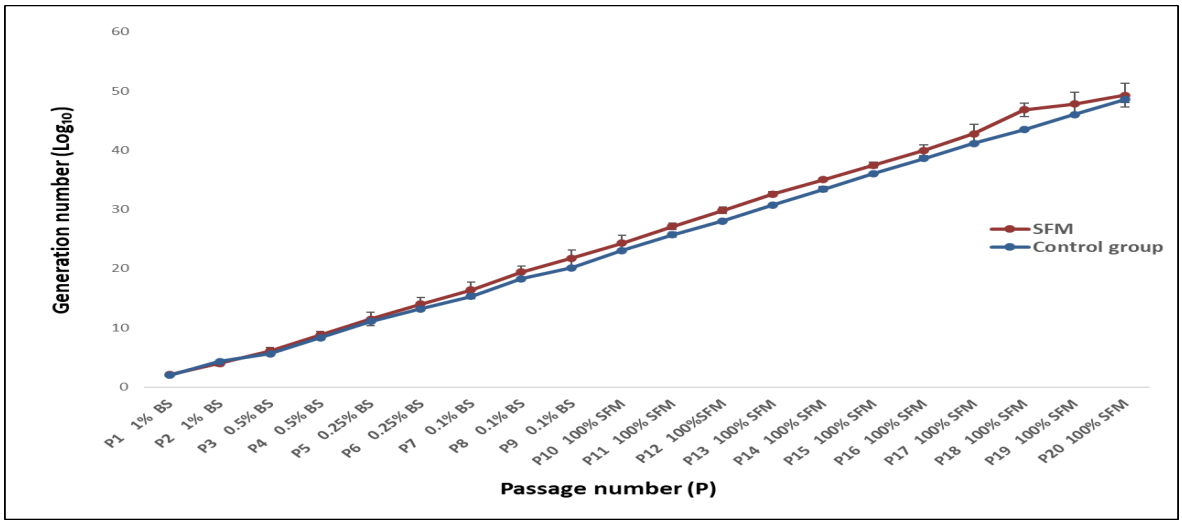

Figure.2 Growth curve based on generation number of suspension BHK-21 cells. Cell growth in SFM was statistically better than control group medium with 10% serum ($p<0.001$).

Cell viability, apoptosis stages and cell cycle phases: Cell viability and apoptosis were plotted and assessed using flow cytometry histograms of Annexin V (Figure 3). Example histograms are given from passage 15 for the control group medium (Figure 3B) and SFM (Figure 3D). Although the results were no statistically significant difference, viability was higher than in the control group, and apoptosis percentages were lower in the SFM during the subcultures in suspension culture in the bioreactor. (Figure 3C). The percentages of cell cycle phases were plotted and assessed using flow cytometry histograms of the DNA content profile histogram (Figure 4). Examples of DNA content profile histograms are given from passage 15 for the control group medium (Figure 4B) and SFM (Figure 4D). No significant difference has been detected between the control group medium (Figure 4A) and SFM (Figure 4C) for all passages.

