EFFECTS OF ACOUSTIC WAVES ON GROWTH, DEVELOPMENT & PHYTOCHEMICALS CONTENTS OF *IN VITRO* RAISED *PICRORHIZA KURROA*

Project report submitted in partial fulfillment of the requirement

for the degree of Bachelor of Technology

in

Biotechnology/Bioinformatics

By

Amrita Mishra (191818), Shikhu (191815)

Under the supervision of

Dr. Poonam Sharma & Dr. Hemant Sood

to

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Candidate's Declaration

I hereby declare that the work presented in this report entitled " **Effects of Acoustic Waves on Growth, Development & Phytochemicals Contents of *In Vitro* Raised *Picrorhiza Kurroa***" in partial fulfillment of the requirements for the award of the degree of **Bachelor of Technology** in **Biotechnology** submitted in the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology Waknaghat is an authentic record of my own work carried out over a period from July 2022 to May 2023 under the supervision of **Dr. Poonam Sharma** (Associate Professor) (Biotechnology and Bioinformatics) & **Dr. Hemant Sood** (Associate Professor) (Biotechnology and Bioinformatics).

I also authenticate that I have carried out the above-mentioned project work under the proficiency stream **Plant Tissue Culture**.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

Amrita Mishra (191818)

Shikhu (191815)

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

Dr. Poonam Sharma
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ACKNOWLEDGEMENT

First, We express my heartiest thanks and gratefulness to Almighty God for His divine blessing to make it possible to complete the project work successfully.

We are really grateful and wish our profound indebtedness to Dr. Poonam Sharma, Associate Professor, Department of BT/BI, Jaypee University of Information Technology, Waknaghat & Dr. Hemant Sood, Associate Professor, Department of BT/BI, Jaypee University of Information Technology, Waknaghat. Deep Knowledge & keen interest of our supervisor in the field of “Biotechnology” to carry out this project. Their endless patience, scholarly guidance, continual encouragement, constant and energetic supervision, constructive criticism, valuable advice, and reading many inferior drafts and correcting them at all stages have made it possible to complete this project.

We would also generously welcome each one of those individuals who have helped me straightforwardly or in a roundabout way in making this project a win. In this unique situation, We might want to thank the various staff individuals, both educating and non-instructing, which have developed their convenient help and facilitated my undertaking.

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ABSTRACT

Picrorhiza kurroa is a plant native to the Himalayas and possesses significant medicinal properties. When consumed before or after exposure to toxins, the herb exhibits potent hepatoprotective (liver-protective) effects attributed to its kutkin molecules. Various toxins or stressors known to impair liver function, as well as situations of intrinsic hepatic failure, have shown protective benefits. The World Health Organization (W.H.O.) estimates that traditional medicine fulfills approximately 80% of the world's fundamental healthcare needs. However, due to ongoing medicinal crises worldwide, it is necessary to develop new technologies not only for the creation of novel medicines but also for the enhancement of existing ones.

Our research focuses on Plant Acoustic Frequency Technology (PAFT), which involves subjecting in vitro cultivated *Picrorhiza kurroa* plants to acoustic waves using an electronic device called an interferometer connected to a speaker. This process releases various sound waves at different frequencies to promote growth and enhance the phytochemical content of the plants. To create a soundproof environment and eliminate external noises, the setup was placed inside a thermocol compartment. Additionally, this study involves measuring various acoustical parameters using plant extracts and examining the presence and content of secondary metabolites in both control plants and plants subjected to the Plant Acoustic Frequency Technology. Throughout the course of our experiment, we discovered how PAFT benefitted both the morphological and anatomical aspects of the plants.

CHAPTER-1 INTRODUCTION

1.1 Introduction

In this section we have given a brief introduction about our plant *Picrorhiza kurroa*, its dosage, medical usage, medications, precautions while consuming, and its respective side effects along with that we have discussed acoustic studies. In reference to our study that is acoustic studies, the Effects of acoustic waves on the growth, development and phytochemicals of *in vitro* raised *Picrorhiza kurroa,* with the help of a function generator we have subjected some acoustic waves of various frequencies on the in vitro raised plants of Picrorhiza kurroa, to foresee the change or enhancement of the phytochemicals in the plant for medicinal beneficiary also we have also performed

1.1.1 Botany

Being sessile, plants constantly navigate their dynamic and complicated environments, recognizing key cues and responding in the right way. Plants have developed the ability to sense a wide range of environmental stimuli as a result, which ultimately assures their effective existence. Plants respond to environmental elements like sunlight, temperature, humidity, and mechanical disturbances (such as wind stimuli, rain, touch stimulus, etc.) by appropriately adjusting their nourishment and development, according to research conducted over the past several centuries. Sound vibrations have been demonstrated to boost plant immunity against diseases and raise the yield of a number of crops.

The plant we used in our research is *Picrorhiza kurroa* [1], an Ayurvedic herb known as Kutaki or Kutki. It consists of a 'bitter principle,' which is a mix of two molecules, the irioid glycosides picroside I as well as picroside II (picroside II is also acknowledged as kutkoside), and the resulting mixture is known as kutkin/picroliv [2]. These are the active components in all.

The herb itself appears to be potently a hepatoprotective (liver-protective) when consumed before or after exposure to a toxin, owing solely to the kutkin molecules. All studied toxins or stressors known to impair liver function, as well as situations of intrinsic hepatic failure, appear to have protective benefits (viral hepatitis and NAFLD caused by a high fat diet).

It is more potent than silymarins at protecting against Tylenol, alcohol, and the deathcap mushroom (the active parts of milk thistle supplementation).

Picrorhiza kurroa's hepatoprotection is hindered by a dearth of human trials [3], with only one study in humans showing that a relatively low dose of the supplement (25mg kutkin) was beneficial against viral hepatitis. Future human research will be required to establish its preventive capabilities, but *picrorhiza kurroa* is a very promising plant right now.

Biology of *Picrorhiza kurroa*

Figure 1: *Picrorhiza kurroa*

The World Health Organization (W.H.O.) has projected that traditional medicine contributes for about 80% of the world's basic healthcare needs [4]. This is primarily due to the fact that medicinal herbs are less expensive than modern pharmaceuticals because they may be cultivated from seeds or harvested for free in natural. India is also called the "World's Vegetable Emporium." Since humanity's origin around 5,000 B.C., Ayurveda, has given aids for terrible diseases. Among many other therapeutic plants, the plant Picrorhiza (Katuki), illustrated in figure 1, is a bitter medicine that has been used in the tr'atment of cancer. Dha"wantari was fam'liar with this plant becausee of India's legendary past. It is an upright, hairy, perennial herb that grows 15-25 cm tall and has a wood rootstock in the Himalayan area at elevations ranging from 3,000 to 5,000 meters. The plant regenerates itself, but unchecked overharvesting has driven it to the brink of extinction. *Picrorhiza kurroa* is now being studied for its hepatoprotective, anti-cholestatic, antioxidant, and immune-modulating properties. For a long time, this plant has been employed in Chinese medicine. This essential plant is occasionally contaminated with other elements. The genus has two species, both of which have distinct qualities, and the plant is mostly found in the Himalayas. Picrorhiza is one such plant that is frequently overlooked in the quest for symptomatic relief. Iridoids, cucurbitacin, and acetophenones are the most significant plant bioactive chemicals.

1.1.2 Medicinal properties of the plant

A. Picrorhiza includes compounds that have been shown to boost body's defense system or immune system, destroy cancer cells, and reduce inflammation or swelling.
B. A skin condition that results in appearing of white spots on the skin (vitiligo). Picrorhiza taken orally for a year in conjunction with a medicine called methoxsalen taken orally and applied to the skin appears to aid in the treatment of vitiligo in people ages.
C. A lung ailment that makes breathing difficult (chronic obstructive pulmonary disease or COPD).

