Coalesce Research Group

Online International Conference on

PHARMACEUTICS & NOVEL DRUG DELIVERY SYSTEMS

April 21-22, 2022 | Virtual Conference

Coalesce Research Group
33 Market Point Dr,
Greenville, SC 29607, USA

Contact Us:

Phone: +1-718-543-9362
Whatsapp: +1-716-320-1018

Scientific Program

Online International Conference on

Pharmaceutics & Novel Drug Delivery Systems

Thursday
April 21, 2022

Day 1 - April 21, 2022	
10:00 - 10:10	Introduction
Keynote Presentation	
10:10 - 10:40	Application of Nanocarrier Delivery Systems For Lung Delivery of Biopharmaceuticals **Imran Saleem,** Liverpool John Moores University in Liverpool, United kingdom
Oral Presentations	
10:40 - 11:05	A alternative Mouthwash Formulation for Oral Care: Co - delivering Quercetin and Mint Oil in Phospholipid Vesicles **Ines Castangia,** University of Cagliari, Italy
11:05 - 11:30	*Development of A Novel Approach of CAR Technology Towards Immunotherapy of Oral Cancer* **Sofia K. Georgiou - Siafis,** Aristotle University of Thessaloniki (A.U.Th.) and University of Thessaly, Greece
11:30 - 11:55	Pharmacological Targeting Of Alpha - Synuclein And Tppp/P25 In Parkinson's Disease: Challenges And Opportunities **Judit Ovádi,** Research Centre for Natural Sciences ELKH, Hungary
11:55 - 12:20	A Combination of Cytokine - Induced Killer Cells with Pd - 1 Blockade And Alk Inhibitors Showed Substantial Intrinsic Variability Across Non - Small Cell Lung Cancer Cell Lines **Yutao Li,** CIO University Hospital of Bonn, Germany
Keynote Presentation	
12:20 - 12:50	Strategic Trends, Current And Future Competitive Landscape In Biologics And Biosimilars(Follow - On Biologics) Drug Development In United States Of America And Emerging Markets **Yavuz Selim Sılay,** Istanbul Consulting Group Managing Partner, Turkey
Lunch 12:50 - 13:30	
Oral Presentations	
13:30 - 13:55	Innovative Colonic Drug Delivery Based on Bacteria Sensitive Polysaccharide Systems **Youness Karrout,** University of Lille, France
13:55 - 14:20	Phytochemical Peculiarities of Some Representatives of The Nepetoideae Burnett. Subfamily (Lamiaceae martinov family) As Sources of New Herbal Medicinal Products **Mariia Shanaida,** Horbachevsky Ternopil National Medical University of Ternopil, Ukraine
14:20 - 14:45	Is Placental Therapy A Significant Tool For Wound Healing Therapy **Madhu Gupta,** Delhi Pharmaceutical Sciences & Research University, India
14:45 - 15:10	Virtual Screening, Synthesis And Biological Evaluation Of Streptococcus Mutans Mediated Biofilm Inhibitors **Lubna Atta,** International Centre of Chemical and Biological Sciences University of Karachi, Pakistan
15:10 - 15:35	Nanobased Diagnosis And Critical Therapeutical Intervention To Identify The Severity Of Mucormycosis In Indian Covid 19 Patients **Subin Mary Zachariah,** AIMS Health Science Campus of Kochi, India
15:35 - 16:00	Quality by Design Based Formulation of Paracetamol Containing Fizzy Granules As Pediatric Alternates **Subh Naman,** Maharaja Ranjit Singh Punjab Technical University of Bathinda, India
End of Day 1	

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Friday
April 22, 2022

Day 2 - April 22, 2022

Oral Presentations

10:00 - 10:25	Development of Orodispersible Tablet Containing Peanut Allergen **Vidya Vswanad,** Amrita Vishwa Vidyapeetham of Kochi, India
10:25 - 10:50	Neuroprotection of Natural Products Against Neurodegenerative Disease **Noureddine Djebli,** Abdelhamid Ibn Badis - Mostaganem Universityof Algeria
10:50 - 11:15	Multivalent Polypeptide - Based Nanovaccine as Anti - Cancer Immunotherapeutic Strategy For Melanoma **Liane Moura,** University of Lisbon, Portugal
11:15 - 11:40	Effects Of Caffeine Ingestion On Psychomotor State And Oxidative Stress Markers After An 8 - Km Run Competition In Sleep - Deprived Recreational Runners **Amir Khcharem,** Sfax University, Tunisia
11:40 - 12:05	Ph Responsive Au - Bsa Encapsulated Exosomes For Cancer Diagnosis and Targeted Drug Delievry **Tanziela,** Southeast University, China
12:05 - 12:30	Adverse Events Following Immunization of Covid - 19 Vaccination Among Health Care Professionals In Ethiopia **Dufera Rikitu Terefa,** Wollega University, Ethiopia
12:30 - 12:55	Screening of antianxiety activity of gingerol on rodents **Chetan Kumar Dubey,** Career Point University, Kota, India
12:55 - 13:20	New Antibacterial Phenolic Compound Isolated From *Agelanthus Brunneus* (Loranthaceae) **Moifo Kuete Thomas Wieland,** University of Yaoundé, Cameroon

End of Day 2

Day-1
Keynote Presentations

APPLICATION OF NANOCARRIER DELIVERY SYSTEMS FOR LUNG DELIVERY OF BIOPHARMACEUTICALS

Imran Saleem

Liverpool John Moores University, School of Pharmacy & Biomolecular Sciences, UK

Abstract

The pulmonary route has been encouraged by the large surface area of the lungs with the thin epithelial membrane and dense vasculature making the lung delivery a suitable route of administration of biopharmaceuticals for local or systemic targets. Delivery of biopharmaceuticals (such as therapeutic proteins, vaccines, genes etc.) to the lungs has many challenges: for example, overcoming the biological barriers at sites of administration and action, recognition and elimination by immune system, stability of the drug. Nanoparticles provide platforms to overcome these challenges of biopharmaceuticals with better outcomes. However, nanoparticle formulation methods face many challenges and is a critical factor, which can affect their physicochemical properties on the pharmacokinetics or the therapeutic effects. We have developed polymer-based nanocarrier systems incorporating biopharmaceuticals (miRNA, vaccine candidates, antimicrobial peptides) using conventional and microfluidic methods. Furthermore, we have incorporated these into dry powder microcarriers using spraydrying technology suitable for lung delivery. We have managed to retain biological stability and activity of biopharmaceuticals, and nanocarrier size following redispersion after spray drying, and performed aerosolisation studies demonstrating suitability for lung delivery. We have applied the technology in the treatment and management of local lung diseases (COPD, lung infections) and vaccination.