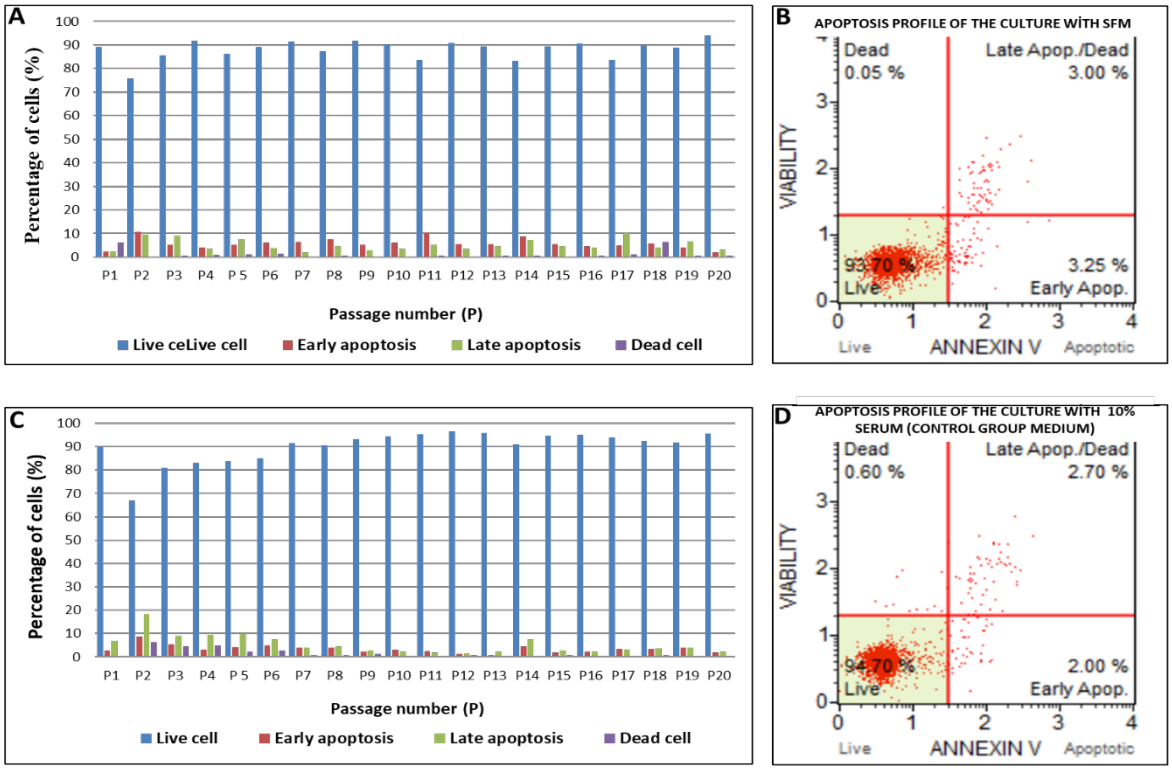

Figure 3. The percentages of viability and apoptosis of suspension BHK-21 cells in the culture with 10% serum-containing medium (control group) (A) and in the culture with SFM (C). Example histograms are given from passage 15 for the control group (B) and SFM (D).

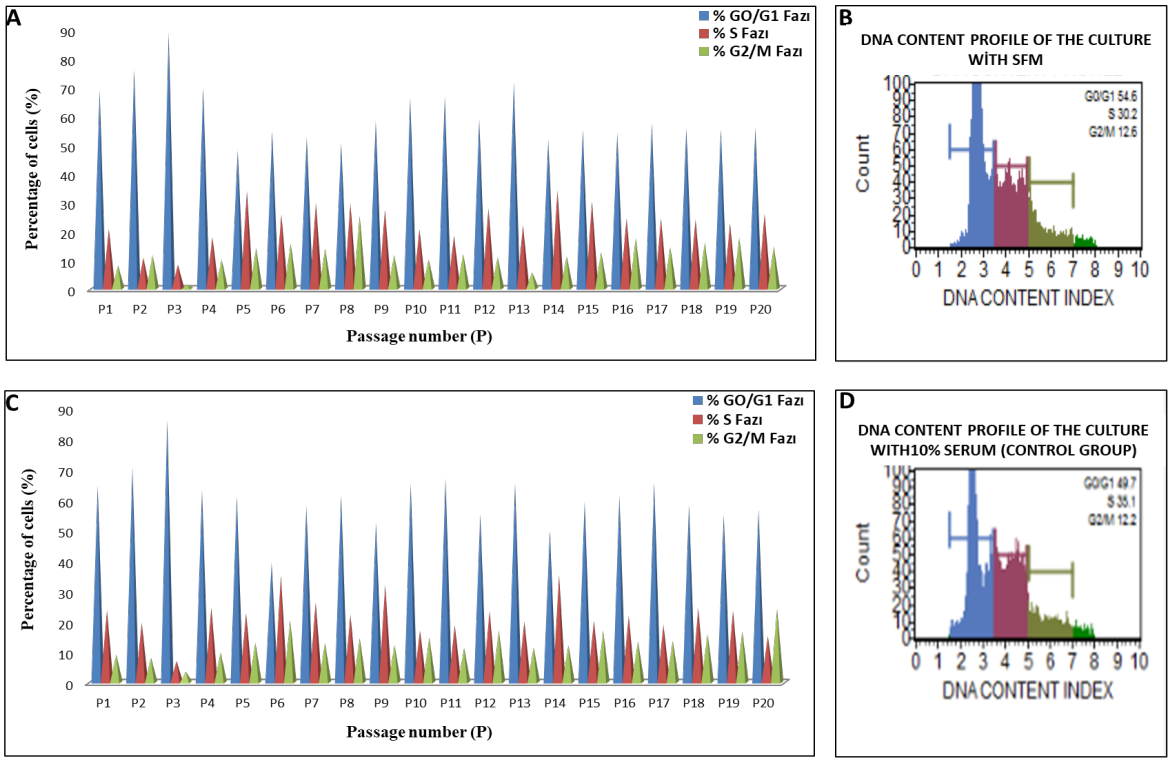

Figure 4. The percentages of cell cycle phases of suspension BHK-21 cells in the control group medium culture with 10% serum (A) and in SFM (C). Examples of DNA content histograms are given from passage 15 for the control group (4B) and SFM (4D).