D. Liver swelling (inflammation) (hepatitis). Early study shows that taking picrorhiza orally for two weeks may alleviate symptoms including anorexia, nausea, and overall discomfort in persons suffering from acute viral hepatitis.
E. Rheumatoid Arthritis
F. Picrorhiza has been shown to stimulate the immune system. Taking picrorhiza alongside drugs that reduce immune system activity may reduce the efficacy of these treatments.
   Some drugs that reduce immune system activity include basiliximab (Simulect), azathioprine (Imuran), cyclosporin (Neoral), tacrolimus (FK506, Prograf), muromonab-CD3 (OKT3, Orthoclone OKT3), mycophenolate (CellCept), daclizumab (Zenapax), sirolimus (Rapamune), prednisone
G. Picrorhiza may aid some people with their blood glucose levels. Diabetes drugs are used to reduce blood glucose levels. Taking picrorhiza along with diabetic drugs may cause your blood sugar to drop to a dangerouss level. Keep a tight eye on your blood sugar levels. Your diabetic medication dose may need to be adjusted.
   Rosiglitazone (Avandia), pioglitazone (Actos), glyburide (DiaBeta, Glynase PresTab, Micronase), Glimepiride (Amaryl), insulin, and other diabetic drugs are available.

1.1.3 Dosage of the plant

Vitiligo is a skin disorder characterized by the development of white patches on the skin. To address this condition, a recommended treatment involves taking 200 mg of Picrorhiza rhizome powder twice daily, in combination with methoxsalen, an oral medication applied to the affected skin.

1.1.4 Interactions with medications

- Picrorhiza has been shown to stimulate the immune system. Taking picrorhiza alongside drugs that reduce immune system activity may reduce the efficacy of these treatments.

Several medications that lower immune system activity are available, such as prednisone, tacrolimus (FK506, Prograf), mycophenolate (CellCept), Orthoclone (OKT3), muromonab-CD3 (OKT3), daclizumab (Zenapax),basiliximab (Simulect), and azathioprine (Imuran).

- Picrorhiza may help some people with their blood glucose levels. In addition to managing blood glucose levels, diabetes medications are prescribed to help reduce them. It's important to be cautious when taking picrorhiza alongside diabetic drugs, as it can potentially lead to a significant drop in blood sugar levels. It is crucial to closely monitor your blood sugar levels and consult with your healthcare provider to determine if any adjustments to your diabetic medication dosage are necessary. Rosiglitazone (Avandia), Glyburide (Micronase, Glynase PresTab, DiaBeta), insulin, Glimepiride (Amaryl), and pioglitazone (Actos), other diabetic drugs are available.

1.1.5 Precautions while consuming

- Picrorhiza may reduce blood sugar levels in certain persons. Diabetes drugs are utilized to control blood glucose levels. Combining picrorhiza with diabetic medications can result in excessively low blood sugar levels. Keep an eye on your blood sugar levels. Your diabetic medication dosage may need to be adjusted. Diabetes drugs include glyburide (DiaBeta, Glynase Pres Micronase), glimepiride (Amaryl), pioglitazone (Actos), rosiglitazone (Avandia), insulin, and others.
- Picrorhiza may help some people with their blood sugar levels. Picrorhiza may, in theory, picrorhiza use should be discontinued at least two weeks prior to surgical procedures to avoid disruptions in blood sugar control both during and after the surgery.

1.1.6 Side effects

When taken orally: Picrorhiza is POSSIBLY SAFE for most people to consume orally for up to one year. Some patients may have vomiting, dermatitis, anorexia, diarrhoea, and itching.

1.1.7 Acoustic studies

The way humans have seen nature through the prism of our wisdom has contributed to our cynicism that plants can have senses akin to ours. As a result, the Aristotelian point of view that differs animals from humans [5].

For so long, plants have prevailed based on their ability to sense. However, given the rapid sensory movements, this argument has been supported by carnivorous plants. Revealed to be false This raises an important question-

Can plants detect temperature and sound if they can detect touch?

Plant acoustics research began in the 1950s, with numerous controversial papers asserting the influence of musical sounds on plants.

To investigate the acoustic responses in plants more, scientists used natural SVs produced by birds chirping, crickets stridulating, bees buzzing, and so on, and obtained intriguing results.

Natural SVs, for example, have been found to boost seed germination rates in zucchini (Cucurbita pepo) and okra (Abelmoschus esculentus). Several researchers have begun to use SVs with variable single frequencies as a further refinement. Collins and Foreman (2001) observed frequency-specific reactions in common beans (Phaseolus vulgaris) as well as impatiens (Impatiens sp.) when various SV frequencies (500, 5000, 6000, 12 000, 14 000 Hz) were applied at the identical pressure level (91-94 decibels, dB). Despite the fact that both examples exhibited enhanced growth, beans reacted best at 5000 Hz, whereas impatiens responded best at 12 000 Hz.

Sound stimulation boosted oxygen absorption and polyamine levels in Chinese cabbage and cucumber, with Chinese cabbage being more sensitive.

Acoustic waves are a type of energy propagation method that uses adiabatic loading and unloading to move through a medium. Acoustic pressure, particle velocity, particle displacement and acoustic intensity are all important quantities for describing acoustic waves.

Acoustics is the science of sound production, control, transmission, reception, and effects [6]. The term comes from the Greek acoustos, which means "heard."

Since its roots in the study of mechanical vibrations and their transmission via mechanical waves, acoustics has found critical applications in practically every aspect of life. It has been crucial to many creative advances, some of which came after significant experimentation by artists and were only much later described as a theory by scientists, notably in the sphere of musical scales and instruments.

Three properties of sound waves—frequency (Hz), strength (dB), and timbre—distinguish one sound from another at the same frequency [7]. The qualities of the medium affect the speed of sound transmission; in particular, it is inversely and directly related to density.

These are the different frequency bands that makeup naturally occurring sound:

- Low-frequency ranges are 20 Hz - 200 Hz
- Medium-low ranges are 200 Hz -1 kHz
- Medium-high ranges are 1 kHz -5 kHz
- High-frequency ranges are 5 kHz -20 kHz

In general, three things influence how sound travels. The first of these the link between the medium's density and pressure, It is impacted by temperature and regulates the speed of sound waves inside the medium. The second element is known as medium motion: as the medium moves, the absolute speed of the sound wave might change depending on the direction of the movement. The third consideration is viscosity. The attenuation produced due to viscosity is minor in water or air.

Furthermore, by directly connecting a sensor to the plant, 20-100 kHz ultrasonic vibrations may be detected. Depending on the stage of growth or the environment, plants generate sound waves from a number of organs. Plants produce sound and faint ultrasound when agitated, according to research using small, extremely sensitive sound receivers.
Though little is known about how plants communicate by sound, it is known that they can generate sound waves at very low frequencies, such as 50-120 Hz. Furthermore, by simply attaching a 20-100 kHz ultrasonic vibration sensor to a plant, ultrasonic vibrations between 20 and 100 kHz may be monitored.

1.1.7.1 Plant acoustic frequency technology (PAFT)

Plant acoustic frequency technology is a cutting-edge area that investigates plant vibrational features [8]. It entails analysing and investigating the patterns and frequencies of acoustic signals released by plants. This developing technology has demonstrated remarkable possibilities in a number of sectors. Scientists may learn about a plant's overall health, stress levels, and growth patterns by decoding its acoustic frequencies. It also provides a unique method for monitoring and detecting illnesses or pests, assisting in early detection and prevention. Furthermore, using specific sound frequencies, this technology has the potential to improve agricultural practises by optimising irrigation, nutrient delivery, and potentially affecting plant development. Plant acoustic frequency technology has a lot of potential for sustainable farming and environmental protection.

1.1.7.2 Interferometer

Figure 2 depicts interferometers, which extract information through interference [9]. They are often used in research and industry to quantify minute displacements, changes in refractive index, and surface defects. Most interferometers split light from a single source into two beams that travel in separate optical channels and are then combined to create interference; however, two incoherent sources can also be made to interfere under specific conditions. Interferometry uses the superposition principle to combine waves in such a manner that the output has some significant quality that is diagnostic of the original state of the waves. When two separate waves with the same frequency combine, the phase difference between the two waves determines the final intensity pattern—waves that are in phase will suffer constructive interference, whereas waves that are out of phase will undergo destructive interference.

Figure 2: Interferometer

1.2 Objective

- To carry out large-scale multiplication of the *Picrorhiza kurroa* plant.
- To see the effect of acoustic waves on the metabolites of *Picrorhiza kurroa*.

