EFFECT OF SURFACE WETTING CHARACTERSTICS ON PHYTOCHEMICAL PRODUCTION IN TISSUE CULTURED PLANTS OF *GENTIANA KURROO.*

Dissertation submitted in fulfilment of the requirement for the degree of Master of Science

In

Biotechnology

By

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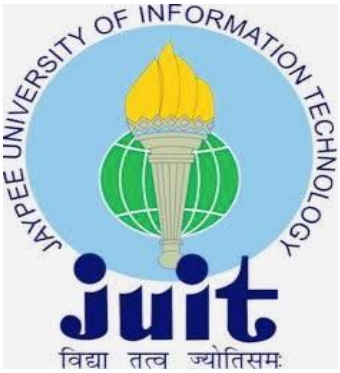

2024

DEPARTMENT OF BIOTECHNOLOGY & BIOINFORMATICS JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT, SOLAN, HIMACHAL PRADESH- 173234

DECLARATION

I hereby declare that the work presented in this report entitled "Effects of the surface wetting characteristics on phyto chemical production in tissue-cultured plants of ***Gentiana kurroo***" in partial fulfilment of the requirements for the award of the degree of "Masters 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 January 2024 to May 2024 under the supervision of Dr. Hemant Sood (Supervisor) and Dr. Poonam Sharma (Co-supervisor).The matter embodied in the report has not been submitted for the award of any other degree or diploma.

Naina Puri

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SUPERVISOR'S CERTIFICATE

This is to certify that the project work titled "Effects of the surface wetting characterstics on phytochemical production in tissue-cultured plants of ***Gentiana kurroo***" by Naina Puri during her end semester in fulfillment for the award of degree of Master's in Biotechnology of Jaypee University of Information Technology, Solan, has been carried out under my supervision. This work has not been submitted partially to any other University or Institute for the award of any degree or appreciation.

Signature of Supervisor- _____ _____

Name of Supervisor- Dr. Hemant Sood Dr. Poonam Sharma

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ABBREVIATIONS

PAFT (Plant acoustic frequency technology)

PGR (Plant growth regulators)

MS Media (Murashige and Skoog)

LAF (Laminar air flow)

TPC (Total phenolic content)

TFC (Total flavonoids content)

DPPH (Diphenyl-1-picrylhydrazyl)

RSA (Radical scavenging activity)

Hz (Hertz)

kHz (Kilohertz)

ABSTRACT

Gentiana kurroo (trayamana) is known for its medicinal properties and therapeutic uses. The plant shows effective results in cases of cancer, leprosy, hepatotoxicity, and diabetes. With the help of the frequency modulator, plant acoustic frequency technique (PAFT), the amount of phytochemical content can be increased by giving plants some fixed sound waves, i.e. three frequencies (500hz, 1Khz, and 1.5Khz) considering them as test samples. Another instrument, a tensiometer, can be used to assess the leaf surface wetting ability. Optical tensiometer used for the surface wetting characteristics specifically how water interacts with the surface of medicinal plants. It also help in identifying the nature of the surface whether it is hydrophilic or hydrophobic. A high contact angle refers to a hydrophobic surface. Surfaces that are super- hydrophobic are mainly charcaterised by means of low adhesion or by friction and are highly useful in industrial applications. There are some plants that show natural hydrophobic surface and possess microstrucrure, microbumps, as well as a thin waxy layer on their leaf surfaces. Thus these are helpful in determining the surface characterization of such kind of leaves and also shows a characterstic behaviour of these leaves . The use of acoustical setups has the potential to influence surface wetting characteristics as well as some other important aspects of plant growth, such as enhanced nutrients and water uptake, optimization of cell growth and metabolism, stress reduction and adaptation, and some more secondary metabolite production. Surfactants like Tween-20 (non-ionic), **SDS** (anionic), and **CTAB** (cationic) are used. Solvents such as dilute HCl, acetone, and water are used. They are very important for measuring how well surfaces stick to each other because they can change the surface tension and how liquids stick to solid surfaces, in assessing contact angle, how much the liquid can penetrate and spread, the surface energy, and more. The tissue- cultured ***G.Kurroo*** plant extract is prepared as control samples (under normal conditions) and test samples (under acoustical conditions) and undergoes physicochemical characterization by considering parameters such as density, viscosity, surface tension, and velocity. These parameters aid in the identification of active compounds, quality control and standardisation, extraction method optimization, formulation and product development, pharmacokinetics , and so on.

CHAPTER 1

INTRODUCTION

INTRODUCTION

1.1Basic Introduction

Gentiana kurroo also commonly known as trayamana is basically known for its therapeutic uses and its medicinal properties. In Sanskrit, it is mainly known by its name kutki, girija, karu. It belongs to family **gentianaceae**. This plant is majorly found in Kashmir, Himachal Pradesh and the adjoining hills of the north-western Himalayas at an altitude of 1500-3000 m. The plant ***Gentiana kurroo*** is mainly characterised by its deep blue colour flowers and lance-shaped leaves. The roots are usually fleshy, thick and branched mainly responsible for storing secondary metabolites such as **Gentiopicrine, Gentianine, C- glucoflavones** etc. The plant shows anti- microbial activities, anti-allergic, and anti- viral, anti- fungal activities. Many other ailments such as leprosy, diabetes, hepatotoxicity, cancer etc. can also be treated. The most effective part of ***Gentiana kurroo*** for medicinal purposes is typically considered to be the roots as well as leaves. In some traditional medicinal practices, the leaves of ***G.kurroo*** are used in herbal formulations for various therapeutic purposes. The leaves of this like other parts of the plant contain bitterness which can stimulate digestion as well as appetite. Due to habitat specificity, overharvesting, complexity in cultivation etc. ***G.kurroo*** now is an endangered plant. Therefore, we performed the experimentation work on tissue-cultured plants instead of field grown.[2]

Fig. 1 Tissue- cultured *G.kurroo*

1.2 Medicinal uses of *G.kurroo*

This plant shows anti- microbial, anti-fungal, anti-diabetic, hepatoprotective, neuroprotective, anti-cancerous, anti- inflammatory and many more other activities that makes this plant most important in the field of research.

