

# Medicinal Examination of Fungal Endophytes in Various Common Mushrooms

Liam Wilson, Dr. Christine MacTaylor, Department of Chemistry and Physics, Salem State University



## Abstract

Button, hen of the woods, oyster, shiitake, and crimini mushrooms are common household mushrooms known for their health benefits and potential use for medicines. This research aimed to explore the connections between the isolated fungal endophytes of the aforementioned mushrooms and their potential medicinal uses.

## Introduction

Mushrooms are one of the most important organisms to any ecosystem. They are valuable decomposers, high in nutrients, and are integral indicators for the health of an ecosystem. Some mushrooms such as button, hen of the woods, oyster, shiitake, and crimini are especially interesting due to their mainstream consumption for their health benefits. The health benefits for most of the mushrooms include anti cancer, immunostimulation effects, angiogenesis, a reduction in benign prostatic hyperplasia, antiviral and antibacterial, lipid metabolism, anti-diabetes, vitality, antioxidant, anti-inflammatory, anti hypoglycemic, and a good source of vitamins and amino acids<sup>3,4,5,6</sup>. One of the reasons any medicinal benefits could be in the organism is from its endophytes. Endophytes are fungal organisms that live within plants for some of its life cycle and are responsible for responding to its environment. This research aims to study the medicinal benefits of these mushrooms by examining the fungal endophytes housed within the organisms.



## Experimental Design

### Sample Collection / Plating:

- Samples of the mycelium of Shiitake, Hen of the woods, Crimini, Button, and Oyster mushrooms were taken.
- The samples were washed with bleach, ethyl acetate, and water and plated on PDA for endophyte growth

### Extraction of Endophyte Metabolites:

- Cuts from the plates were taken with a sterile knife and placed into potato dextrose broth
- The broth was left for weeks to grow and extract the metabolites into the broth.
- The metabolites were extracted out of the broth using separation funnels and ethyl acetate. Then roto-vaped.

### Bioactivity / Inhibition of Bacteria:

- The extracted metabolites, broth, and sample solids were used to test the bioactivity in *E.coli*, *Pseudomonas*, *Staphylococcus*, and *Vibrio*.