1.3 Methodology

The experiment started by preparing 20 jars of media cultures for planting the respective plant that is *Picrorhiza kurroa*, the experiment was carried on in Jaypee University of Information Technology, Solan.

- For PAFT setup was prepared using interferometer and thermocol compartment was prepared to make the environment soundproof
- Later the plants were subjected to sound waves of 500Hz, 1000Hz, and 1500Hz

- Some plants were then used to calculate acoustic parameters like acoustic impedance, adiabatic compressibility, and relaxation time
- On the other hand regular data of both the test plant growth in the set up after 10 days and control plant growth after 10 days were maintained
- For checking the presence of secondary metabolites phenol testing as well as flavonoid testing was performed.

1.4 Organization

There are 5 sections in the project report.

1. The goal of the first chapter resides on introducing the elements of the project and their respective applications
2. The objective of the 2nd chapter is to provide the project's literature review and its respective analysis.
3. The material and methods regarding the project is depicted in Chapter 3.
4. Chapter 4 contains the results related to the method applied.
5. Conclusion as well as future scopes are explained in Chapter 5.

CHAPTER-2 LITERATURE SURVEY

In this section, we have reviewed some research studies, how the researchers have used different methods using different acoustic methods and technologies to get an overview on how it changes plant morphology and anatomy. Over the years various methods were discussed and developed by the researchers, and some of them are discussed.

Cate et al. [10], herbs are still frequently used by the global population due to their higher compatibility with the human body and lower adverse effects. As a result, the globe has shifted its focus to natural products. *Picrorhiza kurroa* is a tiny perennial herb found mostly in the Himalayan area at elevations ranging from 3,000 to 5,000 metres. The plant's leaves are flat, oblong, and highly serrated. The leaf, bark, and subterranean plant components, primarily rhizomes, are commonly utilised in traditional medicine. The main glycoside is 'Kutkin,' a combination of (picroside I as well as II) and has considerable hepatoprotective properties. The plant's main use are owing to its hepatoprotective, anti-cholestatic, antioxidant, and immunomodulatory properties. Many biological activities generate incidental noises, such as heartbeats, breathing, digestive activity, and body movements in animals, to name a few, therefore it is surprising that similar sounds do not exist in plants. Acoustic signals, or sounds that evolved as a result of their impact on receivers, are often differentiated by being structurally distinct from accidental noises, as well as being substantially louder, since they evolved to reach and be noticed by a receiver. Many of the plant sounds recorded, such as drought-related cavitation (e.g., Jackson and Grace 1996; Kikuta et al. 1997; Perks et al. 2004) or other processes (e.g., Laschimke et al. 2006), may only be detected by particular equipment securely linked to the plants. If sounds are to be effective in communication, they must be more than just byproducts.

Masood et al. [11], *Picrorhiza kurroa* Royle ex Benth., sometimes known as Kutki, is a Scrophulariaceae plant. It may be found in China, Pakistan, India, Bhutan, and Nepal's Himalayan areas. It is regarded as a valuable medicinal plant, and it is commonly used in traditional medicine to treat asthama, jaundice, fever, malaria, snake bite, and liver diseases. P. kurroa has been shown to have anti-microbial, anti-oxidant, anti-bacterial, antimutagenic, cardio-protective, hepato-protective, anti-malarial, anti-diabetic, anti-inflammatory, anti-cancer, anti-ulcer, and nephro-protective effects. So far, the phytochemicals isolated from P. kurroa include iridoids (Picroside I and II), cucurbitacins, and phenolic components. The conservation status of P. kurroa in several locations has become threatened due to over-exploitation for therapeutic uses. It is critical to apply various conservation techniques to safeguard this medicinal asset from extinction since it is widely utilised by the local people to cure various maladies, putting enormous pressure on the plant population.

In [12], the authors have the ultrasonic velocity (U) of Ocimum Tenuiflorum leaf extract in distilled water was evaluated experimentally at a range of 2 MHz in binary mixes with concentrations that varied from 0.1% to 0.0125%. Temperatures of 30°C, 35°C, and 40°C were used in the measurements. Various acoustical characteristics, such as adiabatic compressibility (a), relaxation time (), acoustic impedance (z), free length (Lf), free volume (Vf), and internal pressure (Pi), were determined using density, ultrasonic velocity, and viscosity. Wada's constant was discovered to be very beneficial for predicting and validating molecular interactions. Any changes in these parameters give crucial information about the molecular interactions taking place in the solution.

The ultrasonic velocity, density, and viscosity of a solution containing Tridax Procumbens root extract in distilled water were measured experimentally by kamble et al. [13]. The measurements were taken at three distinct temperatures: 298.15 K, 303.15 K, and 308.15 K, at a constant frequency of 4 MHz. Several acoustical metrics, including adiabatic compressibility, intermolecular free length, relaxation time, and specific acoustic impedance, were investigated to obtain insight into molecular interactions. These characteristics are critical in predicting and validating molecular interactions. The ultrasonic velocity, density, and viscosity of the solution were measured to achieve these results. Variations in these parameters reveal important information about the molecular interactions taking place in the solution. The combined data of ultrasonic velocity, density, and viscosity provide a thorough insight of ion, dipole, and hydrogen bonding interactions.

Forrest et al. [C] mentioned when acoustic signals are sent across great distances, they alter significantly from the original sounds created by the sender. Due to the effect of the surrounding environment, these changes include a drop in amplitude, changes in spectral properties, and changes in temporal structure. A significant amount of study in the field of acoustic communication is devoted to comprehending the environmental factors that influence animal transmissions. In this lecture, I will look at the physical constraints that the environment places on auditory communication. In addition, I will look at how anurans (amphibians like frogs) and insects communicate, and how these similarities relate to their various signalling systems in the setting of environmental restrictions. Finally, I will show how signal alterations during propagation impact signal perception during phonotaxis (sound-induced movement), influencing mate choice and sexual selection.

Hence from the research mentioned above we can conclude that:

- The plant *Picrorhiza kurroa* is of great medical importance and is widely used for its therapeutic as well as curative properties, so it is necessary for researchers to dig into this phenomenon
- Acoustic studies have been carried out on many plants and have recorded the efficient results for the same, so by carrying out these studies on *Picrorhiza kurroa* we wanted to observe the changes occurring in these plants when subjected to acoustic waves

CHAPTER-3 MATERIALS & METHODS

In this section, we have discussed the procedure followed for our experimentation as well as the respective materials required for the completion of the method.

3.1 Plant material used

The plant of *Picrorhiza kurroa* was cultured and stored at -80°C in the laboratory of JUIT Solan, later grinded for further use.

3.2 Media Preparation

Material required: Stock A-H, IBA, Kinetin, sucrose, pH meter, agar-agar, distilled water, beaker, hot plate, and autoclave.
The procedure of media preparation is shown in flowchart 1. Figure 3 shows the media prepared.

Take the following material as follows:-
a. Stock A :- 100 ml/l b. Stock B:- 50ml/l c. Stock C-H:- 10ml/l
Mix the above solution order wise

Now mix IBA 1mg/l and further add kinetin 3mg/l
Later add 30gm/l sucrose
Make the volume up to 950 volume
Adjust the pH to 5.6-5.7

Now make the volume up to 1000vol
Now mix agar- agar 9g/l and heat it till the media is properly mixed and further autoclave the media prepared

Flowchart 1: Protocol for preparation of media

Figure 3: Media prepared in the laboratory

3.3 Culturing

The next phase is to cultivate plants in such a medium. Plant tissue culture is characterized as the growth of tissues, organs, explants, cells, plant seeds, or protoplasts on a chemically prescribed synthetic nutritional medium in a sterile and regulated light, temperature, and humidity environment. Flowchart 2 depicts each of the cultivation steps. The cultivated plants are seen in Figure 4.

Turning On LAF and then Turn on UV for 20 minutes before doing culturing in LAF.

Sterlize Laf , forceps , Petridishes, Flasks, with the help of 70%IPA
Start the gas burner and heat sterlize your forceps and petriplates
explants.

Then take a *Picrorhiza kurroa* plant from ptc lab and with the help of forceps divide it into smaller explants.