1.3 **Acoustic waves on plants and its extracts-**

Acoustic waves are kind of energy that propagates through a medium and induce change in constraint and motion. The main aspects of acoustic waves includes: movements of particles, intensity, displacement, variations in constraint measurement. These waves has a specific speed that depends on the medium. Acoustic waves has a significant impact majorly on their roots and leaves. Different ways that shows the impact of acoustic waves on plant growth and development:

- Stimulates the growth and development in plants- Using different frequencies i.e. 500hz, 1Khz, 1.5Khz in acoustical setup (frequency modulator and speaker) can enhance plant growth. The phenomenon known as Sanitation.
- Alteration of Gene expression- Different variations in gene activity in respect to regulation of growth and stress response in different plants exposed to different sound frequencies.
- Enhanced nutrient absorption- The increased ability of plants for taking up nutrients is essential for the growth and health in different plants. Overall, the total nutrient absorption efficiency increased.
- Triggering stress response- Sound vibrations trigger stress responses in different responses that affects stress- related gene expressions and various physiological processes that maximize overall plant health and productivity.[1]

1.4 Surface characterization:

An instrument called optical tensiometer that mainly used to measure surface tension and interfacial tension between two phases i.e. liquid and gas or in between two immiscible liquids. It influences different physicochemical processes and material behaviour. A tensiometer can be utilized for physicochemical characterization:

- Measurement of surface tension: Total net force that causes a molecule on surface to contract and resist being broken/ stretched. This is essential for understanding stability of emulsions, coatings, foams as well as wetting behaviour of water
- Analysis of interfacial tension: In systems of immiscible liquids such as oil-water emulsions, tensiometer help in determining the interfacial tensions that shows the different behaviour of emulsions and multiphase flows and stability of different phases. [14]

It offers certain advantages on plant leaves as well as on their extracts-

- Optimizing different solvents- determining the most suitable solvents in extracting specific compounds from different plants and by analyzing their surface tension and other parameters such as Density measurement, Surface free energy etc thus leads to increase in the extraction efficiency.

- Assurance and Quality control- Surface tension offers uniformity and depenedability across different plant extracts , researchers can ensure the desired quality as well as potency of the end product are maintained.
- Prediction of extract properties- Underlying mechanisms that are mainly involved in plant extraction processes, surface tension provide a deeper understanding by analyzing the relationship between surface properties, solvent properties and plant matrix composition. By this, more advanced and sustainable extraction methods can develop.
- Reducing environmental impact- Extraction processes that are based on surface tension measurements can lead in using more greener solvents rather than depending upon the traditional organic solvents. Therefore, by reducing the impact of organic solvents and energy- intensive processes, the environmental impact of plant extraction can be minimized.

CHAPTER 2

(REVIEW OF LITERATURE)

REVIEW OF LITERATURE

2.1 *Gentiana kurroo*-

G.kurroo also known as ***trayamana*** or ***Kashmir gentian***. It is a species of a flowering plant that is native to Himalayan region particularly in areas of India (Kashmir, Himachal Pradesh), Pakistan and Nepal. It is a perennial herbaceous plant that has bluish violet coloured petals and typically grows up to a height of 30-60 cm. It has trumpet shaped flowers. In family ***Gentianaceae***, this species is mainly found in alpine and sub-alpine regions, growing in meadows, slopes, woodlands. This plant is highly valuable in traditional medicine systems (in ancient time) to treat several chronic ailments including hepatic disorders, dyspepsia, leprosy etc. Several bioactive compounds as well as secondary metabolites such as amarogentin, Gentiopicrine, xanthones, secoiridoids and flavanoids are present that are mainly known for their good as well as potent pharmacological effects. Loamy soil is mainly suitable for *G.kurroo* cultivation. Such type of soil protects as well as provide good drainage while retaining enough moisture as well as by avoiding water logging. The temperature that is mainly suitable for *G.kurroo* is low temperature at higher altitudes.[4]

Fig 2. Field grown *G.kurroo*

Fig 3. Tissue-cultured *G.kurroo*

Ref- Skinder, Bhat Mohd; Ganai, Bashir Ahmad; Wani, Abdul Hamid (December 5, 2017). "Scientific Study of *Gentiana kurroo* Royle". *Medicines*. 4 (4): 74. doi:10.3390/medicines4040074

2.2 Chemical constituents of *G.kurroo*-

Gentiana kurroo (kutki) is rich in various chemical constituents that contribute to its medicinal properties. Here some of the key chemical compounds found in *G.kurroo*:

Table 1. Chemical constituents of *G.kurroo* and their medicinal uses

S.NO	PHYTOCONSTITUENTS	MEDICINAL IMPORTANCE
1.	Xanthones	Shows Anti-allergic, anti-viral and anti-fungal activities.
2.	Iridoids	Hepatoprotective, neuroprotective, and anti-tumor.
3.	Gentiopicrine, Gentianine	Has anti- microbial activities.
4.	Mangiferin	Bioactive compounds used in diabetes, cancer, obesity and other cardiovascular diseases.
5.	Amarogentin	Bitter in taste and used as a good digestive stimulant.
6.	C- Glucoflavones	Used as an alternative therapy and wide range of hormonal disorders as well as menopausal symptoms.

