The Promising Medical Applications of *Verbascum Thapsus* in Relation to Endophytic Species

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ABSTRACT

The endophytic species of *Verbascum Thapsus* and their secondary metabolites, are analyzed for possible medicinal applications. Endophytic Fungi were derived from the root, leaf and stem samples of *Verbascum Thapsus*, better known as Common Mullein, from the Forest River Salt Marsh at Salem State University. These endophytes demonstrated strong antibacterial properties against different kinds of bacteria. Further analysis of the metabolite extracts in LC-MS showed 20 different medicinal compounds.

INTRODUCTION

Drug resistance is a growing problem in various diseases and the issue could be solved by looking closely at drugs used by our ancestors. Plants have been used to treat medical issues since long before illnesses were studied, and the solution to drug resistance may have been available this entire time.

The medical capabilities of plants may lie in the endophyte species that inhabit it. Endophytes are bacteria and fungi that reside in plant tissues and live in symbiosis with its host plant. Endophytes receive nutrients and shelter from the host and the plant gains pathogenic protection and biological stressor management from the endophytes. Mounds of research on a wide variety of plants, has shown that all plants have endophytes and the metabolites produced by these endophytes vary depending on the host plant and the environment. These endophytes produce compounds that have the potential to also benefit humans.

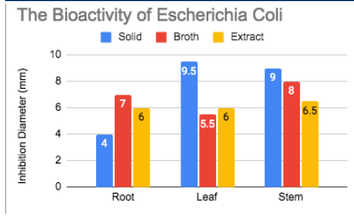
The medical capabilities of *Verbascum Thapsus*, known as common mullein, have been well documented and used for a variety of different applications since ancient times. Dioscorides, in 64 A.D, documented it's effectiveness in wounds, inflammation, and cough (2). Historically, the leaves of Mullein have been brewed into tea and smoked to treat diarrhea and respiratory issues respectively (7). These records indicate to scientists, that mullein holds great medicinal potential.

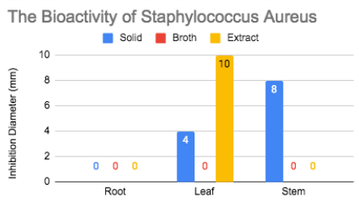
EXPERIMENTAL PROCEDURE

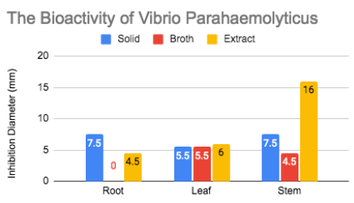
Sample Collection:
Mullein samples collected from two different plants at the Forest River Salt Marsh.
Growth and Isolation of the Endophytes:
Samples cut and washed soap, bleach and ethanol. Placed on a Potato Dextrose Agar (PDA), replated then placed in potato dextrose broth.

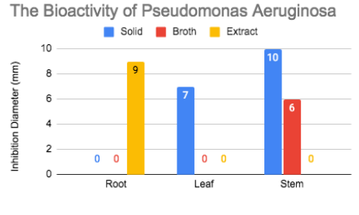
Metabolite Extraction:
Metabolite extracted from the broth using ethyl acetate which was evaporated and left behind the metabolite extract. (Extract of the seed sample was not able to be isolated)
Testing for Bioactivity:
Escherichia coli (E. Coli), *Staphylococcus aureus* (Staph), *Pseudomonas aeruginosa* (Pseudo), and *Vibrio Parahaemolyticus* (Vibrio).
Solid endophyte, broth and extract from each sample placed on PDA plates.
The diameter of the zone of inhibition(ZOI), spot where the endophyte caused the bacteria colonies do not grow, measured to test bioactivity.
Metabolite Identification:
LCMS analyzed the metabolite extract. Drug Bank Database used to identify the compounds within

BIOACTIVITY RESULTS









E. Coli:
The largest ZOI was the leaf solid. The only samples that has no endophytic growth in the ZOI was the root,leaf and stem extracts
Pseudo:
The largest ZOI was the stem solid. However the most effective specimen was the root extract. It was difficult to disconcern the leaf and stem extracts due to the growth obstructing the area.
Vibrio:
The largest ZOI was the stem extract, which was the largest so far and had no endophyte growth. Also the root and leaf extracts as well as the stem broth didn't have any growth in the ZOI.
Staph:
The biggest ZOI was the leaf extract. Both the stem and leaf solids had endophyte growth in the ZOI. the stem extract and broth couldn't be measured due to the growth in the area