After that take media bottles heat sterlize your bottles and caps on gas burner . Sterlize forceps on every use to prevent contamination

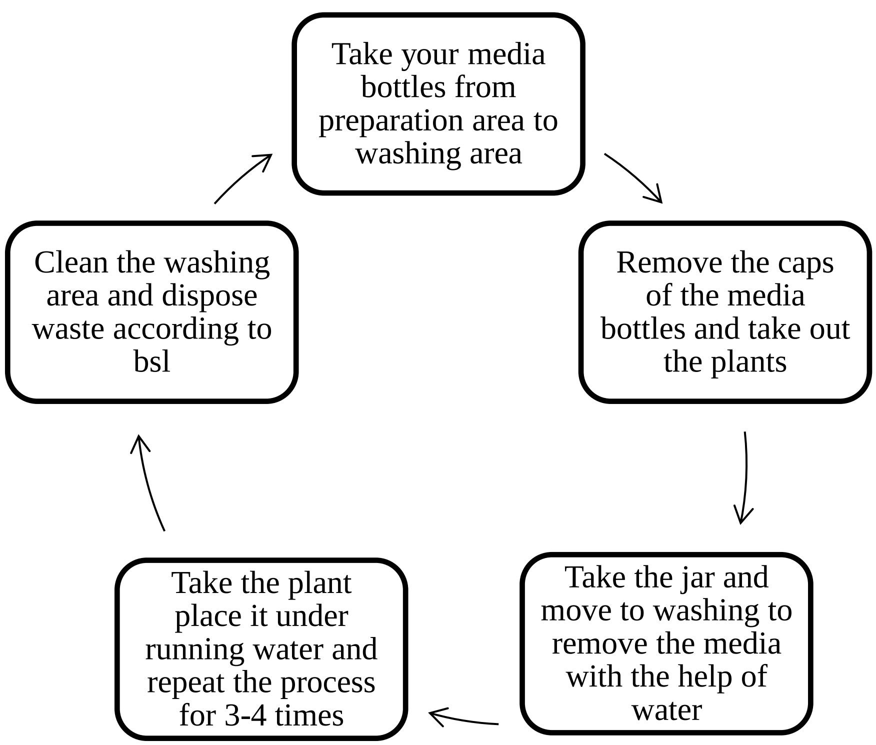
Take one explant and plant it diagonally in that media in such a way that its root get imbedded in the media.

Flowchart 2: Protocol for culturing of plants

Figure 4: Shows the cultured plants

3.4 Removal of plants

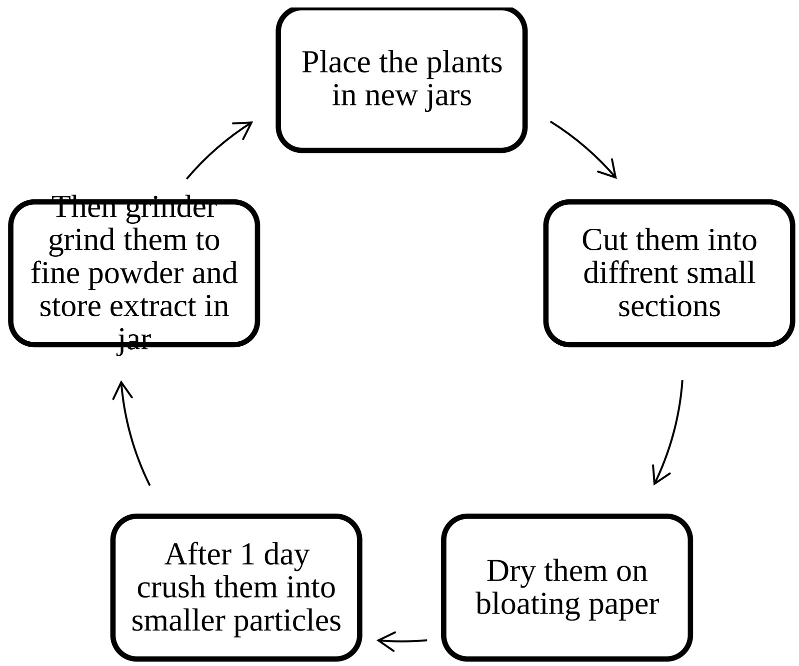
Plants were removed after culturing, the procedure of removal of the same is explained in flowchart 3.



Flowchart 3: Protocol for removal of plants

3.5 Drying and grinding

Protocol for drying and grinding is explained via flowchart 4 and the results of the same are captured in figure 5.



Flowchart 4: Protocol for drying and grinding

Figure 5: Plants dried and grinded for use

3.6 Extract preparation

- Take your media and dissolve it into 80% methanol
- Place it in a shaker for 24 hours
- Filter your extract solution and place it in the refrigerator for further use
- The extract prepared is shown in Figure 6.

Figure 6: Extract dissolved in methanol and then filtered

3.7 Preparations for set up

The material used: Function generator, thermocol sheets, speaker and extract

- Function generator: A function generator is an electronic device that generates a signal waveform with adjustable frequency, amplitude, and waveform shape. It is commonly used in electronics and electrical engineering laboratories for various applications, such as testing and calibration of electronic equipment, waveform analysis, and modulation. Function generators can produce different types of waveforms, including sine, square, triangle, and sawtooth waves. They usually have a built-in frequency counter, modulation capability, and a digital display for accurate frequency and amplitude adjustments. Some advanced models also offer arbitrary waveform generation, which allows users to generate custom waveforms for specific applications.

- For our respective experiment we used it for projecting sound waves in the form of sine waves at a particular frequency to our plants. A speaker was connected to the function generator to act as a media for supplying sound waves to plants and a thermocol compartment was prepared to arrange and make a setup. Figure 7 shows function generator.

Figure 7: Function generator

Figure 8 shows an interferometer connected with the speaker to deflect sound waves on the in vitro plants, the device was placed in the thermocol compartment to make it soundproof properly so that no outer disturbance can interfere in our study.

Figure 8: Themocol setup for soundproof environment

3.8 Phytochemical analysis

- Qualitative analysis is critical for identifying phytochemical components in medicinal plants. Plants have therapeutic potential because of the existence of certain bioactive components.
- Now this analysis will be done by calculating the density, viscosity as well as the velocity of the plant extracts (of both test and control plants)
- This analysis is done to calculate the molecular interactions and chemical analysis of the extracts.
- The extracts will be made by the earlier-mentioned method of drying and grinding.

3.9 Using digital ultrasonic pulse-echo velocity meter

The material used: Velocity meter, water bath and extract

Velocity meter: A velocity meter, also known as a flow meter, is a device that measures the velocity of a fluid within a closed system, often a gas or liquid. It is used in a variety of sectors, including water management, oil and gas, HVAC, and environmental monitoring. To calculate flow rate, and velocity use various concepts such as differential pressure, ultrasonic, electromagnetic, or thermal approaches. These meters calculate the volumetric flow rate or mass flow rate by correctly measuring velocity. To provide precise and consistent measurements of fluid velocities, velocity meters are essential in process control, efficiency optimization, and monitoring systems. Figure 9 shows the pulse echo velocity meter.

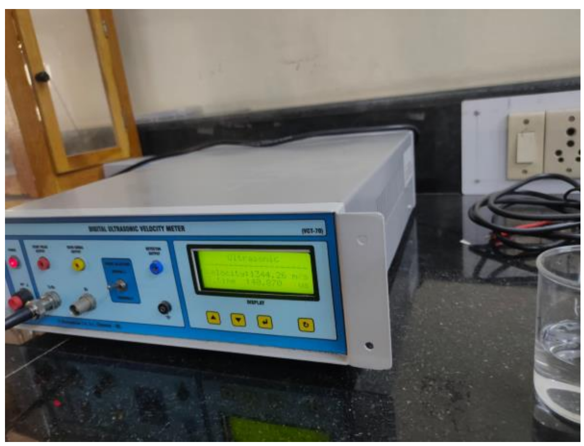


Figure 9: Shows pulse echo velocity meter

Water bath: A water bath is a piece of laboratory equipment used to adjust the temperature of samples. It is made up of a container filled with water that is heated or cooled with the use of a heating or cooling element. The materials are put in a smaller container, such as a test tube or beaker, and submerged in a water bath for precise temperature control during investigations or operations like incubation, melting, or thawing. Water baths are widely utilised in scientific research, medical laboratories, and a variety of industrial applications that require temperature control for sample handling and testing. Figure 10 shows water bath.