Biography

Imran Saleem is a professor in nanomedicine within the School of Pharmacy & Biomolecular Sciences, Liverpool John Moores University, UK. His research is aimed at developing novel delivery systems for targeting therapeutic agents to their site of action, with particular emphasis on lung diseases via pulmonary delivery. He has over 20 years' experience in the area of micro/nanoparticle formulation and drug delivery systems, and has published extensively in peer-reviewed journals, conference abstracts and book chapters. His research group is focused on the design and development of nanocarriers for delivery of biomacromolecules including, genes, peptides, vaccines and drugs.

STRATEGIC TRENDS, CURRENT AND FUTURE COMPETITIVE LANDSCAPE IN BIOLOGICS AND BIOSIMILARS(FOLLOW-ON BIOLOGICS) DRUG DEVELOPMENT IN UNITED STATES OF AMERICA AND EMERGING MARKETS

Yavuz Selim Sılay

Co-Founder & CEO, Fonkolay Chairman, Istanbul Consulting Group Managing Partner, Turkey

Abstract

A presence in Biologics and biosimilars has become a strategic priority for nearly all of the world's leading and emerging pharmaceutical companies. An approximate snapshot of biologic drug sales for top 10 pharmaceutical companies during last five years and future five years will be discussed

Biosimilars or Follow-on biologics are similar terms used to describe officially approved subsequent versions of innovator biopharmaceutical products made by a different sponsor following patent and exclusivity expiry on the innovator product. The following topics during this presentation will be briefly discussed.

- Historical growth of the biologics market
- Leading players
- Changes to the competitive landscape
- Outlook for the biologics market
- Leading players

Subsequently:

Some examples of biosimilars and how it may affect the biologics will be briefly explained.

While the FDA designates interchangeability, states control drug substitution laws. Once an interchangeability designation is acquired, a biosimilar may be substituted for the reference product by the pharmacist at the retail or specialty pharmacy without the intervention of the prescriber in states that have approved legislation or regulation establishing state standards for biosimilar substitution.

- Products classified by technology according to following definitions:
- Biologic (e.g. monoclonal antibody, antibody derivative, recombinant protein, cell therapy, DNA/ RNA therapy, gene therapy)
- Small molecule
- Vaccine
- Other (e.g. synthetic peptide)

Biologics generally exhibit high molecular complexity, and may be quite sensitive to manufacturing process changes,unlike the more common small-molecule drugs.The follow-on manufacturer may not have access to the originator's molecular clone and original cell bank, nor to the exact fermentation and purification process, nor to the active drug substance. They do have access to the commercialized innovator product. Differences in impurities and/or breakdown products may have serious health

implications. This has created a concern that copies of biologics might perform differently than the original branded version of the product. While summarizing above dynamics, the current status and most recent developments in biologics and biosimilar (follow-on) drug development in United States of America and global trends will be explained.

Biography

Yavuz Selim Sılay, graduated from Ankara University Faculty of Medicine in 2000, worked as an academician and research director at Baylor College of Medicine for 5 years. He is one of the top professional working in big pharmaceutical companies in USA. Currently working as Senior Medical Affairs Director, Neuroscience in New Jersey & Ankara on behalf of Global Allied Pharmaceutical as well as Chairman Istanbul Consulting Group providing management consulting services in telecommunication, health, energy, and Finance.

Day-1
Oral Presentations

A ALTERNATIVE MOUTHWASH FORMULATION FOR ORAL CARE: CO-DELIVERING QUERCETIN AND MINT OIL IN PHOSPHOLIPID VESICLES

Ines Castangia

University of Cagliari, Italy

Abstract

The aim of this work was the simultaneous load of quercetin and mint essential oil in phospho- lipid vesicles specifically tailored to obtain an antibacterial and antioxidant mouthwashes. The vesicles were prepared using soy lecithin and Tween 80 as bilayer components and phosphate buffer solution (33%), propylene glycol (33%) and ethanol (33%) as dispersing phase. Cryogenic-transmission electron microscopy analyses confirmed the formation of unilamellar, spherical and regularly shaped vesicles. Similarly, light scattering results disclosed that the size of the vesicles increased as the concentration of mint oil also increased, but in the same time the high amount of mint ensure a high stability, as the size of these vesicles remained unchanged during 12 months of storage. All tested formulations were high biocompatible with epithelia cells, and capable of counteracting oxidative cell' damages caused by hydrogen peroxide. Moreover, the vesicles prepared with the higher concentration of mint oil inhibited the proliferation of the cariogenic *Streptococcus mutans* (*S. mutans*) and *Lactobacillus acidophilus* (*L. acidophilus*).

Biography

Ines Castangia has completed his PhD in Dept. of Life and Environmental Sciences of the University of Cagliari, titled: new lipid nanovesicles as topical delivery systems for anti-inflammatory drugs. The research activity has been carried out in Drug Sciences Section since 2010. The main research topics are as follows: Formulation, preparation, characterization, and evaluation (*in vitro* and *in vivo*) of the anti-inflammatory, antibacterial and antifungal efficacy of innovative vesicular carriers carrying natural and/or synthetic substances and intended for topical administration. Formulation, preparation, characterization of specifically modified phospholipid vesicles (liposomes, glycerosomes, polymer-liposomes, hyalurosomes) for the dermal and transdermal delivery of natural and/or synthetic drugs. Evaluation (in vitro and in vivo) of the antioxidant, antibacterial, anti-inflammatory, and regenerative efficacy. Formulation, preparation, characterization and evaluation of the antioxidant and prebiotic activity of nutriosomes carrying natural or synthetic active ingredients intended for oral administration for intestinal and/or systemic delivery. In vivo evaluation of the anti-inflammatory and antioxidant efficacy of nanoparticle formulations using specific tests on animal models (rats and mice). Scientific Outputs 26peer-reviewed papers, 18 h index, 870 citations (Scopus), 1 European patent.