Virus assays: The effect of SFM on infectivity and 146S antigenic particle amount of virus cultures have been evaluated. Harvested viruses were analysed by plaque assay for infective virus and SDG-UV method for antigenic particle amount.

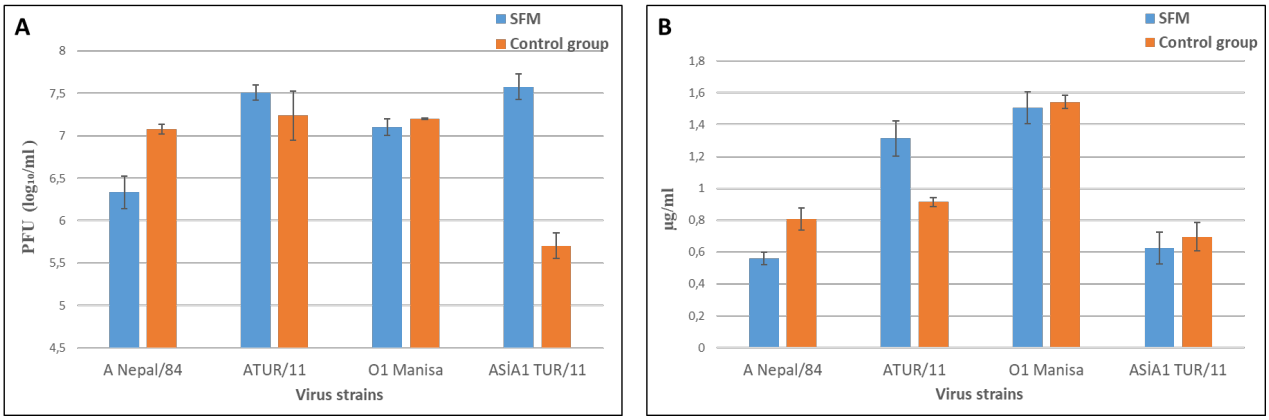

Figure 5. The infective titer (A) and 146S antigenic particle amount (B) of virus strains.

No statistically significant difference existed between the infectivity titer and 146S antigenic particle amounts of virus strains obtained from cell cultures grown in serum and serum-free medium. Infectivity titers of FMDV strains; While ANepal/84 was relatively lower in SFM

than control group media ($p<0.05$, $t(8)=-3.719$), there was no significant difference between SFM and control group medium for ATur/11 ($p=0.41$, $t(8)=0.884$) and O1Manisa ($p=0.40$, $t(8)=-0.891$). In Asia1Tur/11 ($p=0.41$, $t(8)=0.884$), it was higher in SFM than in the control group. 146S antigenic particle amount values; ANepal/84 ($p<0.005$, $t(8)=4.65$) was relatively lower in SFM than in the control group medium, whereas in ATur/11 ($p<0.005$, $t(8)=3.42$) SFM and control group were found to be higher in O1Manisa ($p=0.80$, $t(8)=0.664$) and Asia1Tur/11 ($p<0.005$, $t(8)=3.42$) in SFM compared to the control group (Figure 5).

DISCUSSION

Today, BHK-21 cells adapted to suspension culture systems are widely used in producing inactive FMD vaccines at the industrial scale (Li et al., 2019; Teng et al., 2021; Kim et al., 2021). Suspension cell cultures require specific medium containing serum to protect the cells from mechanical stress. However, using serum in cell culture media brings disadvantages, such as potential contaminants and batch-to-batch quality instability (Merten et al., 2002; Verma et al., 2020). For this reason, SFM studies gained interest in recent years. In viral vaccine production, it is critical to preserve antigenic structures and cell-binding domains of the virion (Kim et al., 2021). Another point to consider is providing the necessities of continuous cell lines. This study aimed to demonstrate the results of SFM and conventional medium with 10% serum in a pilot-scale stirred bioreactor for parameters such as cell viability, growth kinetics of cells, and antigenicity and infectivity titer of the harvested virus.