Figure 10: Water bath

3.9.1 Using temperature controller water bath

Flowchart 5 depicts the usage of temperature-controlled water baths.

Fill the temperature controlled water bath to all levels above the float switch level at first; if the alert sounds, instantly turn off the equipment, add additional water, and try again.

⇩

If the temperature control unit is turned on without water or with water below the float switch level, an alert will sound as soon as the heater bursts.

⇩

After the measures have been taken, turn on the machine and set the temperature in degrees Celsius by adjusting the SET adjust with the PUSH switch.

⇩

When the water reaches the prescribed temperature, the relay turns off and the temperature is maintained; when the temperature drops, the relay turns on and heats the water while maintaining the temperature.

⇩

Now, alter the needed speed of the motor by varying the linear POT supplied for SPEED. Now, water flows to the ultrasonic liquid cell through the INLET and water flows to the temperature controller through the OUTLET.

Flowchart 5: The usage of water bath

3.9.2 Ultrasonic working procedure

For operating with the liquids keep the switch on normal mode

LCD display show:

1. Liquid
2. Solid

After selection following options pop up:

1. Manual mode
2. Auto mode
3. Echo mode

We selected AUTO mode, by pressing increment key twice and then pressing ENTER key. It calculates Velocity and Time.

On MANUAL mode velocity and distance is calculated by pressing the increment key accordingly

On ECHO mode the processor generates a start pulse of 2khz

The readings were observed and collected by putting the device on auto mode and the results are depicted in figure 11.

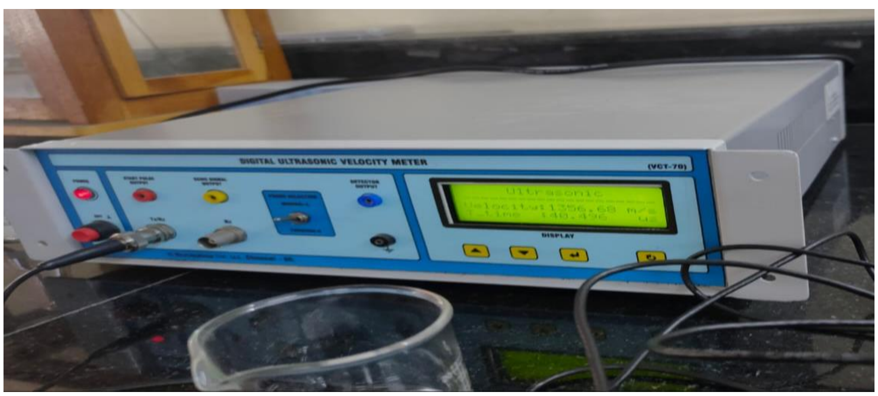


Figure 11: Readings obtained from ultrasonic velocity meter

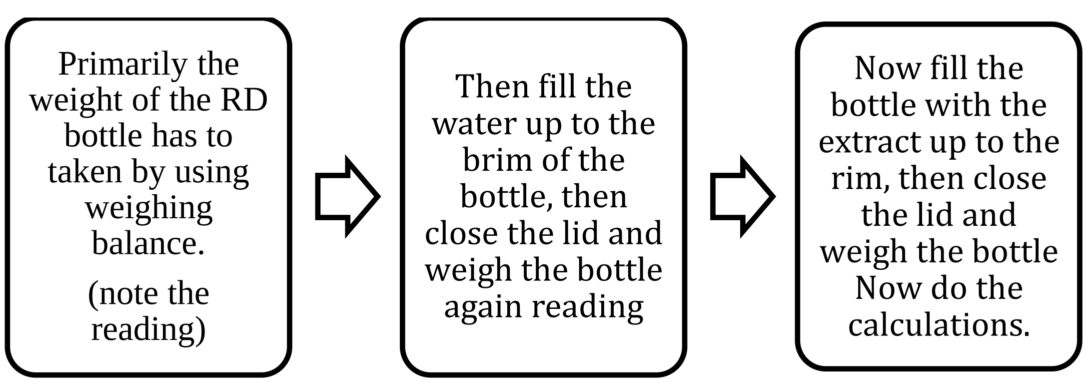
3.10 Calculating the density of the substance

Material used: Weighing balance, extract, and relative density bottle.

Weighing balance: A weighing balance is a basic instrument for properly measuring the mass or weight of items. It is made from a horizontal beam or lever with a fulcrum in the middle that allows two pans to be suspended on each side. The weighing object is placed on one pan, then standard weights are added to the other pan until balance is reached. The balance works on the equal-arm balancing principle, which ensures that the weight of the object is directly proportionate to the weight of the standard weights. Weighing scales are used in a variety of settings, including labs, industries, and commerce, to enable exact measurements and ensure trade fairness.

Relative density bottle: A specific gravity bottle, also known as a relative density bottle, is a specialized device used to assess the relative density or specific gravity of liquids. It is usually constructed of glass, with a thin neck and a ground-glass stopper. The bottle is calibrated to hold a specified amount of liquid, which is generally indicated on the body. To determine the relative density, the bottle is weighed empty first, then filled with the liquid under consideration. The mass of the liquid is determined by the weight difference between the empty and filled bottles. The relative density or specific gravity may be estimated by dividing the mass of the liquid by the volume specified on the container. Quality relative density bottles are extensively employed in scientific research.

- Plant density is calculated in order to perform a phytochemical analysis of the extract.
- The density is calculated by using the RD bottle (relative density bottle).
- In this experiment we have taken distilled water as our respective standard.
- Figure 12 shows RD bottle filled with distilled water and figure 13 shows RD bottle filled with the extract prepared.
- The process is shown in flowchart 6.



Flowchart 6: The process of calculating the density of the extract

Figure 12: RD bottle filled with distilled water

Figure 13: RD bottle filled with extract

3.11 Using viscometer

The material used: Viscometer, extract, and spindles

Viscometer: A viscometer is an instrument used to measure the viscosity or resistance of a fluid to flow. It is commonly used in industrial and research settings to determine the flow properties of various liquids, including oils, paints, adhesives, and food products. The instrument works by measuring the time it takes for a fluid to flow through a capillary tube or spindle, under a specific temperature and shear stress. There are different types of viscometers available, such as rotational, falling ball, and capillary tube viscometers, each with its advantages and limitations. Accurate viscosity measurements are essential for ensuring product quality, consistency, and performance, and for complying with industry standards and regulations.

Spindles: Spindles are cylindrical or conical rods that are utilised in a variety of applications, most notably equipment and manufacturing operations. They function as rotating axes or as supports for things such as wheels, tools, and fabrics. Spindles are used in a variety of sectors, including woodworking, metalworking, textiles, and automotive, to provide stability and facilitate rotational movement. Working method is shown in flowchart 7.

Sample preparation entails preparing and conditioning the sample fluid to the proper temperature and viscosity range for testing.

↓

Calibration: To provide accurate and consistent readings, the viscometer is calibrated with a known viscosity reference fluid.

↓

Loading the sample: The sample is placed in the measurement chamber of the viscometer, which might be a capillary tube, a revolving spindle, or a falling ball.

↓

Shear stress is given to the fluid by turning the spindle or letting the ball to fall due to gravity. The viscometer measures the flow resistance.

↓

The time it takes for the sample to pass through the measurement chamber or the torque necessary to rotate the spindle are both measured.

Flowchart 7: Procedure to measure the viscosity of the extract prepared

3.12 Total Phenol content in the samples

Phenol testing was done on both control as well as PAFT subjected test plants. The procedure to check the phenolic content in the extract prepared is depicted in flowchart 8. The recording of the content taken and measured are depicted in table 1.