DEVELOPMENT OF A NOVEL APPROACH OF CAR TECHNOLOGY TOWARDS IMMUNOTHERAPY OF ORAL CANCER

Sofia K. Georgiou-Siafis[1,2], Androulla N. Miliotou[1,3], Charikleia Ntenti[1], Ioannis S. Pappas[2] and Lefkothea C. Papadopoulou[1]

[1]*Aristotle University of Thessaloniki, Greece*
[2]*University of Thessaly, Greece*
[3]*KES College, Cyprus*

Abstract

Chimeric Antigen Receptor (CAR) technology is a promising cell-based cancer immunotherapy, employing T-cells or Natural Killer (NK) cells, genetically engineered to express CAR receptors against tumor antigens. Oral cancer is highly resistant to conventional therapies and characterized by over-expression of ErbB proteins. ErbB receptors recognition via the ErbB ligand, T1E, fused to a CD28 plus CD3ζ endodomain, results in immune cells-mediated cytotoxicity in various types of cancer. Present work aims at development of CAR-NK-92 cells, through intracellular transduction of *in vitro* transcribed (IVT)-mRNAs, coding the CAR sequence and conjugated to a Protein Transduction Domain (PTD), via our novel, conjugation reaction (PCT/GR2020/000059). CAR- NK-92 cells aim to recognize, target and lyse human tongue squamous carcinoma cells (HSC-3). The CAR sequence was cloned into pGEM vector for *in vitro* transcription into mRNAs, which were then covalently conjugated to PTD. IVT-mRNAs and PTD-IVT-mRNAs were incubated in serum-free medium for 1h at 37oC. PTD-IVT-mRNAs were significantly protected to RNase action. PTD-IVT-mRNAs were then incubated with NK-92 cells for different time intervals (2-72h). RT- PCR depicted that PTD-IVT-mRNAs successfully transduced into NK-92 cells since 2h of incubation, and thereafter. PTD-mediated IVT-mRNA transduction was not accompanied by cell death. Western blot analysis against the CD3ζ epitope showed that PTD-IVT-mRNAs were expressed into the corresponding CAR molecules (35kDa) relatively rapid, persisted thereafter. Subcellular fractionation depicted that CAR molecules located to cellular membranes and cytoplasm, as well. Different co-incubation schemes, employing CAR-engineered NK-92 as the effectors and HSC-3 as the target cells, are now taking place. In conclusion, PTD Technology was successfully exploited for the rapid, efficient transduction and translation of the stable IVT-mRNA, encoding the CAR molecule into NK-92 cells. CAR-NK-92 cells have shown efficient cytotoxicity over HSC-3 cancer cells, leading to encouraging results for the application of our novel PTD-IVT- mRNA delivery platform for CAR immunotherapy.

Biography

Dr. Sofia K. Georgiou-Siafis has completed her PhD in Pharmacology by Aristotle University of Thessaloniki (A.U.Th.) and she is performing post-doctoral studies in A.U.Th. and University of Thessaly. She is a Biochemist and Biotechnologist and hold a master in Biotechnology and Molecular Diagnostics. Among her research interests are CAR technology, free heme/hemin detoxification and cytotoxicity mechanisms. She has worked as an Academic Fellow in Faculty of Veterinary Medicine, Senior Lecturer in KES College, and Programme Leader in Biomedical Sciences (Metropolitan College). She has published several papers in peer-review journals in the field of Biochemical Pharmacology, Cellular Physiology and others, as well as she has several participations in conferences (FEBS, etc.). Deposit (PubChem) of the structures of nine (9) novel chemical structures of biological significance is pending. She was awarded fellowships from Onassis, Bodossakis Foundations as well as Foundation for Education and European Culture.

PHARMACOLOGICAL TARGETING OF ALPHA-SYNUCLEIN AND TPPP/P25 IN PARKINSON'S DISEASE: CHALLENGES AND OPPORTUNITIES

Judit Ovádi

Research Centre for Natural Sciences ELKH, Hungary

Abstract

With the aging of the population, Parkinson's disease (PD) poses a serious socio-economic problem; there is no effective treatment so far for the disease. The hallmarks of PD and other synucleinopathies are the disordered alpha-synuclein (SYN) and Tubulin Polymerization Promoting Protein (TPPP/p25). These proteins have neomorphic moonlighting characteristics by displaying both physiological and pathological functions. Physiologically SYN is involved in neuronal plasticity modulation and synaptic vesicle pool maintenance, while TPPP/p25 regulates the dynamics/stability of the microtubules and is crucial for oligodendrocyte (OLG) differentiation. In a healthy brain, SYN and TPPP/p25 occur predominantly in neurons and OLGs, respectively; however, in the cases of PD and multiple system atrophy, these hallmark proteins are co-enriched and co-localized in both cell types. The pathomechanisms of these diseases are largely unknown; the fatal species are the small, soluble homo- and hetero-associations of SYN, the aggregates/inclusions are formed at the late stage of the diseases. However, both SYN and TPPP/p25 with their high conformational plasticity and chameleon feature are challenging drug targets. Classically, the discovery of small molecules for use as drugs entails targeting individual proteins rather than targeting protein-protein interactions. Nevertheless, we have established a new strategy based upon the validation of the contact surface of SYN-TPPP/p25 associations as a specific drug target, which may overcome the difficulties and limitations and specifically target the homo- and hetero-associations of SYN. This innovative strategy could permit the development of anti-Parkinson drugs with unique specificity and efficacy by targeting the interface of the SYN-TPPP/p25 complex.

Biography

Judit Ovádi is the head of the research team, now as a professor emerita in the Research Centre for Natural Sciences. Dr. Judit Olah is a senior scientist working in the same research team for decades. The objective of their major research is related to the multiple regulatory function of the cytoskeletal microtubule network, and its involvement in the etiology of parkinsonism. They have worked in foreign universities over the world for short and long times and have been invited speakers in international conferences within Europe, Far-East and USA.