LC-MS RESULTS

ANTIBACTERIAL

Common Name	Structure	Samples	Uses
Ethionamide		Stem	Used to treat tuberculosis (TB)
D-Vinylglycine		All Samples	Inactivation of transaminase activity in some bacteria (E. Coli, Bacillus subtilis)
Sorbaldehyde OR 2,4 Hexandienal		Root	Effective antifungal agent (*Aspergillus flavus*)
2-(sec-butyl) thiazole		Leaf	protective food agent by reducing patina bacteria, which can cause dermatitis and allergic and asthma can result

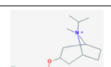
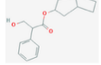
CARDIOVASCULAR

Common Name	Structure	Samples	Uses
Bumetanide		Stem and Leaf	Can treat fluid retention (edema) and high blood pressure
Taurine		Leaf	congestive heart failure, high blood pressure, high cholesterol
3-Picoline		Stem	effective way to treat cardiovascular diseases and atherosclerosis (niflumic acid and copper(II) chloride complex)

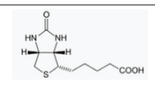
CENTRAL NERVOUS SYSTEM

Common Name	Structure	Samples	Uses
Dihydroxyindole		Leaf	Management of central nervous system disorders (i.e Parkinson's)
Baclofen		All Samples	Treats muscle spasms in many conditions including multiple sclerosis
Ethchlorvynol		Leaf	Sedative and Insomnia Treatment

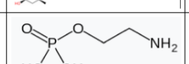
LUNG

Common Name	Structure	Samples	Uses
Ipratropium		Root	(when in a salt with bromine) treats chronic obstructive pulmonary disease and asthma
2-(sec-butyl)thiazole		Leaf	protective food agent by reducing patina bacteria, which can cause dermatitis and allergic and asthma can result

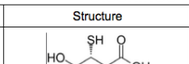
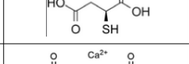
VITAMINS

Common Name	Structure	Samples	Uses
Biotin		All Samples	Vitamin B_7 or Vitamin H Deficiency associated with with malnutrition and rapid weight loss
Halocynthiaxanthin		Leaf and Stem	Antioxidant

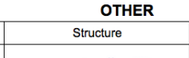
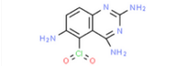
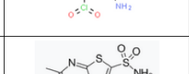
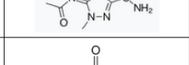
CANCER

Common Name	Structure	Samples	Uses
Halocynthiaxanthin		Leaf and Stem	Carotenoid (breast cancer, leukemia & colorectal)
Phosphoryethano-lamine		Leaf	Being trial tested as an anticancer drug for pancreatic and prostate cancer (Brazil)

POISONINGS

Common Name	Structure	Samples	Uses
Succimer		Leaf and Stem	Used to treat lead and heavy metal poisoning
Calcium acetate		Leaf	Controls high blood levels of phosphorus in people with kidney disease

OTHER

Common Name	Structure	Samples	Uses
5-chloroquinazoline -2,4,6-triamine		Stem	Tested on rats and researched for hyperglycemia. Increased D- glucose inhibition
Neptazane AKA Methazolamide		Leaf	Treat high pressure inside the eye in glaucoma.
Sodium bicarbonate		Root and Stem	Antacid used to relieve heartburn and acid indigestion
1-methyl-2-(nitrosomethylidene) pyridine		Root	controlling tobacco use and alleviating withdrawal symptoms

These antibacterial properties also explain why the ancient world used mullein for wounds. E.Coli showed the best bioactivity, which makes sense with the compounds found. The pulmonary and respiratory relief can be explained by the asthma treatment compounds as well as Ethchlorvynol, the sedative.The stomach relief experienced from drinking mullein tea can be explained by Ethchlorvynol, the vitamins and the antibacterials. The identification of the compounds in mullein not only provided an explanation for the medicinal properties observed before the creation of modern science, but gave incite as to what else this plant could be utilized for. From doing the LC-MS experiments, it was shown that mullein has great potential in cardiovascular issues and central nervous system disorders. It is interesting that there are two compounds, 5-chloroquin azoline -2,4,6-triamine and Phosphoryethanolamine, that haven't been fully researched yet. Particularly interesting is Phosphoryethanolamine , which is being used for pancreatic and prostate cancer in Brazil, but hasn't been approved by the National Health Surveillance Agency or proven effective by U.S scientists, also reported was severe blood clotting as a side effect. Given the compounds identified by the LC-MS, mullein should be further investigated by scientists for it's mental health benefits and more commonly exploited and used for the treatment of medical issues.

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