0.2 ml of sample was diluted with 8.3 ml of distilled water and then mixed with o.5 ml of Folin-Ciocalteu reagent

↓

After mixing 1 ml of 15% (w/v) $Na2CO3$ was added and kept in dark for 30 min at room temprature

↓

The absorbance of gallic acid was measured spectrophotometrically at 760 nm and concentration was calculated using gallic acid astandard

Flowchart 8: procedure to check presence of phenolic content in the extract

Table 1: Recording of the content taken and measured

Gallic acid (in microliter)	**DH_2O (in microliter)**	**DH_2O (in ml)**	**FC (in ml)**	**Na_2CO_3 (in ml**
blank	-	8.5	0.5	1
40	160	8.3	0.5	1
80	120	8.3	0.5	1
120	80	8.3	0.5	1
160	40	8.3	0.5	1
200	0	8.3	0.5	1

3.13 Total flavonoid content in the sample

Flavonoid testing was done on both controls as well as PAFT-subjected test plants. The procedure to check the presence of total flavonoid content in the extract is depicted in flowchart 9. Recordings of the content taken and measured are depicted in table 2.

Five control conc. were made and ouercetin was used as standard with 1mg/ml of methanol.

⇩

500 micrliter extract was taken which was diluted with 2ml distilled water.
after which 150 microliter sodium nitrite and 150 microliter of aluminium chloride was allowed to rest for 5 and 6 mins respectively.

⇩

Later 1M of NaOH was added and kept for 15 mins
Further the absorbance was taken at 510 nm.

Flowchart 9: Procedure to check the total flavonoid content in the extract

Table 2: recording of the content taken and measured

S.No	**Conc.(ul)**	**Methanol (ul)**	**dH2O (ml)**	**NaNO2 (ul)**		**AlCl3 (ul)**		**NaOH (ul)**	
C1	100	900	2	150	Stand For 5min	150	Stand For 6min	1000	Stand For 15 mins
C2	200	800	2	150		150		1000	
C3	300	700	2	150		150		1000	
C4	400	600	2	150		150		1000	
C5	500	500	2	150		150		1000	
Blank	0	1000	2	150		150		1000	
Sample	500	500	2	150		150		1000	

CHAPTER-4 RESULTS AND ANALYSIS

In this section, we have discussed the results of our experimentation with the help of Plant acoustic frequency technology, the results shown below clearly depict the changes that occurred due to applying this technology using our device interferometer connected with the speaker placed in the thermocol setup to make it soundproof.

4.1 Plant height

Below is the recording of the control and test plants before placing them in the setup for 10 days in table 3 in 500 hz. Figure 14 shows the test plants to be kept in setup and figure 15 shows the control plants.

Table 3: Recordings of the control and test plants at 500 hz

Plant height (Control)	Plant height (Test)
C1-2.5cm	T1-2.5cm
C2-3.5cm	T2-3.5cm
C3-3cm	T3-3cm
C4-3cm	T4-3cm
C5-5cm	T5-5cm

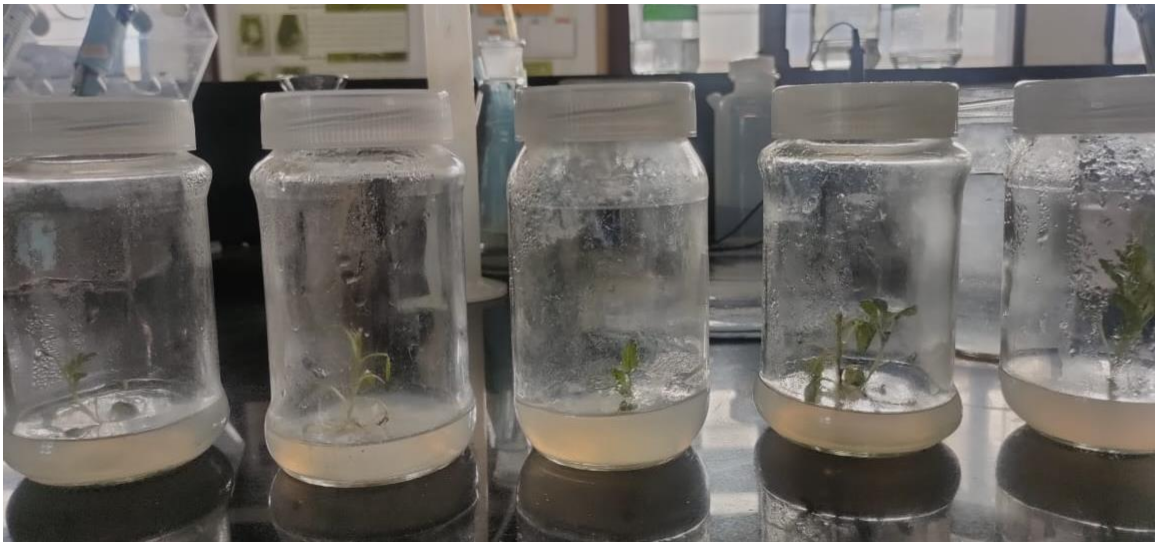


Figure 14: Test Plants before placing them in setup

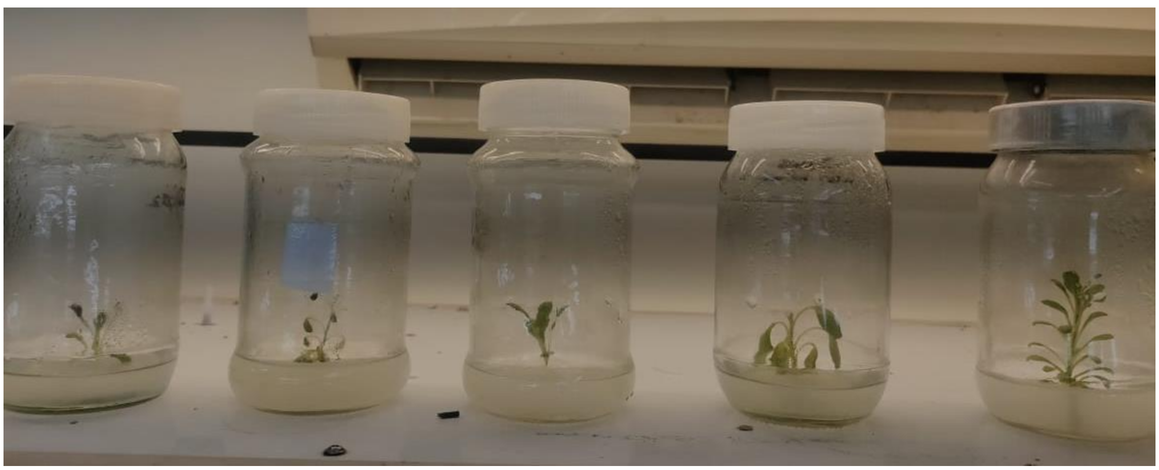


Figure 15: Control Plants

Below is the recording of the control and test plants after placing them in the setup for 10 days in table 4 in 500 hz. Figure 16 shows the test plants after 10 days in the setup and figure 17 shows the control plant after 10 days.

Table 4: Recordings of the control and test plants at 500 hz after 10 days

Plant height (Control)	Plant height (Test)
C1-2.5cm	T1-4.5cm
C2-3.5cm	T2-5cm
C3-3cm	T3-3cm
C4-3cm	T4-7cm
C5-5cm	T5-5cm

Figure 16: Test Plants before placing them in setup after 10 days

Figure 17: Control plants after 10 days

Recordings of the control and test plants are depicted below in table 5 at 1000hz. Figure 18 shows the test plants to be kept in setup and figure 19 shows the control plants.

Table 5: Recordings of the control and test plants are depicted at 1000 hz

Plant height (Control)	**Plant height (Test)**
C1-2.5cm	T1-2.5cm
C2-3.5cm	T2-3.5cm
C3-3cm	T3-3cm
C4-4cm	T4-4cm
C5-5cm	T5-5cm

Figure 18: Test plants before placing them in the setup

Figure 19: Control plants

Recordings of the plants after 10 days is depicted in table 6 at 1000 hz. Figure 20 depicts the test plants after 10 days in set up and figure 21 depicts the control plants after 10 days.

Table 6: Recordings of the plants after 10 days at 1000 hz

Plant height (Control)	**Plant height (Test)**
C1-2.5cm	T1-4.5cm
C2-3.5cm	T2-6cm
C3-3cm	T3-3cm
C4-4cm	T4-7cm
C5-5cm	T5-5cm

Figure 20: Test plants after 10 days in setup

Figure 21: Control plants after 10

Readings of control and test plants are depicted below in table 7 at 1500 hz. Figure 22 shows the control plants and figure 23 shows the test plants before keeping them in the set up.