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A COMBINATION OF CYTOKINE-INDUCED KILLER CELLS WITH PD-1 BLOCKADE AND ALK INHIBITORS SHOWED SUBSTANTIAL INTRINSIC VARIABILITY ACROSS NON-SMALL CELL LUNG CANCER CELL LINES

Yutao Li[1], Amit Shama[1,2], Xiaolong Wu[1], Hans Weiher[3], Dirk Skowasch[2], Markus Essler[2] and Ingo G. H. Schmidt-Wolf [1]

[1]CIO Bonn, University Hospital Bonn, Germany
[2]University Hospital Bonn, Germany
[3]Bonn-Rhein-Sieg University of Applied Sciences, Germany

Abstract

Background: Cancer heterogeneity poses a serious challenge concerning the toxicity and adverse effects of therapeutic inhibitors, especially when it comes to combinatorial therapies that involve multiple targeted inhibitors. In particular, in non-small cell lung cancer (NSCLC), a number of studies have reported synergistic effects of drug combinations in the preclinical models, while they were only partially successful in the clinical setup, suggesting those alternative clinical strategies (with genetic background and immune response) should be considered. Herein, we investigated the antitumor effect of cytokine-induced killer (CIK) cells in combination with nivolumab and crizotinib in vitro on NSCLC cell lines.

Methods: We co-cultured the three genetically different NSCLC cell lines NCI-H2228 (EML4- ALK), A549 (KRAS mutation), and HCC-78 (ROS1 rearrangement) with/without nivolumab (PD- 1 inhibitor) and crizotinib (ALK inhibitor). Additionally, we profiled the variability of surface expression multiple immune checkpoints, the concentration of absolute dead cells, intracellular granzyme B on CIK cells using flow cytometry as well as RT-qPCR. ELISA and western blot were performed to verify the activation of CIK cells.

Results: Our analysis showed that, a) nivolumab significantly weakened PD-1 surface expression on CIK cells without impacting other immune checkpoints or PD-1 mRNA expression, b) this combination strategy showed an effective response on cell viability, IFN-γ production and intracellular release of granzyme B in $CD3^+$ $CD56^+$ CIK cells, but solely in NCI-H2228, c) the intrinsic expression of Fas ligand (FasL) as a T-cell activation marker in CIK cells was upregulated by this additive effect. d) nivolumab induced Foxp3 expression in $CD4^+CD25^+$ subpopulation of CIK cells significantly increased. Taken together, we could show that CIK cells in combination with crizotinib and nivolumab can enhance the anti-tumor immune response through FasL activation, leading to increased IFN-γ and granzyme B, but only in NCI-H2228 cells with EML4- ALK rearrangement. Therefore, we hypothesize that CIK therapy may be a potential alternative in NSCLC patients harboring EML4-ALK rearrangement, in addition, we support the idea that combination therapies offer significant potential when they are optimized on a patient-by-patient basis.

Biography

Yutao Li is a MD/Ph.D. candidate in the Department of Integrated Oncology (CIO), University Hospital Bonn. Her recent main research work includes cytokine-induced killer cells (CIK)-mediated immune therapy combination with PD-1/PD-L1 inhibitor targeting non-small lung cancer, lymphoma. CIK cells are harvested from PBMC and expanded in vitro, composed of $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^+CD56^+$ subsets. $CD3^+CD56^+$ subsets of CIKs have cytotoxic dual function of effector T cells and NK cells. Multiple clinical trials have been conducted effectively, however, CIK from healthy individuals show differential susceptibility to PD-1/PD-L1 inhibitor. Her recent work is to better understand the PD-1/PD-L1 resistance mechanism. She also conducts research on primary macrophages` inflammation and the impact of long interspersed nuclear (LINE-1) elements on tumorigenesis.

Yutao Li received her bachelor`s degree in clinical medicine degree from Capital University of Medical Sciences in Peking, China. Her professional career started in Peking Union Medical College Hospital in China as a radiologist. She received a master`s degree in intern medicine in the Department of Respiratory Medicine, Peking University, where she was first involved in research. She studied the role of NF-κB and AP-1 in cigarette smoke-induced chronic inflammation and emphysema in murine model. She was first person who built chronic obstructive pulmonary disease mouse model in her department. She also had experience as a technician in the monoclonal antibody production in a bio-company.

INNOVATIVE COLONIC DRUG DELIVERY BASED ON BACTERIA SENSITIVE POLYSACCHARIDE SYSTEMS

Youness Karrout

University of Lille, France

Abstract

The site specific delivery of drugs to the colon can be highly advantageous for various applications, including: (i) the local treatment of inflammatory bowel diseases (IBD), and (ii) the oral administration of protein drugs, which are to be absorbed into the blood stream. In the first case, premature drug release into the stomach is likely to lead to complete and rapid drug absorption into the systemic circulation. Thus, the risk of undesired side effects can be considerable, and at the same time the resulting drug concentrations at the site of action (in the colon) are low, leading to poor therapeutic efficacies. In the second case, fragile protein drugs need to be effectively protected against the low pH and enzymatic degradation within the upper gastro intestinal tract (GIT). Thus, in both cases, premature release into the contents of the stomach and small intestine must be avoided. In contrast, once the colon is reached, the drug should be released (in a time-controlled manner) to allow for local drug action in the case of inflammatory bowel diseases or to allow for drug absorption into the blood stream in the case of protein drugs with systemic effects. Thus, this novel type of colon targeting system based on polysaccharide is adapted to the pathophysiology of the patient. It is to emphasize that polysaccharides used in this novel technology should be resistant in the upper GIT but once arriving into the colon they should be degraded by bacterial enzymes located only in the colon in order to trigger drug release at the site of action (inflamed area by IBD patients).

Biography

Youness Karrout studied pharmacy and did his Ph.D. in Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology at the Freie Universitaet Berlin. After his Pharmacist "diploma", he worked as a Pharmacist in retail Pharmacy, Pharmaceutical Industry as well as in medical Centre (Microbiology). Since 2009, he is Associate Professor in Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology at the University of Lille. He was visiting scientist at the university of New Mexico (USA, 2012) in the research group of Prof. L. Felton. He obtained 2013 his postdoctoral lecture qualification on Pharmaceutics and Pharmaceutical Technology at the University of Lille. His research focuses on controlled drug delivery systems, in particular on the development of innovative drug delivery targeting the distal part of GIT. So far, he published his work in more than 25 articles in peer-reviewed, international scientific journals, 4 patents, 82 poster presentations and 25 oral presentations at national and international scientific meetings.