The direct adaptation or semi-direct or step-wise adaptation method can be used to adapt the cells to the serum-free media (Cruz, 1998; Kallel, 2002; Chun, 2007). In our study, semi-direct adaptation with the minor modification described by Yılmaz et al. 2009, has been used. It was observed that cell proliferation in cultures with SFM was better than the control medium with 10% serum. (Figure 1 and Figure 2). The viable cell rate were equal or higher in SFM than in the control medium with 10% serum. During the sequential subcultures, viable cell numbers for both media were low and apoptotic cell numbers were high in the second passages, critical adaptation steps. However, after the cell was adapted to the suspended culture and conditions, the viability rates of the cells were 80% and above, especially in SFM, 90% and above after the sixth passage, and the apoptosis rates were lower than 10% in the advancing passages. (Figure 3).

The cell cycle which consists of interphase (G0/G1 phase, S phase and G2 phase) and mitosis differs according to cell types, cellular environments, and metabolic stages. They can therefore be considered part of the phenotype of a particular cell (Weber et al., 2014). Flow cytometry makes it possible to distinguish the phases of the cell cycle to predict the growth rate of cells. Cells in the S+G2+M phase are called the S phase fraction (SPF) or G2M; When this rate is high, the proliferation rate of cells will also be high (Kim et al., 2015; Kanev, 2016). The G2M rate is about between 10%-20% in the cell population. In the study of Cecchini et al. (2012), in which in breast epithelial cells, the percentage of the G2M phase of cells about 15-20% were measured. In the current study, during adaptation to the suspension state, the percentage of G2M was low up to the sixth passage in both SFM and control group medium with 10% serum (Figure 4). The low percentage of G2M in the first five passages may be due to the adaptation of the cells to the suspension culture condition, and this process may have stressed the cells. After the sixth passage, the percentage increased gradually, especially in SFM. However, no significant difference was found in G2M rates between the media after adaptation to suspension culture.

A Nepal/84, ATUR/11, Asia1 TUR/11 and O1 Manisa FMD vaccine strains were used for 125 ml spinner flask virus culture with SFM and control group virus culture medium without serum. No statistically significant difference has been observed for 146S particle amounts and infective titers of harvested viruses except for serotype Asia1 produced in SFM and control group virus culture medium (Figure 5). FMDV serotype Asia1's infectivity titer in SFM was higher than the control group medium, albeit without statistical significance. FMDV serotypes may have differential behaviors in cell culture systems and conditions (Hassan, 2016; Dill et al., 2019). The results are also coherent with the study of Gulde et al., (2016) and Dill et al. (2018), in which the O1 Manisa FMD virus was grown in BHK-21 cells using Cellvento™. The viral titer and 146S antigen of the FMD virus was obtained better in Cellvento™ BHK-200 SFM than in the control group medium.

CONCLUSION

The cell numbers obtained with SFM was similar to that produced in the conventional medium with 10% serum. No difference have been observed in viability, apoptosis rate and cell cycle parameters of the cells. The infectivity titer and 146S antigenic particle amount of viruses harvested from a culture with SFM were the same or better than the conventional

medium. These results show that BHK-21 cells and vaccine viruses can be produced in industrial volumes without compromising from quality and quantity in SFM. In addition, the absence of serum-derived foreign proteins will contribute to the quality of the vaccine produced with SFM.

ACKNOWLEDGMENTS

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Inflammatory bowel disease in a horse: clinical presentation and cecum microbial profile

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ABSTRACT

In the clinical examination of the 11-year-old horse brought to the hospital with complaints of chronic diarrhea, pain and weight loss, it was observed that the heart rate increased, the respiratory rate and rectal temperature were normal. Anemia, dehydration and neutropenia were detected in the hemogram findings, while a decrease in total protein and albumin values and an increase in liver enzymes were detected in the blood biochemistry findings. The horse died on day 4 after being treated for suspected inflammatory bowel disease (IBD). In order to clarify the etiology of the disease and to investigate the taxonomic bacterial composition, DNA extraction was performed for metagenomic analysis by taking samples from three different parts of the cecum. Primers that amplify the V3-V4 region of the 16S rRNA gene were used for bacterial profiling. Amplicon readings from the Illumina MiSeq System were analyzed using quantitative insights into microbial ecology 2 QIIME2 (2022.11) software. *Campylobacter rectus* (36.1%) and *Roseburia inulinivorans* (18.3%) were higher in the cecum areas showing hemorrhagic lesions compared to the less inflamed parts. As a result, clinical examination findings, hemogram and biochemistry changes and cecum bacterial profile of a horse with IBD were determined. In addition, the study also allowed the comparison of different regions of the infected horse cecum.