2.3 Acoustic waves on plants

Acoustic waves are particularly high ultrasound waves that sows some advantageous as well as interesting effects on plants at different frequencies as well as on its extracts. These refers to a sound waves having frequencies more than the upper limit of human hearing. It shows impacts on plants as well as on its extracts[1]

1. Stimulation in growth: ultrasound frequencies at low intensitities shows stimulation of plant growth by promoting cell division, cell growth and cell elongation. Thus, this effect enhance the total nutrient uptake as well as all the major physiological processes within the cell.
2. Sterilization of culture: these waves eliminate microbial contamination without the need for harsh chemicals as well as high temperature that could damage delicate plant tissues.
3. Enhancement in secondary metabolite production: on treatment with acoustic setup can induce stress responses in plant cell cultures in vitro, leading in production of secondary metabolites such as phenolics, alkaloids and flavanoids.

4. Reduced aggregation in suspension cultures: These sound waves thus reduced aggregation and promote uniform distribution of cells.
5. Seed and embryo germination: ultrasound waves ruptures seed dormancy and promote seedling development .[6]

   A sound source emits vibrations into the medium around it, which in turn produces sound waves. As, the particular medium continues to vibrate in response to sound waves. Longitudinal as well as transverse waves are both used for transmission of sounds through solid objects or through air, water and maybe some other media. The main three characterstics of sound waves that is, frequency (Hz), strength (dB) and timbre to distinguish and validate one sound from another at same frequency. In fact, the plants respond to sound by generating more mRNA suggesting that sound alters plant genes i.e. rubisco small sub-unit (rbcs) and fructose (1, 6-biphosphate) aldolases that are essential for the process of photosynthesis in plants. Constant sound exposure thus, believed to improve plant development by encouraging carbon dioxide fixation. Sound vibrations can modify any kind or any activity of certain proteins, increase level of polyamines and soluble carbohydrates recognizing microfilaments and shows the regulation as well as transcription of specific genes. It also alters the activity of enzymes, anti-oxidants, gene expression, protein synthesis as well as different stress related hormones.[3]

2.31 Plant acoustic frequency technique-

(PAFT) Plant acoustic frequency technology is a technique that is used to stimulate the growth and development of plants using acoustic waves. This technique is majorly used to stimulate the opening of stomatal pores in plants. By the use of this technique, there occurs the enlargement of substances that are required in the process of plant growth. Researchers show significant changes. This technique includes a device that produces different frequencies that can be provided to the plants through which it can be detected whether the plants shows significant changes when comes in contact with sound frequencies or not. A frequency modulator (function generator) and a speaker are connected to produce different frequencies. Function generator thus, creates several waveforms as an output. It shows waveforms in the form of square waves, triangular waves, sine waves, and more. The range of frequencies in the acoustic setup varies between few Hz and several hundred Khz

Fig 4. PAFT (Plant acoustic frequency technology)

2.4 Acoustic waves on plant extraction

Acoustic waves can show some good and interesting effects on plant extracts specially in context of extraction processes. In acoustic setup sound waves are used in conjunction with plant extraction, they can show enhancement in efficiency of extraction. By promoting the release of secondary metabolites as well as the release of different bioactive compound from plant materials. The process by which high sound frequencies passing through the plant material is called Sonication . By this phenomenon, hot spots as well as high pressure zones involves in the cell disruptions that can break down cell as well as release the release of the intercellular compounds into the required medium. The liquid extract of plant can be made to study the physiological properties of plant with the help of which the molecular interactions can be studied. The extracts are further studied by exposing them to different instruments that is, ultrasonic interferometer, viscometer , densitometer etc. And through this measurement of different acoustical parameters can be studied for researching liquid states.

Thus, with the help of acoustic setup mainly on extracts involves following changes that include:

1. Increased extraction efficiency by enhancing the extraction of bioactive compounds such as polyphenols, flavanoids, and essential oils from plant material.
2. Reduced extraction time.
3. By preserving thermo labile compounds by generating minimal heat unlike the conventional extraction methods.
4. Known as green technology as it typically requires less solvents as well as energy in comparison to traditional extraction methods.

2.41 Ultrasonic interferometer:

An instrument that can accurately measure the ultrasonic velocity in liquid thus, help in understanding intermolecular as well as intramolecular interactions. The basic principle on which it depends involves splitting of wave front into two or more separate paths thus, resulting in waveform interference pattern.

The major key components of the interferometer involves:

1. Beam spitter- A component that splits a wave front into two or more separate beams . the beam splitter can be a prism, reflecting mirror or diffraction grafting
2. Arms – Before recombining the split beams, interferometer have multiple paths, which also known as arms. Each arms/paths have different lengths that may further interact with different samples.
3. Recombination- recombination can be achieved by using reflecting mirror or by allowing the beams to overlap with each other
4. Interference patterns: the patterns that are formed due to overlapping of spectral beams with each other, thus creating interference band patterns of light and dark fringes that can be observed and analyzed.

