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University of Mississippi Botanical Dietary Supplements Research Center

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Arthrospira/Limnospira oral Supplement for Enhancing Host Resilience to Virus Infection

Established in 2020 (www.umbdsrc.org)

The University of Mississippi Botanical Dietary Supplements Research Center (UM BDSRC) is a component of the NIH Consortium for Advancing Research on Botanicals and other Natural Products (CARBON) Program. It was created to foster collaborations between scientists at the University of Mississippi housed at the main campus in Oxford and the Medical Center in Jackson.

Public health relevance and significance

Arthrospira/Limnospira (commonly known as spirulina*)* is a top selling botanical for improving immune health. This center's research is directed towards generating sufficient data to optimally design future human intervention studies to evaluate the utility of *Limnospira*-derived oral supplements in promoting resilience against and/or recovery from respiratory viral infections such as influenza. The use of a *Limnospira*-derived oral supplement may provide an important complementary approach to currently available antiviral therapies that is inexpensive, safe and readily available to the public.

Influenza virus infection is a continual, worldwide public health problem that has challenged western society for centuries. The CDC estimates that the burden of illness during the 2017–2018 flu season included 48.8 million symptomatically infected people in the U.S., 959,000 patient hospitalizations, and 79,400 deaths. Since the modern flu vaccine program has moderated but not eliminated infection risk, enhancement of host antiviral immune response through the use of botanicals may provide an important complementary approach.

What is the rationale for selecting *Limnospira* as the botanical product for our research?

Limnospira is a cyanobacterium that has been used as a food for centuries and more recently as a health supplement by a large segment of global society. Although early interest in commercial production of *Limnospira* was focused mainly on its nutrient and protein content, it has emerged as a popular dietary supplement due to scientific evidence supporting various human health benefits such as immune-enhancing and antiviral properties.

About 25 years ago the UM National Center for Natural Products Research (NCNPR) established a unit to investigate the immune-enhancing properties of botanicals and dietary supplements. Numerous products that are traditionally used to enhance immune function were evaluated, and extracts from *Limnospira* were found to be hundreds of times more active than all others tested. Based on this discovery, the NCNPR invested substantial research effort to investigate the immune-enhancing properties and therapeutic applications of *Limnospira*.

A growing body of evidence generated from the NCNPR and the literature indicate that oral consumption of *Limnospira* products are particularly useful natural products for providing resilience against influenza viral infection. Research demonstrates that a major mechanism by which *Limnospira* products provide anti-viral resilience is through its impact on immune function. Through this research effort Braun-type lipoproteins were identified as the predominant macrophage-activating principal within *Limnospira,* and a patented extract (Immulina™) was developed that preferentially enriches for the level of these active macromolecules from the raw material. The Immulina extract has been commercially available for the last 15 years and is the product research focus for our UM BDSRC.

Administrative Core

Dr. Ikhlas Khan
Director

Dr. Nirmal Pugh
Associate Director

Gray Dale
Program Manager

Administrative Supplement (10/1/22 – 6/30/23)
The objective is to advance research on the identification of chemical marker(s) to monitor subject adherence (the extent to which they consume the Immulina product). To identify adherence/surrogate marker candidates, the Botanical Core team will implement hyphenated chromatographic methods (viz., GC- or LC coupled withQToF-MS) to evaluate biological fluids from human volunteers (collected before and after Immulina consumption) for differential levels of compounds using both targeted and non-targeted approaches.

Acknowledgements

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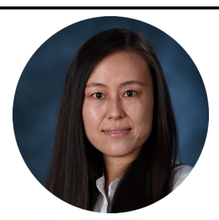
Botanical Core

Dr. Amar Chittiboyina
Core Leader

Dr. Nirmal Pugh
Senior Investigator

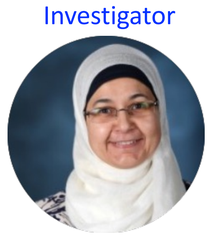


Dr. Jin Zhang
Investigator

Dr. Jungmoo Huh
Postdoc

Dr. Iffat Parveen
Investigator

Dr. Bharathi Avula
Investigator



Dr. Mona Haron
Investigator

The overall purpose of the Botanical Core is to ensure product integrity and advance the chemistry research on the *Limnospira*-based product, Immulina, by using a combination of bioassay- and chemical-based approaches.