PHYTOCHEMICAL PECULIARITIES OF SOME REPRESENTATIVES OF THE *NEPETOIDEAE* BURNETT. SUBFAMILY (*Lamiaceae Martinov* FAMILY) AS SOURCES OF NEW HERBAL MEDICINAL PRODUCTS

Mariia Shanaida

Horbachevsky Ternopil National Medical University of Ternopil, Ukraine

Abstract

The comprehensive phytochemical investigation of some unofficial medicinal plants belonging to the genera Basil (*Ocimum* L.), Dragonhead (*Dracocephalum* L.), Hyssop (*Hyssopus* L.), Giant hyssop (*Agastache* Gronov, syn. *Lophanthus* Adans.), Bee balm (*Monarda* L.) and Savory (*Satureja* L.) (subfamily Nepetoideae Burnett., family *Lamiaceae* Martinov) gave the possibilities to develop new herbal medicinal products with certain pharmacological activities. The standardization parameters for the raw materials and obtained medicinal products were established. The analysis of the dependence of biological activity of the developed herbal medicinal products on presence and content of their main biologically active compounds was conducted.

Biography

Mariia Shanaida Current Position: D.Sc. (Pharmacy, 2021), Ph.D. (Biology, 2002) Associate Professor at Pharmacognosy and Medical Botany Department, I. Horbachevsky Ternopil National Medical University (Ukraine). Scientific Interests include Pharmacognosy, phytochemistry, pharmacological activity of herbal medicinal products. Professional Skills include Essential oils and polyphenols (isolation, investigation), in vitro evaluation of the antioxidant and antimicrobial properties of essential oils and herbal extracts; *in vivo* study the sedative and anti-inflammatory activities of herbal medicinal products.

IS PLACENTAL THERAPY A SIGNIFICANT TOOL FOR WOUND HEALING THERAPY

Madhu Gupta

Delhi Pharmaceutical Ssciences & Research Uuniversity, India

Abstract

The placenta maintains and regulates the growth of foetus and consists of various biologically active nutrients such as cytomedines, vitamins, trace elements, amino acids, peptides, growth factors and other biologically active constituents. The therapeutic effectiveness of the placenta can be well defined with respect to several biochemical mechanisms of various components present in it. The placental extract derived from biomedical wastes has also shown a great potential for treatment of various diseases. Placental therapy has been reported specifically to have potent action on treatment of diseases and tissue regeneration. Placental bioactive components and their multi-targeting identity prompted us to compile the précised information on placental extract products. However, some findings are needed to be explored by scientific community to prove their clinical potential with significant statistical validation. In the light of available information and the usefulness of the placental extract, it is necessary that the formulations of various desirable properties may be developed to meet the clinical requirements in several treatment paradigms. It is also a matter of exploration that the short- and long-term adverse effects to be explored by advanced scientific techniques.

Biography

Madhu Gupta is working as an Associate Professor in Delhi Pharmaceutical Science and Research University, New Delhi. She has research experience pertaining to drug delivery to nanoformulations for magical molecule delivery, bioligands for targeting of bioactives and drug moiety, biopolymers, cancer nanomedicine as well as topical delivery. She has over 80 research publications to her credit published in journals of high scientific impact and contributed 30 chapters in various renowned books with h index 20 and more than 1000 citations.. She has the recipient of Research Excellence of the Year 2020, Youth Education Icon of the Year 2018, Young Scientist Award, Best Administrative Service Award, IDMA-G.P. Nair award and Prof. C.S. Chauhan award, BioAsia Innovation Award – 2012, Grace India awards. She has also filed the PCT patent for effective wound healing therapy.

VIRTUAL SCREENING, SYNTHESIS AND BIOLOGICAL EVALUATION OF *STREPTOCOCCUS MUTANS* MEDIATED BIOFILM INHIBITORS

Lubna Atta

H.E.J Research Institute of Chemistry, International Centre of Chemical and Biological Sciences, University of Karachi, Pakistan

Abstract

Dental caries, a global oral health concern, is a biofilm-mediated disease. *Streptococcus mutans*, the most prevalent oral microbiota, produces extracellular enzymes, including glycosyltransferases responsible for sucrose polymerization. In bacterial communities, the biofilm matrix confers resistance to host immune responses and antibiotics. Thus, in cases of chronic dental caries, inhibiting bacterial biofilm assembly should prevent demineralization of tooth enamel, thereby preventing tooth decay. A high throughput screening was performed in the present study to identify small molecule inhibitors of *S. mutans* glycosyltransferases. Multiple pharmacophore models were developed, validated with multiple datasets, and used for virtual screening against large chemical databases. Over 3000 drug-like hits were obtained that were analyzed to explore their binding mode. Finally, six compounds that showed good binding affinities were further analyzed for ADME (absorption, distribution, metabolism, and excretion) properties. The obtained *in silico* hits were evaluated for *in vitro* biofilm formation. The compounds displayed excellent antibiofilm activities with minimum inhibitory concentration (MIC) values of 15.26–250 µg/mL.

Biography

My name is Lubna Atta, born in Karachi, Sindh, Pakistan. I have received my early education from White House Grammar School (W.H.G.S.) and completed my S.S.C. examination in 2009 from the same school. I completed my H.S.C. education from Begum Amna Majeed Malik (B.A.M.M.) P.E.C.H.S. Government College for Women in the year 2011. I completed my M.Phil from H.E.J Research Institute, International Center of Chemical and Biological Sciences, as a M.Phil. student. My research in the field of Computational Drug Design. I won poster prize at ISNPC-14 title "Molecular Investigation of calcium binding domain of *Streptococcus mutans*.

NANOBASED DIAGNOSIS AND CRITICAL THERAPEUTICAL INTERVENTION TO IDENTIFY THE SEVERITY OF MUCORMYCOSIS IN INDIAN COVID 19 PATIENTS

Subin Mary Zachariah

Amrita Vishwa Vidyapeetham, AIMS Health Science Campus of Kochi, India

Abstract

The COVID-19 infection caused by the new SARS-CoV-2 virus has been linked to a broad spectrum of symptoms, from a mild cough to life-threatening pneumonia. As we learn more about this unusual COVID-19 epidemic, new issues are emerging and being reported daily. Mucormycosis, causes severe fungal illness to individuals with a weakened immune system. It is a devastating fungal infection, and the most frequent kind is the rhino cerebral type. As a devastating second wave of COVID-19 sweeps India, doctors report several instances involving a strange illness-sometimes known as the "black fungus"-among returning and recovered COVID-19 patients. This paper analyzes the existing statistical data to address the severity of prevalence and further notes the nano-based diagnostic parameters, clinical presentations, its connection with other conditions like diabetes, hypertension, and GI disorders, and the importance of anti-fungal therapy in treating the same. Anti-fungal therapies, as well as surgical interventions, are currently used for the treatment of the disease. Proper and timely diagnosis is necessary, along with the reduction in the spread of COVID-19. From the review, it was found that timely pharmacologic interventions and early diagnosis by using a nano-based diagnostic kit can help control the disease. This paper provides novel information about the nanotechnology approaches such as fungal detection biosensors, nucleic acids-based testing, point-of-care tests, and galactomannans detection, in the diagnosis of mucormycosis, and thereby reinforces the need for further research on the topic.