Keywords: IBD, bacterial, clinical, cecum

INTRODUCTION

Inflammatory bowel disease (IBD) in the horse is an important digestive system disease that causes infiltration of the mucosa and submucosa with malabsorption. The disease may be associated with many different etiologies such as bacterial, viral, parasitic or dietary abnormal antigens (Kalck 2009). The disease can develop in horses in eosinophilic enteritis and multisystemic eosinophilic epitheliotrophic, granulomatous, lymphocytic/plasmacytic enteritis and lymphosarcoma types. Clinical examination has an important place in the diagnosis of the disease. Although the horses have a good appetite, there is weight loss. Diarrhea occurs when the large intestines are affected and edema due to hypoproteinemia. However, there is intermittent colic (Southwood et al., 2000). Broadly speaking, treatment

can be classified as diet modification, elimination of parasitic causes and environmental antigens (Barr 2006).

The existence of different but few studies has been reported in the literature (Vitale 2022). Especially in digestive system diseases, it is important to determine the bacterial flora present in the disease and to distinguish it from healthy flora in order to clarify the prognosis and treatment. The microbiome analysis we included in the study relies on rapid and accurate bioinformatics tools to characterize the taxonomic composition of samples based on the 16S rRNA gene. Quantitative Insights Into Microbial Ecology 2 (QIIME2) are one of the most popular tools available to perform this task which is an open-source software for the analysis of microbiomes. The aim of the study was to determine the clinical findings, hemogram and biochemistry changes and taxonomic bacterial composition of the horse with IBD. In addition, the study also allowed the comparison of different regions of the infected horse cecum.

CASE REPORT

Sample Collection

Diarrhea, pain and weight loss were reported in the anamnesis of the 11-year-old horse brought to the hospital. It has been reported that these complaints have recurred in recent months, but have lost their effect from time to time. The horse died on day 4 after being treated for suspected IBD. To clarify the etiology and investigate the taxonomic bacterial composition, DNA extraction was performed for metagenomic analysis by taking samples from three different parts of the cecum (Figure 1).

DNA Extraction and Quality Check

Metagenomic DNA was extracted using the Qiagen DNeasy Blood and Tissue kit following the manufacturer's instructions for Gram-positive bacteria (Qiagen). Tissue samples were cut into small pieces (25mg) to enable more efficient lysis and they were effectively disrupted using a rotor–stator homogenizer. Briefly, they were suspended by vortexing in 90 µl TET buffer (TE supplemented with 0.2% [v/v] TritonX- 100). 90 µl of TET supplemented with 40 mg/ml egg white Lysozyme were added and incubated at 37℃ for 2h. Proteinase K digestion of the sample was performed at 56℃ for 1h. Samples were further incubated at 90℃ for 5 min and after adding AL buffer, they were loaded in the Qiagen column. Elution was performed in

50 µl of AE buffer. Extracted DNA was stored at -80℃ until further use. Sample quality control was performed on the isolated DNA samples using three methods. A NanoDrop spectrophotometer (Thermo Fisher Scientific) was used to test the DNA purity and yield (OD260/ OD280). An agarose gel electrophoresis test was used to analyze DNA degradation and potential contamination. Then, a Qubit 2.0 fluorometer (Thermo Fisher Scientific) was used to precisely quantify the DNA concentration.

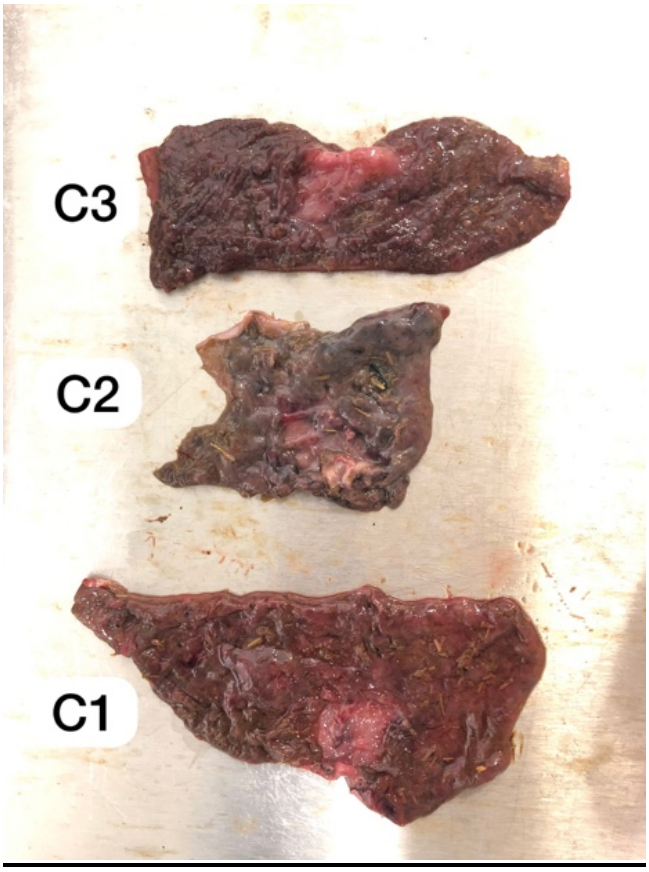

Figure 1. Parts of the caecum of the horse with IBD (C1: most hemorrhagic, distal part; C2: medial part; C3: less hemorrhagic proximal part)