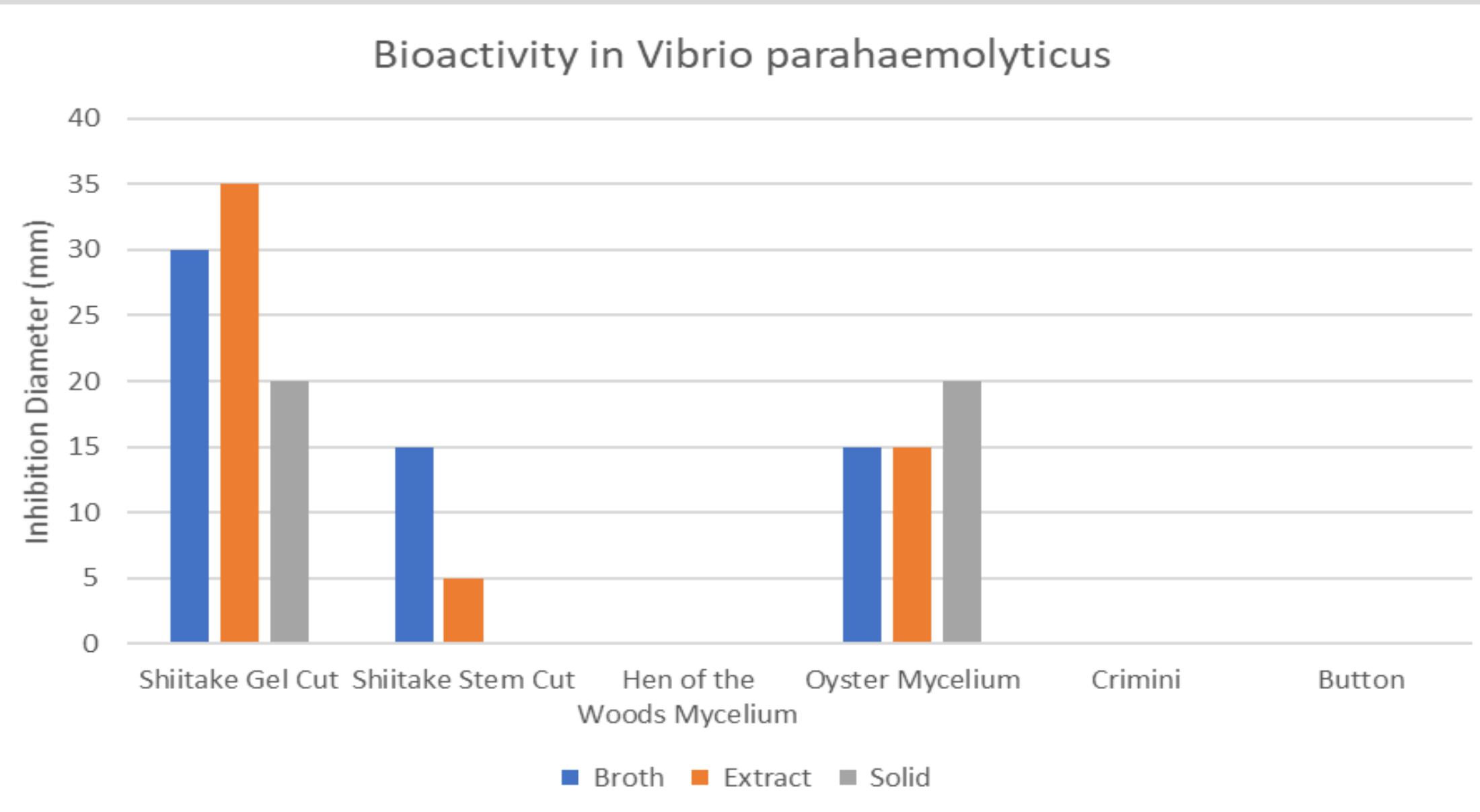
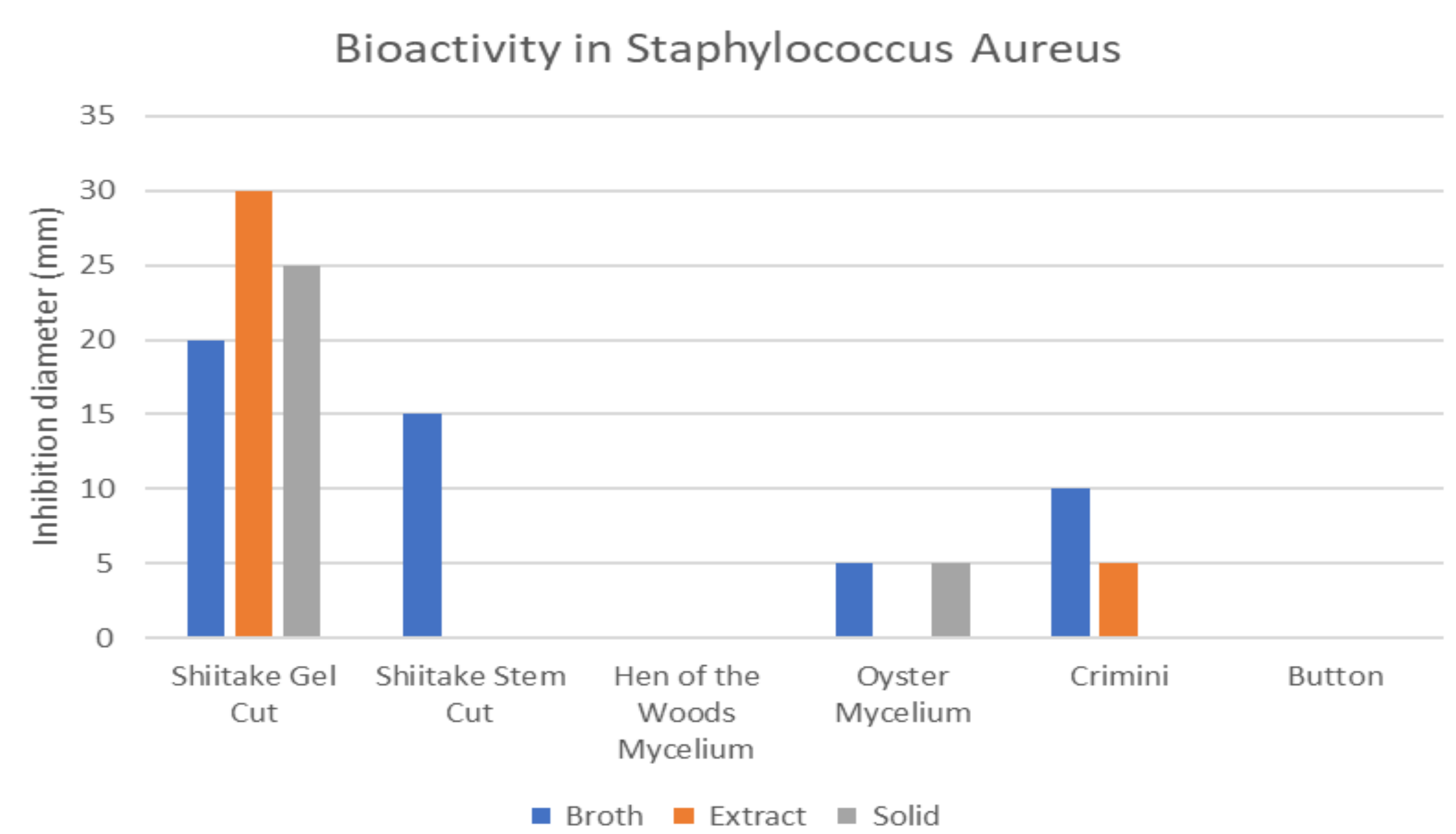