Biography

Subin Mary Zachariah is working as Associate Professor in the Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, AIMS Health Science Campus,Kochi-682041,Kerala,India. She is having around 20 years of teaching experience and 13 years of research experience in the field of pharmaceutical education and research. She has published more than 55 papers in several reputed national and international journals with an average impact factor of 5.

QUALITY BY DESIGN BASED FORMULATION OF PARACETAMOL CONTAINING FIZZY GRANULES AS PEDIATRIC ALTERNATES

Subh Naman

Maharaja Ranjit Singh Punjab Technical University, India

Abstract

Present day's conventional pediatrics doses forms are not very attractive towards the children. Due to various reasons such as larger size, bitter taste etc. pediatrics patients reject the present conventional doses form in many cases. So, there is need for development of some attractive and effective dosage form for children. In Fizzy granules, medicament is given in form of effervescence beverage with variety of flavor which increases the chance of acceptance of dosage form and have required therapeutic effect. In present study fizzy granules of paracetamol as pilot study are prepared by non-aqueous wet granulation method. Concept of Quality by Design (QbD) including the total quality risk management has been applied while formulation of fizzy granules. Various important parameters such as Ishikawa fishbone diagram, risk hazard assessment techniques have been included for developing a formulation that is more favorable for industrial acceptance and also have great chance of acceptability at target patients (Pediatrics). Critical Process Parameters, Critical Quality Attributes & Critical Material attributes has been identified for fizzy formulation. Placket Burman design for screening and Central composite design has been applied for the optimization of various critical factors that can affect the quality of our formulation. 2D and 3D response surface plots have generated for depicting the relationship among the various selected factors. Developed QbD model were verified by comparing the expected results to the results at laboratory scale and results were found to be satisfactory.

In conclusion, this study can provide an alternate to current available dosage form to pediatrics as well as extended knowledge for implementation of concept of QbD during the formulation of a dosage form.

Biography

Subh Naman, is currently pursuing PhD at Department of Pharmaceutical Sciences and Technology, MRSPTU Bathinda under the guidance of Prof. Ashish Baldi. He is also working as Senior Research Fellow in SERB, Department of Science (DST), Government of India funded project. He published several quality articles in national and international journal. He has obtained patent in design modification of Pharmaceutical Soxhlet apparatus. With several best presentation and research award, he is presently working on quality certification of spices using machine learning.

Day-2
Oral Presentations

DEVELOPMENT OF ORODISPERSIBLE TABLET CONTAINING PEANUT ALLERGEN

Vidya Vswanad

Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, India

Abstract

Allergen immunotherapy by orodispersible sublingual tablets has immense potential in the treatment of asthma and allergic diseases. Peanut is one of the most common allergen and can triggers the allergic reactions at very low doses of allergen. Ara h1, Ara h2 and Ara h3 are the major allergens in peanut. Sublingual tablet containing allergenic extract of peanut is an alternative to subcutaneous immunotherapy. As subcutaneous administration results in repeated systemic reactions, oral administration of allergen is of great interest in inducing the allergen specific tolerance. It is well known that that oromucosal contact of the allergen induces tolerant responses by T cells, dendritic cells, langherhans cells. We hypothesis that the SLIT tablet of peanut extract may be an alternative too. In the present study we developed sublingual orodispersible tablet containing peanut extract in gradually increasing doses starting from micrograms upto 2 mg. The efficacy of the tablet is dose dependent which includes a sensitisation, maintenance and disease modifying effect.

Biography

Vidya Viswanad has completed her PhD in Pharmacy at Vinayaka Missions Univesity. She is currently working as Associate Professor in Department of Pharmaceutics at Amrita School of Pharmacy.She has more than 20 national and International papers to her credit.

NEUROPROTECTION OF NATURAL PRODUCTS AGAINST NEURODEGENERATIVE DISEASE

Noureddine Djebli

Abdelhamid Ibn Badis-Mostaganem-University, Algeria

Abstract

Alzheimer's disease is a progressive neurodegenerative disease characterized by cognitive and functional decline, leading to behavioral disturbances and progressive and irreversible loss of cognitive functions, including memory, with installation of a lethal course of dementia. It is characterized by progressive cortical atrophy due to death of cortical neurons (and other territories). To date, few pharmacological solutions are available in the treatment of Alzheimer's disease. Although some drugs can provide symptomatic improvement, they are not very effective and they do not work to slow the progression of the disease. Among the medicinal plants widely used in herbal medicine, we have chosen the young shoot of wheat Triticum aestivum. The aim of our work is to characterize the effect of a hypothetical treatment of a model of Alzheimer's disease in adult mice after eleven weeks of chronic treatment with ingestion of AlCl3 (10 mg/kg) and D -galactose by intraperitoneal (120 mg/kg) grass Triticum eastivum on memory by assessing the effects of this herb on spatial memory performance in the test twice H, however, the potential effects on anxiety were tested in an elevated plus maze, those in a depression on the forced swimming test. The results show an attenuation of the behavioral disturbance and impaired spatial memory caused by AlCl3 and D-galactose, after treatment with wheatgrass. In addition, histological examination of the cerebral cortex confirms cognitive attenuation.

Biography

Noureddine Djebli has completed his PhD in Neuro-Biochemistry by Es-senia ORAN University and postdoctoral studies from University of Mostaganem. He has worked as professor of Pharmacology and Pharmacognosy at Abdelhamid ibn badis-Mostaganem- University, Algeria. He has published more than 80 papers in reputed journals and has been serving as a director of pharmacognosy & Api-Phytotherapy laboratory and Chairman of Master-pharmaco-Toxicology–Mostaganem University.