Applications includes: Metrology, spectroscopy, astronomy , laser interferometry etc.

2.42 Viscometer

Viscometer is a device that is mainly used to find out the viscosity of any liquid. It is used to measure the viscosity of any fluids by operating controlled stressor the fluid and then measuring the resulting deformation or flow rate of any liquid. Rotational viscometer are widely used to find out the viscosity of any liquid by rotating a spindle or bob within the desired fluid as well as by measuring the torque required to overcome the viscous resistance that is often offered in path. The whole principle is based on the principle that the torque exerted on spindle is directly proportional to the viscosity of the fluid. The torque that is mainly required to maintain the spindle rotation at any constant speed is measured and used to calculate the velocity of any fluid.

It can be by means of single viscometers or by means of multiple viscometers. Thus different spindles are also present for different liquids depending upon the torque required to spin the cone at constant speed used to calculate the viscosity. The units of viscosity are centipoises (cP) or millipascal- seconds (mPA- s) totally depends upon the calibration and settings of the instrument. Viscometers are widely used in areas of cosmetics, pharmaceuticals, food processing, paints and coatings that are mainly used to validate the instrument's performance.

Fig 5. **Brookfield viscometer**

2.5 **Tensiometer**:

An optical tensiometer also known as surface tension tensiometer is a special kind of instrument that is mainly used to measure to surface tension as well as the contact angle of liquids. Surface tension is a parameter that is force per unit length acting on the interface in between a gas or a liquid that tends to minimize the surrounded surface area of the liquid. In optical tensiometer several methods such as: drop pendent, sessile pendent can be used for the analysis and determining surface tension. In pendent drop analysis, a drop is hang from the needle to the surface area where the contact angle a well as the surface tension can be

determined. In sessile drop analysis, a drop is placed on the solid surface and the contact angle is further used to calculate the surface tension of any liquid. Tensiometer is used in various researches as well as industries including surface coatings, paints formulation, pharmaceuticals as well as biomedical research for studying interfacial interactions of fluid in relevance to drug discovery and biological interactions, in monitoring environment by analyzing as well as determining surface interactions in material processing.

Optical tensiometer particularly in context with plant leaves as well as their extracts plays a crucial role in determining the surface wetting characterstics as well as water- related behaviour / characterstics of plants. In tissue- cultured apart from measuring surface tension as well as the contact angle, optical tensiometer can be used to optimize or monitor the moisture content as well water availability that is required for growth medium in tissue-culturing. Thus, by maintaining optimal moisture and by continuously monitoring the moisture tension or the potential of growth medium, tissue- culturing can be further adjusted into irrigation by ensuring consistent and required optimal conditions for growth and development of the plant. Optimization of nutrient uptake can be very useful as water availability affects the presence of nutrients uptake and the transport movement within the plant tissues. It is also required for further controlling the osmotic potential by monitoring or optimizing the concentrations of sugars or osmotic agents in the growth medium that is equally important to maintain turgor pressure by preventing osmotic stress potential. An optical tensiometer help in studying the hydrophobocity and hydrophilicty of any leaf surface as more high contact angle shows hydrophobicity and help in determining how the surface shows interaction with different solvents. Hydrophobic surfaces are mainly characterised by adhesion and friction, that makes the surfaces super-hydrophobic. Therefore, this instrument help in analyzing the surface characterstics of the in-vitro ***Gentiana kuroo*** leaves as well as shows the contribution of different microstructures that are present on the surface to determine their hydrophobic and hydrophilic nature.

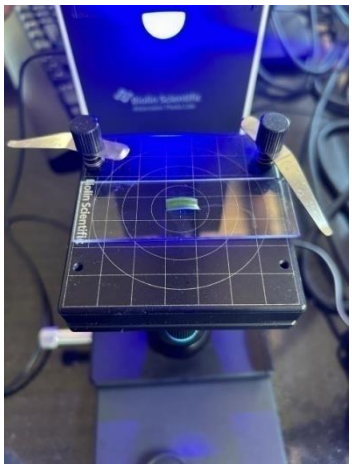


Fig 6. Optical tensiometer **Fig 7. Leaf under tensiometer**

2.6 **Physico-chemical parameters:**

Velocity, viscosity, surface free energy, density and surface tension are the parameters that are mainly required for physic-chemical characterization. Each parameter is equally important in context with plant extracts-

1. **DENSITY**- It is defined as mass per unit volume of a substance. It is a very fundamental property that helps in identifying and differentiate between materials based on their composition as well as compactness. By measuring density of plant extracts one can determine or assess the quality and consistency of plants extracts, resulting in formulation as well as standardization of herbal products.
2. **VELOCITY**- It refers to the speed or direction in which the object is moving or it refers to the rate of flow of any liquid. By understanding about the velocity parameter one can optimize the design of extraction machines or equipments by ensuring good or efficient extraction and processing of plant material.
3. **VISCOSITY**- Viscosity refers to the fluid's resistance or flow. By measuring viscosity one can optimize the extraction processor parameters thus by selecting suitable solvents and design various delivery systems (examples. creams, gels) for plant extraction resulting in making plant-extract based products.
4. **SURFACE FREE ENERGY (SFE)**- It mainly shows the wetting and spreading behaviour of plant extracts on surfaces. It helps in optimizing the plant-based formulations for showing enhancement in the efficacy, stability or bioavailability.
5. **SURAFCE TENSION**- In making of plants products such as herbal teas, tinctures, cosmetic preparation, in pharmaceuticals it provides as well as ensures uniform distribution.