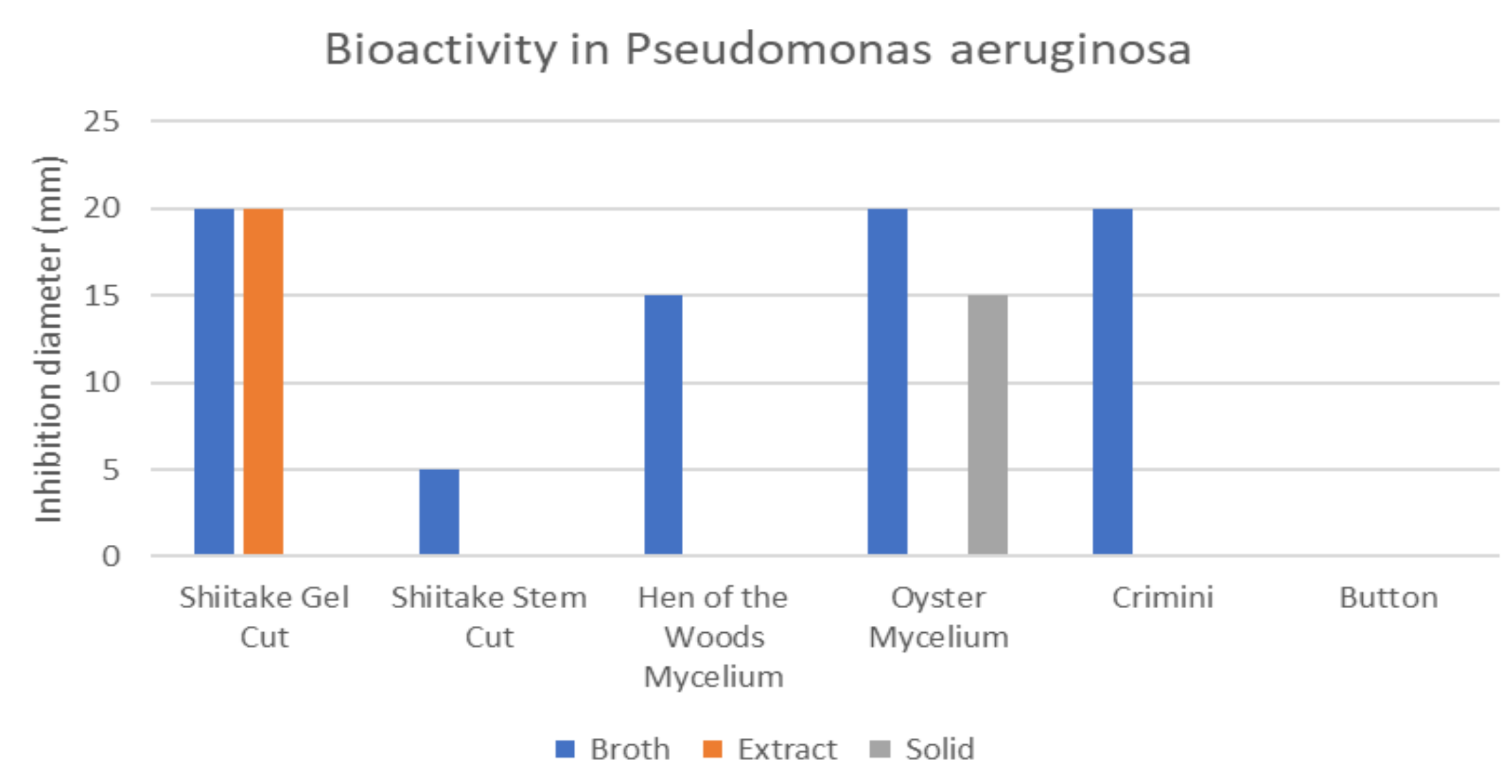
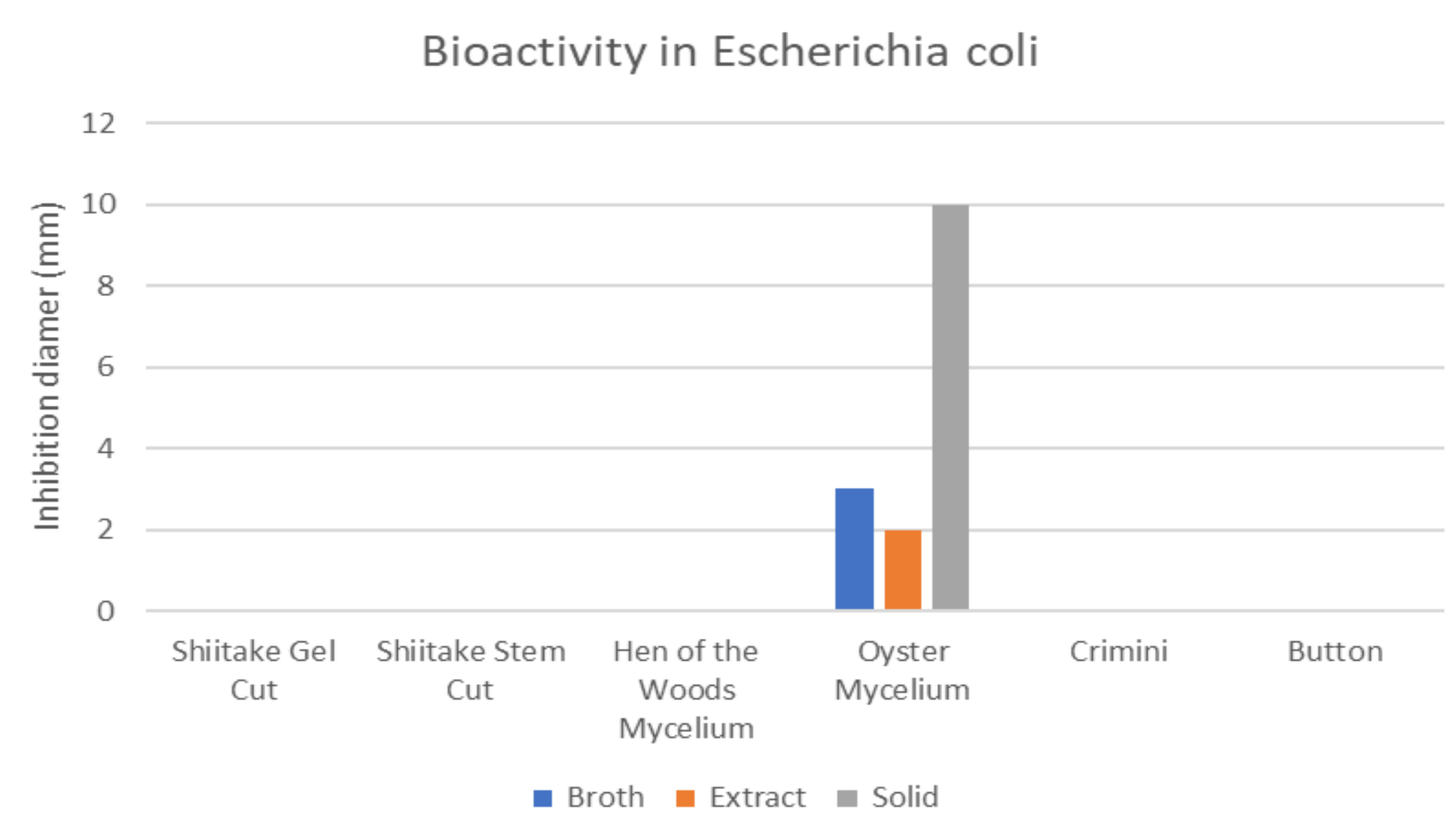
- Measurements of the inhibitory rings were recorded.