MULTIVALENT POLYPEPTIDE-BASED NANOVACCINE AS ANTI-CANCER IMMUNOTHERAPEUTIC STRATEGY FOR MELANOMA

Liane Moura

University of Lisbon, Portugal

Abstract

Introduction: Melanoma is the most aggressive skin cancer and novel treatments are needed. Branched polypeptides (BP) exhibit advanced engineered complexity and have unique structural properties to be employed as drug delivery systems. As BP activate immune cells, they constitute potential nanoplatforms to modulate the release of tumor associated antigens and adjuvants. This work aims to evaluate in vivo immune-mediated anti-tumor effect induced by BP delivering melanoma-associated peptide antigens Major Histocompatibility Complex (MHC) class I (MHCI) and MHC class II (MHCII) ([pept-MHCI-pept-MHCII-BP]).

Materials and Methods: BP were synthesized and conjugated with the MHCI and MHCII- peptide ([pept-MHCI-pept-MHCII-BP]). To evaluate the conjugate effect on melanoma tumor growth, B16F10 cells were implanted subcutaneously into 8-week-old C57BL/6J mice. At day 7, mice were injected with two doses (1-week apart) of PBS, free antigens/adjuvants (MHCI/MHCII antigens + Toll-like receptor ligands (CpG and Poly I:C) in solution, [pept-MHCI-BP], [pept- MHCII-BP], and [pept-MHCI-pept-MHCII-BP] mixed with adjuvants. Mice weight and tumor growth were followed regularly. At day 21, mice were sacrificed, and tumors were collected. The expression of functional markers of different subtypes of tumor-infiltrating immune cells was quantified by FACS.

Results: A significant reduction on the tumor size of [pept-MHCI-pept-MHCII-BP]-treated mice was observed when compared with the other groups, which presented significantly increased levels of tumor-infiltrating immune cells (CD8, CD4, CTL) and pro-inflammatory cytokines (IFN- γ, TNF-α, IL-2).

Conclusion: [pept-MHCI-pept-MHCII-BP] is a promising nanoconjugate for TAA delivery and constitutes an effective anti-tumor immune strategy able to overcome tumor growth.

Biography

Liane Moura obtained her Ph.D. degree in 2013 (University of Coimbra). Liane is currently a Researcher Associate in BioNanoScience group at Faculty of Pharmacy, Universidade de Lisboa, Portugal and she is focused on the characterization of the immune modulatory effect of nanovaccines in different cancer models. Additionally, Liane is interested in the development of advanced nanocarriers, such as polypeptide-based nanotechnologies, as immunotherapeutic approaches able to conjugate and deliver different active biomolecules to specific cells, as immune and cancer cells. Liane is first author of one invited book chapter and various peer-reviewed international papers. She has 1510 citations and an h-index of 15 (by Scopus).

EFFECTS OF CAFFEINE INGESTION ON PSYCHOMOTOR STATE AND OXIDATIVE STRESS MARKERS AFTER AN 8-KM RUN COMPETITION IN SLEEP-DEPRIVED RECREATIONAL RUNNERS

Amir Khcharem

Sfax University, Tunisia

Abstract

Background: Various pharmacological aids are used to counteract the detrimental effects of sleep loss on exercise, such as caffeine. However, studies examining the possible protective impact of caffeine against reactive oxygen species (ROS) at the level of cell and tissue culture as well as *in vivo* during exercise are very limited and showed controversial results.

Objective: The current study aimed to investigate the effects of caffeine intake on psychomotor state and oxidative stress markers after an endurance race following 26 hours of sleep deprivation.

Methods: Ten recreational runners performed four test sessions in a randomized order at 09:00 h after placebo or 5 mg/kg of caffeine ingestion during a reference night (RN) (bedtime: from 22:30 h to 07:00 h) or a night of total sleep deprivation (TSD). At each session, they performed an 8-km running competition around a 400 m outdoor athletic track. Before and after the race, blood samples were taken and psychomotor tests were performed. Concentrations of oxidative stress markers (e.g. glutathione peroxidase (GPX) and superoxide dismutase (SOD)) were quantified and ratings of psychomotor state (e.g. feelings of good-being (FG), stress (FS), and fatigue (FF)) were measured.

Results: In comparison with RN, FF increased while GPX, SOD, and FG decreased after the TSD condition. As compared to placebo, caffeine ingestion decreased FS by 12.5% following RN, reduced FF by 16.7%, raised FG by 25%, and did not affect levels of oxidative stress markers following TSD.

Conclusion: Caffeine is an effective strategy to mitigate psychomotor adverse effects induced by TSD without being pro-oxidant during endurance running competition.

Biography

A Doctor in biological sciences applied to physical and sporting activities. Also a teacher of biological sciences in the High Institute of Sport and Physical Education (University of Sfax-Tunisia). I became a teacher of physical education and sports in 2004 and I obtained a Master's degree in biological sciences in 2006. I have published three articles in impacted journals in the field of pharmacology in connection with physical activity. I am dynamic, polyvalent, curious, and motivated in work. I have good skills and a good analytical mind to approach the field of scientific research. In addition, I have a good mastery of the sciences of physical activities in relation to the biological sciences and current research trends in this field.

PH RESPONSIVE AU-BSA ENCAPSULATED EXOSOMES FOR CANCER DIAGNOSIS AND TARGETED DRUG DELIEVRY

Tanziela

Southeast University, China

Abstract

Background: Drug resistance remains one of the major hurdles to the effective cancer chemotherapy. Exosomes have recently come into focus in the field of nanotechnology which holds great promise for effective and target specific conveyance of therapeutic tools to malignant site. Albumin-based nanoparticles (NPs) as a drug delivery system have attracted much attention owing to their nontoxicity, non-immunogenicity, great stability and ability to bind to many therapeutic drugs.

Objective: Here, we develop a biocompatible exosome based on DOX loaded Au-BSA nanoparticles exocytosed by HepG2 cells (E@DOX@Au-BSA) for overcoming drug resistance in cancer chemotherapy.

Methods: Exosomes generated by exocytosis of *in situ* biosynthesized DOX@Au-BSA from cancer cells were isolated from cell culture medium by Ultracentrifugation and characterized their size, shape, drug release, intracellular ROS production and *in vitro* and *in vivo* antitumor efficiency. Cytotoxicity was determined by MTT assay.

Results: The as prepared exosomes (E@DOX@Au-BSA) with ability of bioactive transportation are sphere-shaped with size of 30-150 nm. In addition, the enhanced rate of drug release under acidic environment, DOX@AgNCs-Exo^{U87} independent of their source, possess significant increased cellular uptake of DOX as compared to free DOX and DOX@Au-BSA and cytotoxicity involved ROS mediated DNA damage in bulk cancer cells and have no activity on healthy cells. *In vivo* studies reveal enhanced accumulation of E@DOX@Au-BSA in tumor, escaped from blood vessels and penetration into deep root of tumor tissues following intravenous inoculation which led to overcoming the drug resistance in cancer cells without off-site effects of DOX.