2.7 Pharmacological activities of *G.kurroo*:

- **ANTI-BACTERIAL ACTIVITY**- The extracts of *G.kurroo* provides a good growth of both the gram positive as well as gram negative bacteria especially in leaves and roots of the plant thus, possessing higher anti-bacterial as well anti-oxidant activity. It shows high anti-bacterial properties due to the presence of flavanoids in high content typically in the leaves of the plant. The anti-bacterial nature of the extract shows highest against the ***Micrococcus luteus*** and lowest in case of ***Salmonella enteriditis.[4]***

• *Micrococcus luteus*	• 0.15mg/ml
• *Salmonella enteriditis*	• 0.75mg/ml

- **ANTI-OXIDANT ACTIVITY**- The methanolic extracts that are prepared from the leaves as well as from the roots shows high anti-oxidant activity due to the presence of high phenolic as well as the flavanoids that are present. Thus, high phenolic content (TPC) can be used for the rapid screening of anti- oxidant activity, whereas the flavanoid content shows protective anti-oxidant defences.

- **ANTI- ARTHRITIC AND ANTI-INFLAMMATORY ACTIVITY**- Methanolic extracts of *G.kurroo* is more effective in comparison to other plants as it shows excellent results against the edema formation also shows inhibition against inflammatory mediators which shows good arthritic control. The anti-inflammatory property of *G.kurroo* shows excellent relations with the secondary metabolites (terpenoids and flavanoids) as well.[4]

- **ANTI- DIABETIC ACTIVITY**- The methanolic as well as the hydroethanolic extracts has found to fight against diabetes. It is mainly used to control the high glycemic levels in blood. The major anti- diabetic activity is majorly shown by the bioactives that are present in ***G.kurroo*** are lipeol, gentiopicrine, magiferin. Thus, ***G.kurroo*** shows great scientific validation and can be used in many parts of the country for the treatment of diabetes.

- **HEPATO-PROTECTIVE ACTIVITY**- Bioactive compounds such as **Iridoids** (such as gentiopicroside), **Xanthones , flavanoids and other phenolic** compounds possess hepatoprotective properties which may contribute their beneficial effects to liver health. It also supports the liver from damage and disfunctioning. It also supports liver- enzyme regulation such as ALT, AST,ALP etc. The extracts of ***Gentiana kurroo*** also protects the hepatocytes from injuries, promote tissue-repairing and shows considerable histological improvements in tissue of liver.

- **ANTI-CANCER ACTIVITY**- The main bioactive compound that is present in ***G.kurroo***. Amarogentin provides chemopreventive as well as chemotherapeutic effects on the health of a individual. These bioactive compounds induces apoptosis, has the ability to inhibit cancerous or tumor cell growth and shows considerable improvements as well as modulate cell signalling.

- **GASTROPROTECTIVE ACTIVITY**- The extracts of *G.kurroo* activity shows enhancement in the secretion of protective factors such as prostaglandins and mucin that helps in maintaining the integrity of mucosal barrier and protects tissue repair.

2.8 **Objectives-**

1. To carry out in-vitro propogation of ***G.kurroo*** by using shoot apices

2. to check the effect of acoustic waves on growth and development of in-vitro raised ***G.kurroo***

3. To study the effect of acoustic waves on biosynthesis and accumulation of metabolites in ***G.kurroo*** by means of tensiometric studies.

4. By evaluating different parameters involving physico-chemical characterization.

2.9 Gaps-

1. There are limitation in production of biomarkers compound from field grown plants that's why alternate techniques are required.

2. No report on growth and phytochemical production in ***G.kurroo*** by using surface wetting and tensiometric studies

CHAPTER 3

MATERIALS AND METHODS

3.1 **PREPARATION OF MS MEDIA-**

Media was prepared by using stock solutions, PGR'S, sucrose, and agar.

COMPONENTS	VOLUME
STOCKS (A-H)	
STOCK A STOCK B STOCK C STOCK D STOCK E STOCK F STOCK G STOCK H	100 ml/l 50 ml/l 10 ml/l 10 ml/l 10 ml/l 10 ml/l 10 ml/l 10 ml/l
PGR'S IBA Kn	 3mg/l 1mg/l
SUCROSE	30g/l
AGAR-AGAR	9g/l
Ph	5.6-5.7

1. Stock solutions (A-H) were measured one by one and added to a beaker and then PGR' i.e. IBA (3mg/l) and kn (1mg/l) were added in it.
2. 30 g sucrose was added into solutions
3. Solutions was made 900 ml by adding water and then pH was checked.
4. Then agar was added to solution and volume was raised to 1000ml
5. Solutions was boiled and after boiling 50-50ml of it was put into jars
6. Autoclaving was done at 121 degree and 15 psi
7. After autoclaving, jars were kept at room temperature.