### Identification of Metabolites Using UPLC-MS:

- The endophyte metabolites previously extracted were analyzed using LC-MS to find the medicinal compounds within.



## Bio Activity



## LC-MS Results

### Medicinal Compounds: Antibiotics/Anesthesia/Muscle Relaxers

Compound Name	Uses	Sample	Compound Structure
L(-)-Menthol	A drug, usually applied topically, that relieves pruritus (itching), and suppresses the cough reflex. Has a connection to the metabolism of E.Coli.	Crimini Mycelium Oyster Mycelium	<chem>CC1C(C)C(C)C1O</chem>
ethionamide	A substance that kills or slows the growth of Mycobacterium tuberculosis and is used in the treatment of tuberculosis. A substance that suppresses Mycobacterium leprae, ameliorates the clinical manifestations of leprosy, and/or reduces the incidence and severity of leprosy reactions.	Shiitake Gel Cut Crimini Mycelium Button Mycelium	<chem>CC1=CC=C(C=C1)NC(=O)N</chem>
8-Iodo-guanine	Methods of treating a metabolic syndrome by modulating heat shock protein (hsp) 90-beta, used in Biomedical device implantable in bone and/or cartilaginous tissue	Hen of the Woods Mycelium Crimini Mycelium	<chem>O=C1NC(=O)N(C1=O)I</chem>
Lignocaine	Used as a local anaesthetic... topical, intravenous, epidural or spinal block.	Oyster Mycelium Cut	<chem>CCN(CC)CC(O)C1=CC=C(C=C1)C</chem>
benzenediol	A drug used to treat or prevent bacterial infections. A substance used locally on humans and other animals to destroy harmful microorganisms or to inhibit their activity	Crimini Mycelium Cut Oyster Mycelium	<chem>Oc1ccc(O)cc1</chem>
Imiquimod: Aldara	Antifungal medicine used to treat the symptoms of Actinic Keratosis, Superficial Basal Cell Carcinoma and External Genital Warts.	Shiitake Stem Cut Button Mycelium	<chem>Cc1nc2c(nc1)nc2</chem>

## Results / Figures

### Medicinal Compounds: Cardiovascular/Endocrine

Compound Name	Uses	Sample	Compound Structure
Taurine	congestive heart failure, high blood pressure, high cholesterol, cystic fibrosis, and to prevent the buildup of plaque in the arteries throughout the body. Taurine occurs naturally in the human brain.	Hen of the Woods Mycelium Crimini Mycelium Cut Shiitake Stem Cut Button Mycelium Cut	<chem>CC(N)S(=O)(=O)O</chem>
Neptazane	An agent that promotes the excretion of urine through its effects on kidney function.	Crimini Mycelium Cut	<chem>CC(=O)N1C=NC(S1)S(=O)(=O)N</chem>
Calcium acetate	used to control high blood levels of phosphorus in people with kidney disease who are on dialysis	Hen of the Woods Mycelium	<chem>CC(=O)[O-].[Ca+2].CC(=O)[O-]</chem>
Metirosine	Cardiovascular antihypertensive drug... Any drug used in the treatment of acute or chronic vascular hypertension regardless of pharmacological mechanism.	Oyster Mycelium Button Mycelium	<chem>CC(O)C(N)Cc1ccc(O)cc1</chem>