Table 7: Recordings of control and test plants at 1500 hz

Plant height (Control)	**Plant height (Test)**
C1-2.5cm	T1-3cm
C2-3.5cm	T2-3cm
C3-3cm	T3-2.5cm
C4-4cm	T4-3.5cm
C5-5cm	T5-3cm

Figure 22: Control plants

Figure 23: Test plants before placing them in the setup

Recordings of control and test plants after 10 days are depicted below in table 8 at 1500. Figure 24 depicts the test plants after 10 days in the setup and figure 25 shows the control plants after 10 days.

Table 8: Recordings of control and test plants after 10 days at 1500 hz

Plant height (Control)	Plant height (Test)
C1-2.5cm	T1-4.5cm
C2-3.5cm	T2-5cm
C3-3cm	T3-2cm
C4-4cm	T4-7cm
C5-5cm	T5-5cm

Figure 24: Test plants after 10 days in the set up

Figure 25: Control plants after 10 days

4.2 Acoustical Parameter

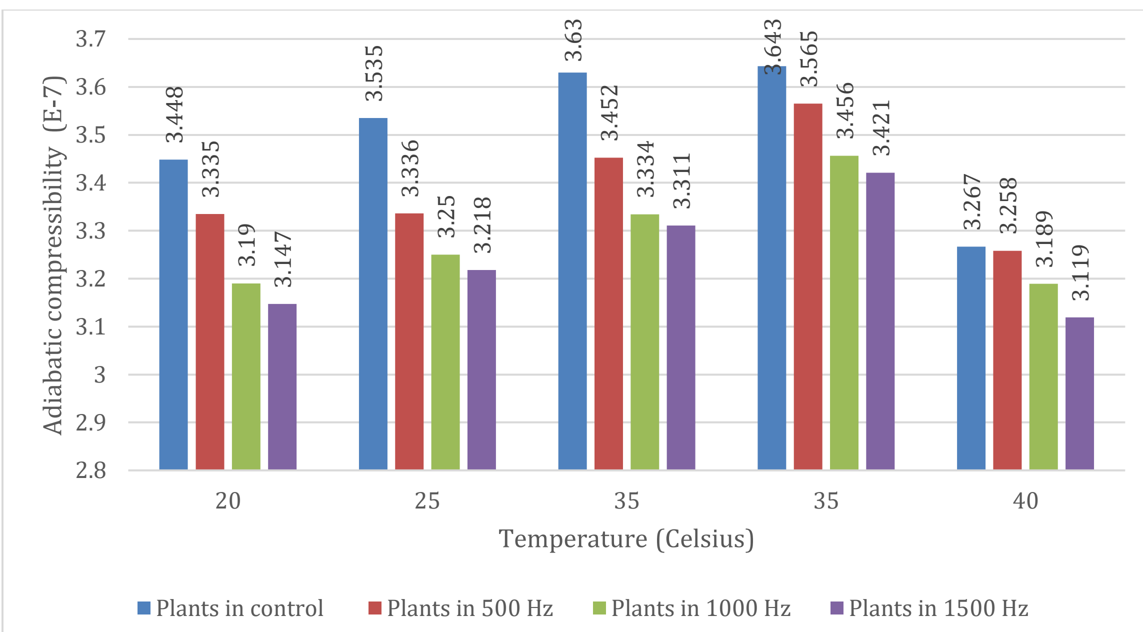
Acoustical parameters are depicted in the following table 9.

Table 9: Depicts the acoustical parameter of both test and control plants

Acoustical Parameter	**Temperature**	**Plants in control**	**Plants in 500 Hz**	**Plants in 1000 Hz**	**Plans in 1500 Hz**
Ultrasonic Velocity	20° 25° 35° 35° 40°	1306.76m/s 1309.40m/s 1328.86m/s 1352.70m/s 1442.92m/s	1312.74m/s 1325.12m/s 1341.23m/s 1362.65m/s 1478.54m/s	1323.34m/s 1333.56m/s 1348.89m/s 1369.90m/s 1481.89m/s	1328.65m/s 1339.98m/s 1356.76m/s 1375.17m/s 1494.81m/s
Density	20° 25° 35° 35° 40°	1.70(8.5%) 1.65(5.5%) 1.56(5.2%) 1.50(5%) 1.47(4.9%)	1.74(8.5%) 1.69(5.5%) 1.61(5.2%) 1.58(5%) 1.54(4.9%)	1.79(8.5%) 1.73(5.5%) 1.65(5.2%) 1.66(5%) 1.57(4.9%)	1.80(8.5%) 1.76(5.5%) 1.66(5.2%) 1.68(5%) 1.61(4.9%)
Viscosity	25° 35° 35° 40°	0.8803gm/cm3 0.879gm/cm3 0.8777gm/cm3 0.8711gm/cm3	0.8813gm/cm3 0.881gm/cm3 0.8787gm/cm3 0.8731gm/cm3	0.8837gm/cm3 0.889gm/cm3 0.8790gm/cm3 0.8751gm/cm3	0.8853gm/cm3 0.895gm/cm3 0.8801gm/cm3 0.8781gm/cm3
Adiabatic compressibility	20° 25° 35° 35° 40°	3.448 E^{-7} 3.535 E^{-7} 3.630 E^{-7} 3.643 E^{-7} 3.267 E^{-7}	3.335 E^{-7} 3.336 E^{-7} 3.452 E^{-7} 3.565 E^{-7} 3.258 E^{-7}	3.190 E^{-7} 3.250 E^{-7} 3.334 E^{-7} 3.456 E^{-7} 3.189 E^{-7}	3.147 E^{-7} 3.218 E^{-7} 3.311 E^{-7} 3.421 E^{-7} 3.119 E^{-7}

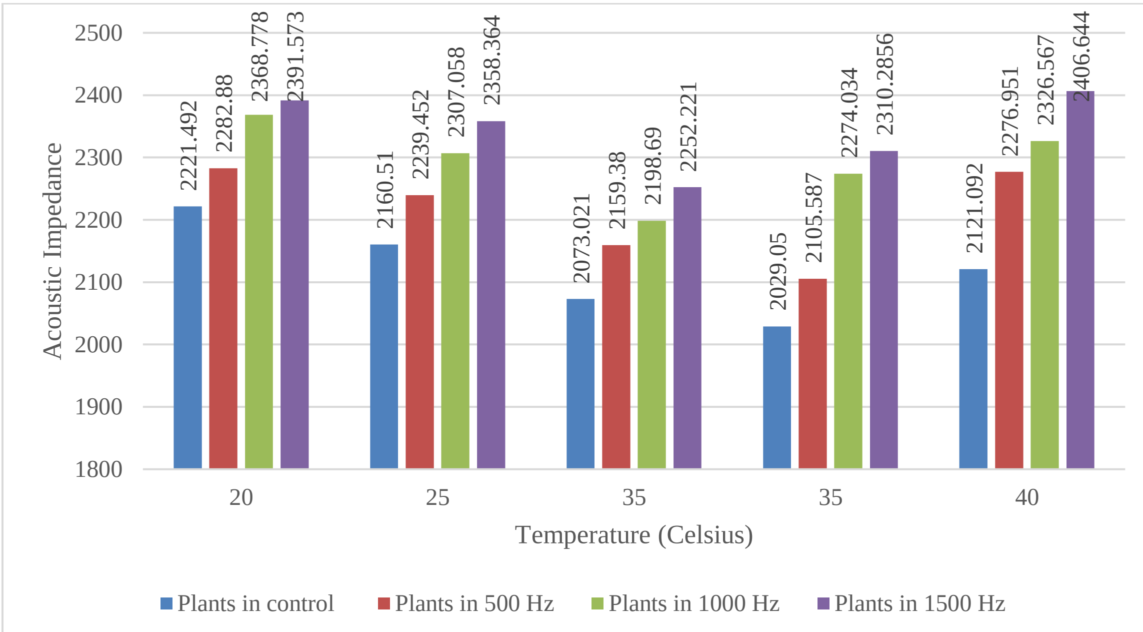
Acoustic Impedance	20° 25° 35° 35° 40°	2221.492 2160.510 2073.021 2029.050 2121.092	2282.880 2239.452 2159.380 2105.587 2276.951	2368.778 2307.058 2198.690 2274.034 2326.567	2391.573 2358.364 2252.221 2310.2856 2406.644
Relaxation time	25° 35° 35° 40°	4.03691 E^{-8} 4.13266 E^{-8} 4.23744 E^{-8} 3.78502 E^{-8}	3.91022 E^{-8} 4.04481 E^{-8} 4.16631 E^{-8} 3.78326 E^{-8}	3.81979 E^{-8} 3.94202 E^{-8} 4.04030 E^{-8} 3.71162 E^{-8}	3.78903 E^{-8} 3.94124 E^{-8} 4.00439 E^{-8} 3.64259 E^{-8}

The graph 1 depicts the difference in adiabatic compressibility of the control as well as various test plants. As shown in graph 1 the compressibility is less in test plants as compared to control plants at various temperatures.