Aim 1.
Ensure the unambiguous identification and perform additional safety testing on *Limnospira fusiformis* raw material that will be used in the production of sufficient quantities of Immulina for the proposed research projects.

Aim 2.
Validate a selective *in vitro* bioassay for quantitation of the toll-like receptor 2-dependent activity exhibited by the Braun-type lipoproteins in Immulina extracts.

Aim 3.
Advance the chemistry research on Immulina by establishing chemical-based authentication and standardization approaches, characterization of immune-inhibitory substances, and detailed structural analysis of the active immune-enhancing Braun-type lipoproteins.

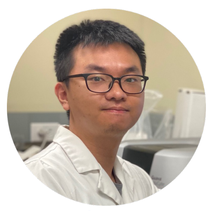
Aim 4.
Perform additional stability studies, provide sufficient quantities of well-characterized and safe Immulina for use in the proposed UM BDSRC research projects, and explore collaborative opportunities with other CARBON units.

Project 1

Dr. Chalet Tan
Principal Investigator

Nan Ji
PhD Student

Mingja Wang
PhD Student



Yusheng Li
PhD Student

Pranav Pankshe
PhD Student

<u>Unraveling Immune Enhancement by Immulina</u>

Aim 1.
Develop an optimized liquid formulation for Immulina.

Aim 2.
Evaluate the pharmacodynamics of Immulina and identify *in vivo* biomarker(s).

Aim 3.
Investigate the molecular mechanism on immune enhancement by Immulina.

Statement of Potential Impact
The successful completion of this project will result in the development of an improved formulation of Immulina and the identification of *in vivo* biomarkers for Immulina treatment.

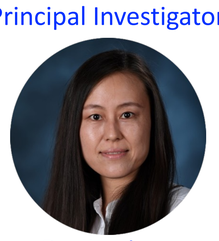
Project 2

Dr. Gailen Marshall,
Principal Investigator

Dr. Khalid Ashfaq
Senior Investigator

Dr. Tahir Mir
Investigator

Dr. Shabana Khan
Senior Investigator



Dr. Jin Zhang
Investigator

Dr. Siddharth Tripathi
Investigator

Dr. Kashif Shamim
Postdoc

John Trott
Research Staff

<u>Examining the Effects of Immulina to Increase Immune Resilience against Influenza Virus Infections</u>

Mouse model (Years 1-2)
Aim 1. Evaluate oral administration of Immulina in three non-lethal mouse models of resilience against influenza A virus infection (prophylaxis, prodrome and recovery) to determine the most effective utility of Immulina for enhancing host immunity to improve antiviral resilience.

Aim 2. Confirm that activation of the TLR2 signaling pathway by Braun-type lipoproteins is a causal mechanism through which Immulina enhances host immunity against antiviral infection.

Human model (Years 3-5)
Aim 3. Determine the optimal form and dosage of the Immulina-based supplement that will maximize effects on increasing lymphocyte cell numbers and/or activity, increased supporting cytokines, ADCC against influenza-infected target cell lines and influenza-specific antibody titers.

Aim 4. Establish the timeline for optimal lymphocyte, cytokine and antibody responses in terms of both initial changes and maximal changes and duration of the change once the Immulina is discontinued in normal and immune compromised (elderly) human research participants.

Aim 5. Examine the effects of routine influenzas vaccine given before, during, or after Immulina use to investigate influenza antigen-specific antibody responses in individuals receiving Immulina supplement vs placebo.