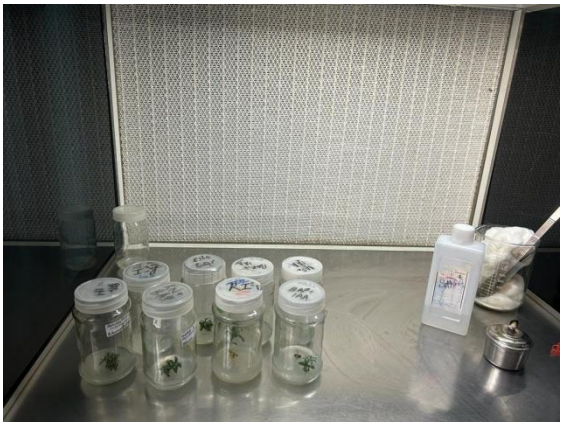


Fig 8. sub-culturing of *G.kurroo* in LAF

3.2) **Plant collection and Sub-culturing of *G.kurroo-***

Sub-culturing of micropropogated ***G.kurroo*** was done to increase all the no. Of plantlets for checking the surface and texture of the leaf.

1. All objects that are needed in for culturing such as forceps, scalpel, media, jars, petri plates, were placed in LAF for sterilization (15-20min) before working in it. UV light was switched on.
2. After 20 minutes, switch off the UV light and then LAF floor was cleaned.
3. 2 jars of ***G.kurroo*** were taken and from them the plants were removed. Shoots were further put into the 20 media jars.
4. Jars were labelled and put into the culture room.

Fig 9. sub-culturing of *G.kuroo*

Fig 10.On different medias, sub-culturing *of G.kurro*

3.3 Sound exposure to *in-vitro* raised *G.kurroo*

A setup was made to expose the plants under sound to check the growth and development of the plants. A frequency modulator was connected to a speaker in order to get the sound of different frequencies from the acoustic setup. The plants were exposed under sound frequencies 500 Hz(0.686 m wavelength), 1kHz (0.343 m wavelength), 1.5 kHz (0.228) for 4-5 hours for 10-15 days in each frequency.[6]

Fig 11. *In - vitro* plant under acoustic setup of sound waves

3.4 **Extract preparation**-

Extracts of the *G.kurroo* plant kept in culture room as (control) and in sound setup with 3 different frequencies as test samples). Plants were washed and dried and then grind in pestle & mortar with the help of liquid nitrogen. Powder formed put in 100 ml of 80% methanol and kept for shaking for 24 hours. After 24 hours, with the help of whatman filter paper followed by syringe filter. 4 different extracts were prepared, one (control sample) and three for (test samples) and kept under 4°C.

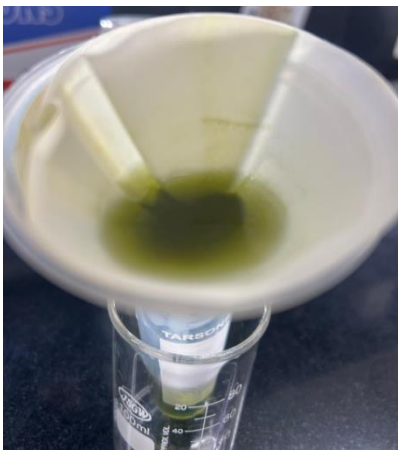


Fig 12 Filteration of extract by whattman paper

Fig 13. After shaking for 24 hours

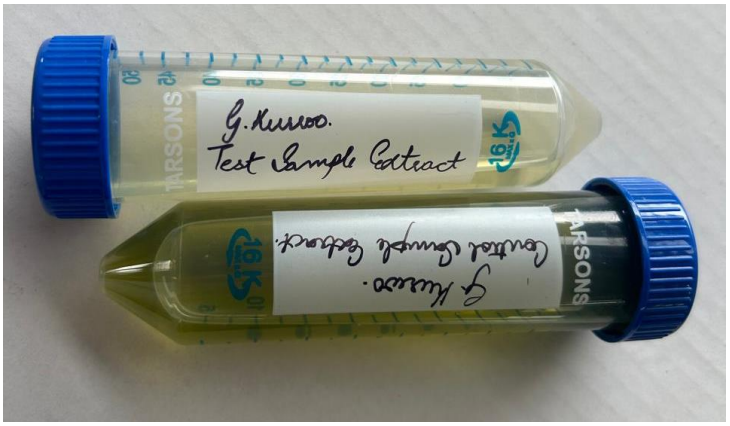


Fig 14. Storage of ***G.kurroo*** extract at -4℃

3.5 Phytochemical analysis of in vitro raised *G.kurroo* extracts

Quantitative and qualitative analysis was done to check the presence of phytochemicals and their quantity in all 4 extracts using standard methods.

3.51 Qualitative analysis of *in vitro* raised *G.kurroo* extracts

1. Alkaloids test-

Mayer's test- first of all, Mayer's reagent is prepared in which two solutions were prepared individually i.e. 1.35 g mercuric chloride (Hgcl) dissolved in 60 ml distilled water and potassium iodide dissolved in 10 ml distilled water. After this both solutions were mixed and volume was raised to 100 ml. Then, the 4 test tube were taken of 4 extracts (1 control and 3 samples exposing to different frequencies at 500 Hz, 1Khz and 1.5 Khz). In each test tube 5-6 drops of Mayer's reagent is added thus, resulting in yellow precipitate formation gives positive results.

2. **Flavanoids test**-

Alkaline reagent test- in 4 different test tubes, 2ml of a mixture of 2.0% NaOH added to plant extract resulting in yellow colour solution. After adding few drops of Hcl to it, the yellow solution become colourless. The presence of flavanoids was demonstrated by this.