Amplicon Sequencing

Intestinal microbiome was analysed by mass sequencing of the V3-V4 region of 16S rRNA gene (fwd: TACGGGAGGCAGCAG rev: CCAGGGTATCTAATCC (Turner et al., 1999). The 16S rRNA amplicon reads obtained from an Illumina MiSeq System (Eurofins Genomics, UK) were analysed using QIIME2 (2022.11). Illumina results include both the nucleotide sequence of the reads and a quality score (Q-score) associated to each nucleotide in each read.

Bioinformatic Analysis of the Sequences

Unindexed paired endpoint sequences were exported from Miseq to continue their analysis in FASTQ format. The SILVA reference database (SSU, release 132) was used in the taxonomic annotation of reads. Sequence reads imported into QIIME2 version 2022.11 (https://qiime2.org) for further analysis. FastQ files were filtered to 260 (forward) and 200 (reverse) bp using the DADA2 package. The DADA2 pipeline (2) was used to pair forward and reverse reads and for quality control; phiX reads and chimeric sequences were filtered using a pooled consensus method. The resulting feature table was used for taxonomic assignment based on the Greengenes version 13.8 reference database by training a Naive Bayes classifier with the QIIME2 q2-feature-classifier plugin (Mc Callum et al., 1998; Metsis et al., 2006). For all comparisons and statistical tests, α=0.05 was set as the threshold for significance.

RESULTS

Clinical examination and blood findings

In the clinical examination, it was observed that the heart rate increased, the respiratory rate and body temperature were normal. Anemia, dehydration and neutropenia were detected in the hemogram findings, while a decrease in total protein and albumin values and an increase in liver enzymes were detected in the blood biochemistry findings (Table 1).

Amplicon Sequencing Data Summary

Demultiplexed sequences were imported into QIIME2, and sequencing results were summarized. For 3 samples, the raw data resulted in a total of 342,181 reads (mean per sample: 114,060). A feature table was constructed with DADA2 to map feature identifiers to representative sequences. Within the 3 samples, there were 362 total unique features, with a total frequency of 123,896. For downstream analysis, the frequency tables were rarified at an even sampling depth of 32,906 reads per sample. At this level, 98,718 of the original sequences (79.68%) and all of the 3 samples were retained in the data set.

Table 1. Clinical and laboratory findings of horse with IBD

Parameters	**IBD**	**References***
Heart rate /min	48 ↑	28–40
Respiratory frequency /min	11	10–14
Rectal temperature	37.8	37.3–38.2
RBC × 10^6/mcL	4.36 ↓	6.0–10.4
Hgb g/dL	8.1 ↓	10.1–16.1
Ht (%)	21 ↓	34 - 46
Neutrophils (segmented) × 10^3/mcL	2.68 ↓	2.9–8.5
Neutrophils (band) × 10^3/mcL	0	0–0.1
Total protein g/dL	4.1 ↓	5.6–7.6
Albumin g/dL	1.8 ↓	2.6–4.1
AST (U/L)	494 ↑	160–412
ALP (U/L)	292 ↑	88-261
GGT (U/L)	8	6–32
Total bilirubin (mg/dL)	0.6	0–54.7

* (Kahn and Line, 2010)

Microbial Community Diversity and Taxonomic Composition

Alpha (α) and beta (β) diversity metrics were determined for the sample microbial communities in order to estimate species number (richness) and distribution (evenness) within a particular sample, and similarity score between populations of different samples respectively. The observed effect of sample region on α-diversity was significant (P-value 0.001). Differences in β-diversity on comparison to region of the samples, represented by weighted UniFrac distances, were not significant (P-value 0.683). Results revealed in the

caecum parts registering totally 8 phyla. 2 phyla including Firmicutes and Proteobacteria represented ≥98% of relative abundance especially for the most hemorrhagic part of the cecum (Figure 2).