Conclusion: These results prove the perception for the use of drug and nanoclusters conjugated exosomes exocytosed from tumor cells as a promising drug carrier for competent cancer chemotherapy.

Biography

I am pleased to write this in favor of Dr. Tanziela. She will complete her PhD in June, 2022 from Southeast University. She has 6 publications in well reputed journals and knows about biological techniques. She earned a devoted repute and everyone admire her for her character and decency. She owns a very sound behavior with lab mates. Actually, what makes her different is her zeal for research work and practically of any sort of field; she has ability to work in a team and has capabilities to develop appropriate response to various issues in her field of expertise.

ADVERSE EVENTS FOLLOWING IMMUNIZATION OF COVID-19 VACCINATION AMONG HEALTH CARE PROFESSIONALS IN ETHIOPIA

Dufera Rikitu Terefa

Wollega University, Ethiopia

Abstract

Background: COVID-19 Vaccine is a vaccine intended to provide acquired immunity against severe acute respiratory syndrome coronavirus. Vaccines are not free from adverse events (AEFI). However, evidence on the AEFI of COVID-19 vaccination among health care professionals in our country was not addressed. Therefore, we aimed to assess AEFI of COVID-19 vaccination among health professionals in Ethiopia.

Methods: An online cross-sectional survey was conducted on AEFI of COVID-19 vaccination among HPs in Ethiopia from June 1 to 30, 2021. Data was collected using a semi-structured questionnaire which was created on Google forms and disseminated online. Both descriptive and inferential analyses were performed using SPSS Ver.25. AOR along with 95% confidence level and variables with a P value < 0.05 were considered to declare the statistical significance in the multivariable analysis.

Results: Of those participants, 243(75%) of them had experienced AEFI of COVID-19 vaccination. The major AEFI experienced were injection site (inching, pain, warmth, swelling and redness) and 69.1%. Being married [AOR=4.19, 95% CI: 2.07,8.45] ,family size >5 [AOR=5.17, 95% CI: 1.74, 15.34], family not tested for COVID-19 [AOR=0.39, 95% CI: 0.15,0.97], Lack of family support to take vaccine [AOR=3.58, 95% CI: 1.75, 7.33], heard anything bad about vaccine[AOR=4.17, 95% CI: 1.90,9.13] were statistically associated with AEFI of COVID-19 vaccination.

Conclusion: The study found that about three fourth of respondents had experienced AEFI of COVID-19 vaccination. Marital status, family size, family tested for COVID-19, lack of family support to take the vaccine, heard any things bad about the vaccine and concerned about the vaccine could cause AEFI were factors associated with AEFI of COVID-19 vaccination. So, we recommend health managers at different level of health system in the country to encourage full vaccination to any health care professionals across the nation as the benefit outweighs the AEFI.

Biography

Dufera Rikitu Terefa has completed his Msc in Health Economics from Jimma University. He has worked as a lecturer of Health economics, Management and policy at Wollega University. He has published 5 papers in reputable journals and he has been serving as a lecturer at Wollega University.

SCREENING OF ANTIANXIETY ACTIVITY OF GINGEROL ON RODENTS

Chetan Kumar Dubey
Career Point University, Kota, India

Abstract

Anxiety is a state of excessive fear which is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes. Anxiety disorders are psychiatric disorders affecting nearly 25% of the adult population at some point in their life. The prevalence of anxiety disorders is 30.5% and 19.2% in women and men respectively. The present study was undertaken to evaluate the anxiolytic activity of Gingerol. Gingerol is a main active constituent of Ginger and it's isolated from herbal extract of Zingiber officinale Roscoe (Ginger). Ginger, (Zingiberacae) is one of the important medicinal plant which naturally occurs in various country like India, China, South East Asia, West Indies, Mexico and other parts of the world.

The anxiolytic activity was evaluated in elevated plus maze (EPM), open field method and hole board test. The efficacy of Gingerol was compared with the standard anti-anxiety drug (diazepam 2 mg/kg). It was observed that Gingerol at the dose of 200 mg/kg is effective and showing significant anti-anxiety activity in mice by increasing time spent in open arm and entries to open arm in EPM model.

NEW ANTIBACTERIAL PHENOLIC COMPOUND ISOLATED FROM *Agelanthus brunneus* (LORANTHACEAE)

Moifo Kuete Thomas Wieland

University of Yaoundé, Cameroon

Abstract

Agelanthus brunneus (Loranthaceae) is a hemiparasitic plant growing on *Senna siamea* (Fabaceae). The chemical investigation of its leaves and flowers led to the isolation of one new phenolic compound namely (–)-brunneusine (1), together with thirteen known compounds. The crude leaves and flowers extracts (CLE and CFLE) with their ethyl acetate fractions (EAFL and EAFFL) and some isolated compounds (1-3; 8-9; 11-14) have been tested on four bacterial species of sanitary importance isolated in an aquatic environment. All the samples except compound 3 showed antibacterial activity with MICs ranging from 0.43 to $8.88.10^3$ µg/mL and MBCs from 0.43 to $3.55.10^3$ µg/mL. Compounds 9 and 14 showed better activity on all bacterial species tested with MICs ranging from 0.43 to 27.77 µg/mL. Only CLE, EAFL, compounds 14, 2, 8, and 9 showed bactericidal effects on all bacterial species tested.

Biography

Moifo has completed is Masters in medicinal chemistry at the University of Yaounde 1 in 2017 and registered to PhD the next year on the topic: isolation of antibacterial natural compounds from Agelanthus brunneus and Phragmanthera polycrypta, plants of Loranthaceae family. He isolated a new antibacterial natural compound namely Brunneusine and publish in the German journal Zeitschrift für Naturforschung C. in 2021(https://doi.org/10.1515/znc-2021-0143). He is now working as fellow guest scientist at the university of Bielefeld, in the frame of the DAAD - project Bilateral Graduate School YaBiNaPA.

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Coalesce Research Group
33 Market Point Dr,
Greenville, SC 29607, USA

Contact Us:

Phone: +1-718-543-9362
Whatsapp: +1-716-320-1018