3. **Cardiac glycoside Test-**

Legal test or Keller- Killani test- To 10ml aq. Plant extract, 1ml of concentrated H_2SO_4 and 4 ml of glacial acetic acid solution were added. After this a drop of a 2% $FeCl_3$ is added.

There occurs the formation of reddish- brown ring that shows the presence of cardiac glycosides between the layers.

4. **Phenolic compound test**-

Braymer's test- 1 ml of each extract was put in different test tube. Then 1ml of water is added followed by 3 drops of 10% $FeCl_3$ added in each test tubes. Occurrence of deep dark blue colour appears if the test is positive.

5. **Saponin test**-

Foam test- 2 g powder of alcoholic extract was prepared and tested for foaming. Saponins were detected by the persistence of frothing. After that, a few tablespoons of olive oil/ coconut oil were added to the foam. The presence of saponins was suggested by the formation of an emulsion.

6. **Tannins test**-

5 ml of extract were taken in different 4 test tubes followed by 2ml of 5% $Fecl_3$ solution in each test tube containing extracts. Red, blue, green or purple colour indicate the presence of tannins.

7. **Terpenoids test-**

5 ml of each aq. Plant extract was taken in test tubes and afterwards followed by 2ml of chloroform was added in each of them. Then on evaporating it on water bath combining with 3 ml of concentrated H_2SO_4 to boil. Appearance of reddish brown colour shows the presence of terpenoids.

8. **Carbohydrate test-**

Benedict's test/ Molisch test/ Fehling test- This test is mainly for detection of reducing sugars (eg. Glucose, fructose, maltose). Positive brick red colour indicates the presence of carbohydrates.

3.52 Quantitative analysis of in vitro raised *G.kurroo* extracts:

3.5.2.1 Total phenolic content (TPC)

50 mg of Gallic acid was dissolved in 5ml of methanol (10mg/ml). Different dilutions of gallic acid were made in order to plot a standard graph of gallic acid and were dissolved in methanol. In different 4 test tubes, extracts were added. After this, water is further added in each test tube containing extracts. The 100ul FC reagent were added in each test tube and then kept for 6 min of incubation. After this, 1 ml of Na_2CO_3 was added and kept for 30 min incubation. After that, absorbance was recorded at 760 nm. Experiments were performed in triplicates.

3.5.2.2 Total Flavanoids content (TFC)

50 mg Quercetin is taken and further added to 5 ml methanol(10mg/ml). Different dilutions for quercitin was prepared in order to plot a standard graph and were dissolved in methanol. By taking 4 different test tubes, 4 different extracts were added. 4 ml of distilled water was added followed by 300ul sodium nitrate. Kept for 5 min incubation and 300ul Alcl3 added. Then again after 5 minutes incubation addition of 200ul NaOH was added. Absorbance was recorded at 510 nm. Experiment was done in triplicates.

3.6 Antioxidant activity of in vitro raised *G.kurroo*

3.61 DPPH Free radical scavanging activity of in vitro raised *G.kurroo*

Gallic acid solution was prepared by addition of 50 mg in 5ml methanol (10mg/ml) and 0.002% DPPH (diphenyl-1- picrylhydrazyl) solution were prepared by dissolving 10 mg DPPH in 5ml methanol. Different dilutions of quercitin were made for standard graph having conc. 50ul, 100ul, 200ul, 400ul that were dissolved in methanol with 400ul, 350ul, 200ul conc. Respectively, in 4 different test tubes, 3.6 ml of DPPH was added to each test tube. Then afterwards kept for incubation and then absorbance was recorded at 517 nm. Experiments were performed in triplicates.

3.7 Optical tensiometric studies of *G.kurroo*

Optical tensiometer is crucial for understanding the wetting behaviour of liquids and their interactions with surfaces. Sessile drop method can be used to calculate the surface tension as well as the contact angle. By taking the leaves of ***G.kurroo*** of control as well as the test samples under the tensiometer to check its surface tension as well as its contact angle on both the abaxial as well as adaxial sides of the leaf. By means of different solvents as well as different surfactants. The solvents that are used are: water, ethanol, toluene, 0.1N Hcl and liquid NH_3. If the angle θ is less than 40 is super hydrophobic , θ is less than 90 it is highly wettable, if θ is less than 110 it is termed as wettable, if θ is greator than 130 and 150 it is super hydrophobic. Measurement of contact angle can be done by means of 3 surfactants i.e. SDS(anionic), CTAB(cationic) and Tween - 20(non-ionic) at different concentrations i.e.0.0001, 0.0005, 0.001, 0.005 and 0.01 the contact angle on both the surfaces of leaves i.e. adaxial and abaxial can be calculated. When the cationic as well as the anionic surfactants comes in contact with the leaf, their charge influence the surface interactions as well as induce some changes on the leaf due to the presence of charge groups over them. With increase in the concentration of surfactants leads to induce some changes in the adsorption of the surfactant molecule over the surface due to which alteration of the surface wettability , surface free energy as well as the surface wetting characterstics may be affected due to which contact angle changes. From lower to higher concentration can affect the stronger interactions between the surfactant molecule and the surface leading to higher contact angle. This usually

happened in case of control samples but when we take plants that are under acoustic setup (test samples)some different kinds of changes can be seen in case of contact angle.[14]

With increase in surfactant concentration, the contact angle increases in the abaxial region and thereby decrease in adaxial region of the leaf.