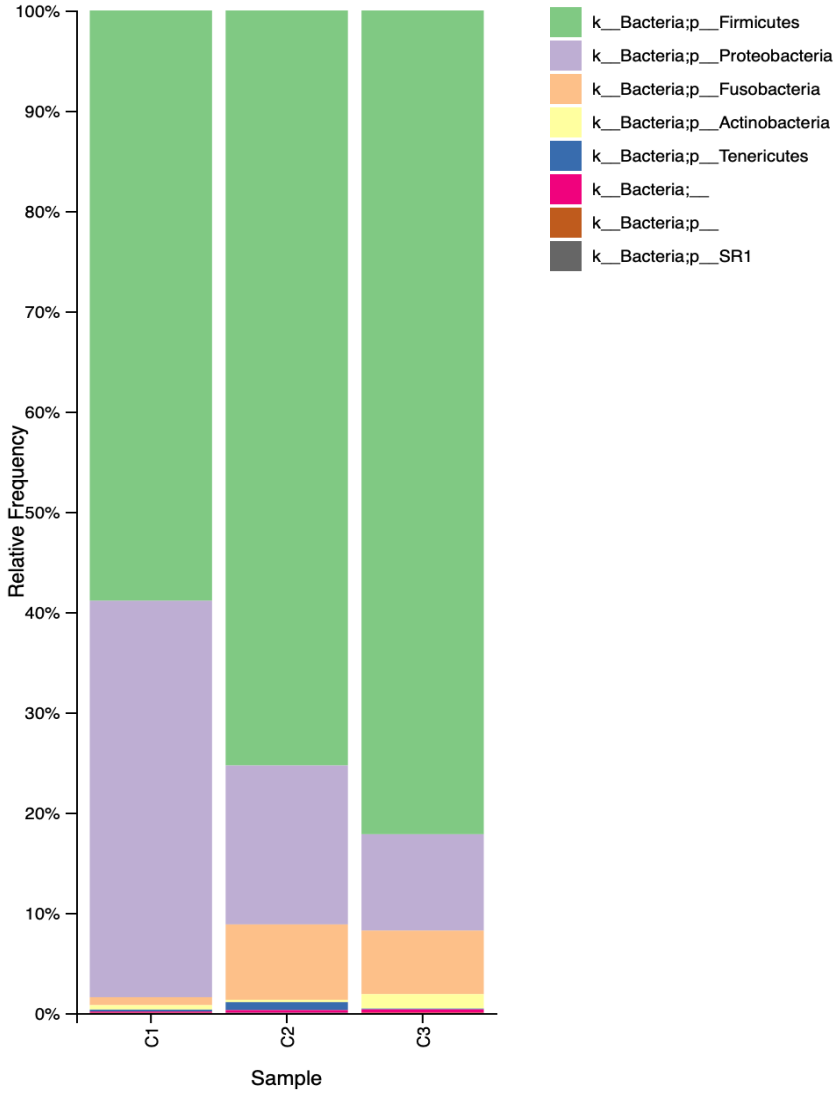

Figure 2. Phylum-level taxonomic composition of horse with IBD

Although, Fusobacteria abundance in the most non-hemorrhagic part of the cecum was highest when compared with the other parts. On average, the most abundant genera detected were *Lachnospiraceae Campylobacteraceae Ruminococcaceae Clostridiaceae* and *Fusobacteriaceae.* At the species level, the most abundant bacteria detected were *Campylobacter rectus* and *Roseburia inulinivorans* (Figure 3).