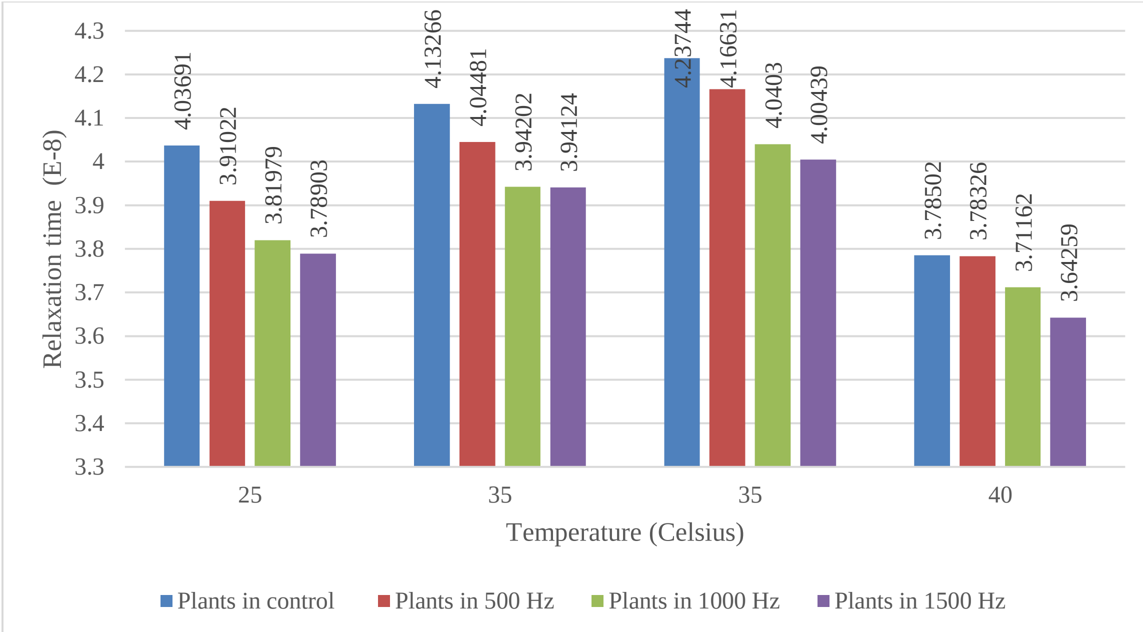
Graph 1: Depicts the difference between the adiabatic compressibility between test and control plants

The graph 2 depicts the difference in acoustic impedance between control and test plants. As shown in graph 2 acoustic impedance in more in test plants as compared to control plants at various temperatures.



Graph 2: Depicts the difference between acoustic impedance between test and control plants.

The graph 3 depicts the difference in relaxation time between test and control plants. As shown in graph 3 relaxation time is less in test plants as compared to control plants at various temperatures.



Graph 3: Depicts the difference between relaxation time between test and control plants.

4.3 Phenol Testing

Phenol testing was done to ensure the presence of secondary metabolites in both the control and test plants**.** Below are recordings of the phenol testing in the samples in table 10.

Table 10: Recordings of phenol testing

Sample	**OD**
blank	0.047
C1	0.134
C2	0.303
C3	0.384
C4	0.615
C5	0.711
S1(control)	0.096
S2(Test)	0.191
S3(-80^0 Test)	0.480

4.4 Flavonoid Testing

Flavonoid testing was done to ensure the presence of secondary metabolites in both the control and test plants. Below are recordings of the Flavonoid testing in the samples in table 11.

Table 11: Recording Flavonoid Testing

Sample	**OD**
blank	0.084
C1	0.268
C2	0.337
C3	0.281
C4	0.363
C5	0.290
S1(control)	0.176
S2(Test)	0.249
S3(-80^0 Test)	0.722

CHAPTER-5 CONCLUSIONS

5.1 Conclusions

Sound stimuli may influence germination rates and enhance plant growth and development, improving the yield of some crops. Through our project, we are going to validate the meaning and effect of sound waves on plants.

In this experiment, we have attempted to generate some changes in our respective plants in order to observe any phytochemical and molecular changes.

Morphological and phytochemical changes are observed by measuring and comparing the heights of control and test plants. The velocity, viscosity, and density of plant extracts from both test and control plants are calculated for phytochemical analysis.

Acoustic parameters such as adiabatic compressibility, intermolecular free length, relaxation time, and specific acoustic impedance can be used to predict and confirm molecular interactions. Ultrasonic velocity, density, and viscosity can provide detailed information about ion, dipole, and hydrogen bonding interactions. This data will help in pharmacological studies in order of novel drug formation as well as existing drug development.

To calculate time, distance, and velocity of the material can be calculated by using a velocity meter. To procure the experiment firstly the velocity and time of water were calculated and then the same was calculated for methanol and extract. Then plant density is calculated in order to perform a phytochemical analysis of the extract. After that, the viscosity of test and control plants is calculated by using a device named a viscometer. After that, all the other acoustic parameters like adiabatic compressibility, Intermolecular free length, etc are calculated with the use of the above. Throughout the experiment, we also ran the test for secondary metabolites. To conclude the above subjecting acoustic waves to the plants has resulted in exponential studies in both physiochemical studies and metabolites. This experiment carries great potential in the future as during the course of our experiment we have seen a great prospective in acoustical studies related to plant growth and its metabolites. The use of PAFT technology has enabled in better morphological growth of the plant (with respect to height) and also resulted in increased presence of secondary metabolites in acoustic waves subjected test plants.

5.2 Future Scope

To unveil the hidden buried elements of this understudied branch of botany, a more concerted effort is necessary. The moment has come to advance our understanding of plant communication beyond the argument over whether plants can detect and convey sound waves.

Scientists should now focus on sharing the exciting features that are now hidden in the research of plant acoustics. We should be excited about the potential of this new and rising field of plant study.

The future scope of plant acoustic investigations has the potential to significantly advance our understanding of plant physiology, communication, and environmental reactions. Here are a few major areas where plant acoustic research can have a significant impact:

1. Plant Communication: Acoustic research can shed insight on plant communication systems by investigating how plants produce and receive sound waves to communicate information. Investigating the function of auditory signals in plant interactions, such as interactions between plants and beneficial insects or interactions between plants in response to stress, might reveal novel communication networks.
2. Stress Detection and Monitoring: Plant stress reactions may be detected and monitored using acoustic methods. Researchers can create non-invasive strategies for early stress detection and prompt intervention by analysing the acoustic fingerprints given by plants under various stress circumstances such as drought, disease, or nutrient deficits.
3. Precision agricultural: By using acoustic technology into precision agricultural practises, farmers can monitor crop health and optimise irrigation, pest control, and fertiliser management. Farmers may make data-driven decisions to increase crop yields and resource efficiency by analysing plant acoustic responses.

4. Exploration of the acoustic characteristics of plants can have transdisciplinary applications. Studying the acoustic characteristics of plant materials, for example, might inspire the creation of innovative biomimetic materials or lead to advances in audio engineering and sound insulation.

As technology advances and our understanding of plant acoustics grows, the future of acoustic studies on plants holds immense potential for expanding our knowledge of plant biology and its applications in diverse fields to provide us with a new perspective on plants as a perceiving organism: much smarter and more sensitive to its surroundings environmental stimuli than we might believe.

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