### Medicinal Compounds: Neurological/Psychological

Compound Name	Uses	Sample	Compound Structure
tenocyclidine	anesthetic drug with stimulant and hallucinogenic effects. It is more potent than phenacyclidine and hence, this drug was classified under the schedule 1 in 1970.	Shiitake Gel Cut Hen of the Woods Mycelium Shiitake Stem Crimini Mycelium Button Mycelium Oyster Mycelium	<chem>C1CCN(C1)C2=CC=CC=C2S</chem>
Biotin	used for preventing and treating biotin deficiency associated with pregnancy, long-term tube feeding, malnutrition, and rapid weight loss. It is also used orally for hair loss, brittle nails, skin rash in infants (seborrheic dermatitis), diabetes, and mild depression. Has a role in the metabolism of E. Coli.	Shiitake Gel Cut Hen of the Woods Mycelium Shiitake Stem Crimini Mycelium Button Mycelium Oyster Mycelium	<chem>CC1=NC(=O)NC(C1)CCCC(=O)O</chem>
N,N-Diethyltryptamine (DMT)	It is used as a recreational psychedelic drug and prepared by various cultures for ritual purposes as an entheogen.	Shiitake Gel Cut	<chem>CCN(CC)c1ccc2c(c1)c[nH]2</chem>
Bulbarbital	A central nervous system depressant used to induce drowsiness or sleep or to reduce psychological excitement or anxiety.	Shiitake Gel Cut	<chem>CC(C)C(O)C(=O)N1C=NC(=O)N1</chem>

### Medicinal Compounds: Other Compounds of Interest

Compound Name	Uses	Sample	Compound Structure
chlorotrianisene	A substance that inhibits or prevents the proliferation of neoplasms. A hormone that stimulates or controls the development and maintenance of female sex characteristics in mammals by binding to o-estrogen receptors.	Shiitake Gel Cut Hen of the Woods Mycelium Shiitake Stem Crimini Mycelium Button Mycelium Oyster Mycelium	<chem>COc1ccc(cc1)C2=CC=CC=C2C3=CC=CC=C3OC</chem>
Succimer	Any protective agent counteracting or neutralizing the action of poisons. chelator A ligand with two or more separate binding sites that can bind to a single metallic central atom, forming a chelate. A substance administered to aid diagnosis of a disease.	Shiitake Gel Cut	<chem>CC(O)C(S)C(S)C(=O)O</chem>
Sorbaldehyde	used as a food additive for flavor... used in spices, extracts, colorings, etc for human consumption. Antifungal properties	Button Mycelium Oyster Mycelium	<chem>CC=CC=O</chem>
2,3-Dihydroxybenzoate	A potential treatment for cystic fibrosis <sup>1</sup> , could have been created by the E.Coli aerobacter aerogenes <sup>2</sup>	Shiitake Gel Cut Hen of the Woods Mycelium	<chem>OC(=O)c1cc(O)ccc1</chem>
Delavirdine	An antiviral agent that destroys or inhibits the replication of the human immunodeficiency virus. A substance that destroys or inhibits replication of viruses.	Shiitake Stem Cut	<chem>CC1=CC=C(C=C1)N2C=NC(=O)N2C3=CC=CC=C3</chem>
Nabumetone	Analgesic agent capable of relieving pain without the loss of consciousness or without producing anesthesia. In addition, analgesic is a role played by a compound which is exhibited by a capability to cause a reduction of pain symptoms. A substance that reduces or suppresses inflammation.	Hen of the Woods Mycelium	<chem>CCOC1=CC=C(C=C1)C(=O)CC</chem>

## Conclusion

Research conducted on mushrooms over many years is largely supported by the compounds found in the LC-MS. Some examples include, psychedelic properties of mushrooms possibly be due to the DMT found in the Shiitake mushrooms.

Mushrooms are notably very heart healthy foods, and this may be due to the taurine or metirosine found in most of the mushroom samples<sup>3</sup>. Another property of mushrooms that is confirmed by the compounds found via the LC-MS is the antimicrobial resistance that they provide. This could be due to the presence of benzenediol or sorbaldehyde. The research has also shown possible evidence for the anti-cancer and human immunodeficiency virus (HIV) properties of shiitake mushrooms, due to the presence of the compounds delavirdine, imiquimod: Aldara, and chlorotrianisene.

Overall, this study supports the common traditional uses for most of these mushrooms based on the constituents found in comparison to the literature. However, the compound 2,3-Dihydroxybenzoate that has properties to potentially treat cystic fibrosis has not previously been recorded in literature but was seen in Shiitake and Hen of the Woods. Future studies on mushrooms should explore the possibilities of treatments for cystic fibrosis

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