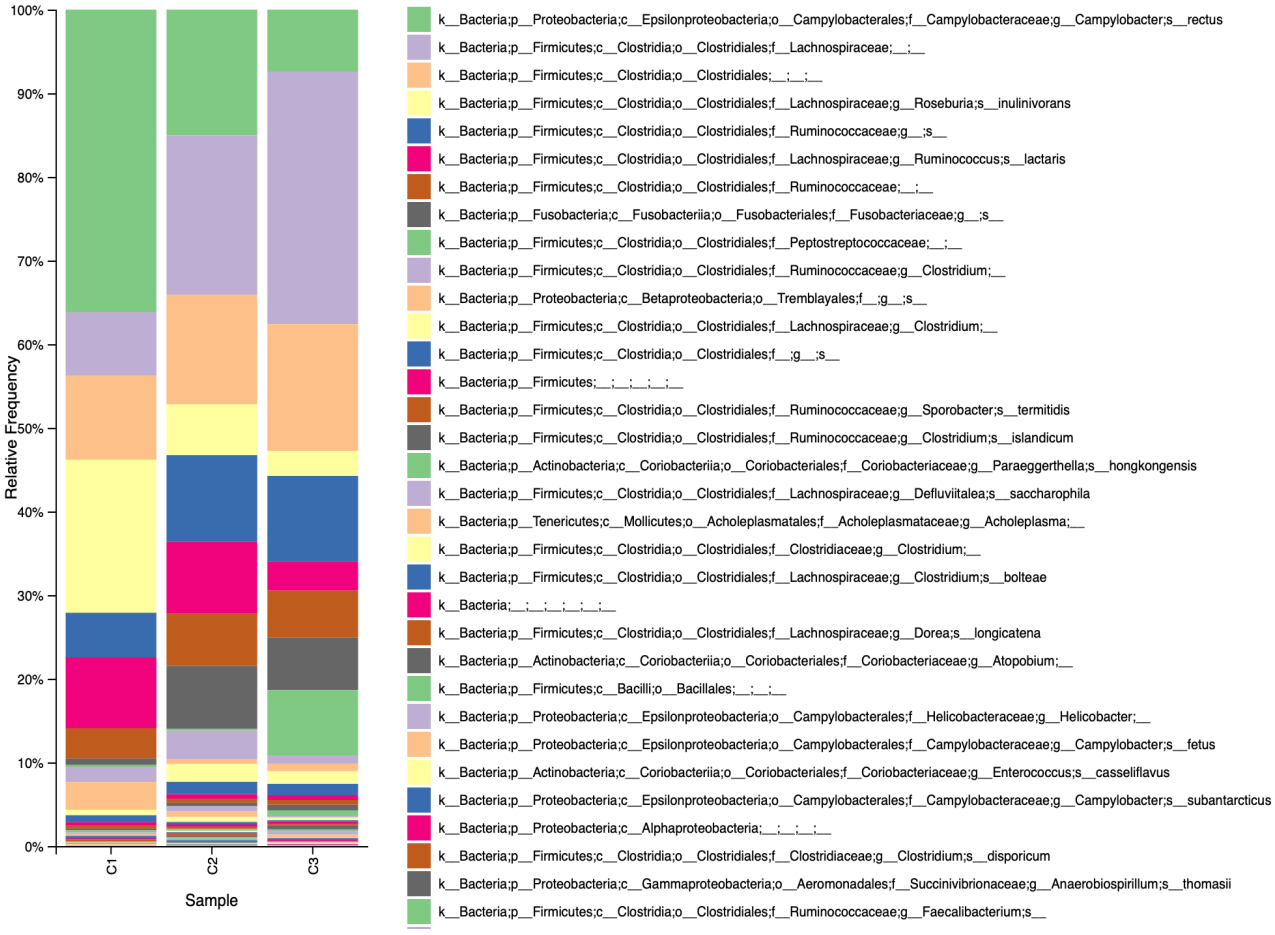

Figure 3. Species-level taxonomic composition of horse with IBD

DISCUSSION

IBD in horses is a disease with complex etiology, requiring long treatment and often with a poor prognosis. Although it is similar to many other gastrointestinal diseases, researchers have reported that it is formed as a result of an abnormal host inflammatory reaction with altered bacterial composition (Kalck 2009). Clinical and laboratory findings are described in the case report and show similarities with the studies (Kalck 2009; Kaikkonen et al., 2014; Boshuizen et al., 2018).

There were small differences between microbial populations at the different locations in the cecum, suggesting a different micro-environment. Although they were different, they related. Recent studies have reported that Firmicutes represent the largest phylum of the equine intestinal bacterial community ranging from 40% up to 90% in different compartments, including Clostridia and Bacilli (Dougal et al., 2013). In our study, microbiome is dominated by Firmicutes, in particular by Clostridia. It is natural that these obligate anaerobic bacteria

are abundant, especially in the intestinal flora. In view of the overall diversity of residing Proteobacteria, various functional activities can be assumed, which are not entirely known yet. In some studies, an overabundance is reported to be associated with inflammatory intestinal diseases and dysbiosis like colic in horses (Costa et al., 2017). In our study, significant part of the population is taken over by Proteobacteria. Additionally, foals with diarrhea have shown a less rich microbiome composition in comparison with healthy foals together with decreased abundances for Lachnospiraceae and Ruminococcaceae (Schoster et al., 2017). In our study, it was seen that Lachnospiraceae and Ruminococcaceae were found at a lower rate in the sample taken from the most hemorrhagic part of the cecum compared to the other parts. In this respect, our study was found to be related to the literature data.

Conflict of interest: The authors declared that there is no conflict of interest.

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