- This can be due to physiological responses, plants under acoustic setup shows some different physiology as well as growth patterns that might affect their surface wetting characterstics or wettability of the leaf. This can vary contact angle measurements between adaxial and abaxial surfaces due to differences in structure or function.
- Acoustic stimulation might affect the presence of microstructures that are present over the leaf surface or there occurs the alteration in surface chemistry of the leaves due to which it leads to varying contact angle between the two sides of the leaf.
- The other and the most important factor is the localized effect on the leaf surfaces. It is due to the differential changes in contact angle between adaxial and abaxial surfaces, where one side shows higher contact angle and the other side experience lower contact angle.

  While taking the third surfactant i.e. Tween 20, the contact angle on the (control as well as the test samples) increases with increase in concentration. Due to its non-ionic nature, Tween-20 is the mildest of all, it does not show strong electrostatic interactions over the leaf surfaces. As the concentration of tween-20 increases it tends to cause more surfactant molecules on the surface. This increase in changes in surface properties is due to increases adsorption leads to decreased surface tension as well as increased surface coverage. It also gives a uniform effect, as Tween-20 acts via hydrogen bonding and hydrophobicity, the interactions are not wholly dependent upon the surface energy and likely to give more uniformity on both the adaxial as well as the abaxial surfaces of the leaf and thus leads to show similar type of changes on both the surfaces of the leaf.

3.8 **Physicochemical characterisation by using different parameters:**

3.81 **Density Measurement:**

Density of a particular liquid can be checked by means of RD bottle (Relative density bottle). Density is defined as mass per unit volume. Density is represented as φ .Density of all 4 liquid extracts was checked with the help of RD bottle at different temperature i.e. 20℃, 25℃, 30℃, 35℃ & 40℃ . Weight of the empty relative density bottle is taken by using weighing balance. After that, the weight of RD bottle filled with water was taken. Laterly, weight of the RD bottle filled with extract was taken.

Then, density of all the 4 extracts can b calculated by using the formula-

Density of the extract= (weight of extract/ weight of water) x Density of water

= (weight of extract- weight of empty bottle/weight of water-weight of empty bottle) x density of water.

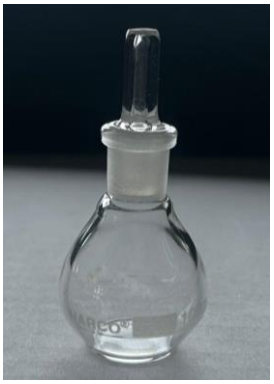


Fig. 15 Empty RD bottle

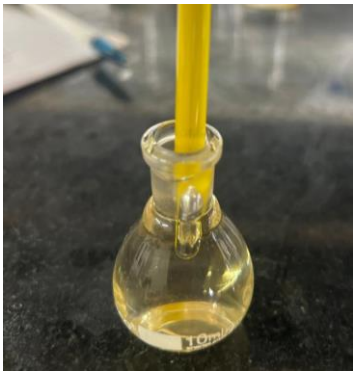


Fig 16. Temperature regulation

Fig 17.Weight of ***G.kurroo*** extract

3.82 **Viscosity Measurement-**

Fluid resistance to flow is known as viscosity. It also refers to the thickness or stickiness towards the flow or fluid. Viscosity is represent by centipoise units (cp units). It is measured by an instrument name Brookfield Viscometer with the help of which the viscosity of each sample at different temperatures can be checked. The temperature can be maintained by water bath and can be checked by means of thermometer. At different temperatures (20℃, 25℃, 30℃, 35℃ & 40℃) the viscosity of all 4 samples was checked with the help of viscometer spindle s 61 . spindle started rotating after switching it on. Readings were recorded for different samples at varying temperature with spindle no. s 61.

Fig 18. Brookfield Viscometer

3.83 **Velocity Measurement-**

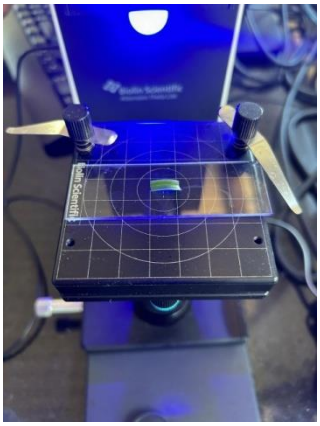
Velocity is defined as the rate of change of change of objects with respect time. A setup was arranged called Digital ultrasonic velocity meter that is connected to a water bath precision with the help of which temperature can be regulated i.e. (20℃, 25℃, 30℃, 35℃ & 40℃). The digital ultrasonic velocity meter measured the velocity of the fluid by means of a pipe or a channel using ultrasonic waves. At different temperatures, the time and the velocity of different extract samples can be calculated. The velocity that shows on the velocity meter screen provides a real time information regarding the fluid flow or extract flow velocity.

Fig 19. Ultrasonic velocity meter

Fig. 20 Precision water bath

3.84 **Surface Tension Measurement**-

Surface tension is a physical property that arise due to the cohesive forces between the molecule and the surface of a liquid. It can be calculated through optical tensiometer by sessile drop method. Surface tension can be calculated for the *G.kurroo* leaves i.e. control as well as the test samples (under different frequencies- 500 Hz, 1Khz, 1.5Khz). The surface tension can be calculated at the Adaxial and the Abaxial region of the leaf. By taking different concentrations (0.0001, 0.0005, 0.001, 0.005, 0.01) of surfactants and solvents to calculate the contact angle by means of which the surface tension can be calculated. The units of surface tension is N/m (Newton /minute).[19]



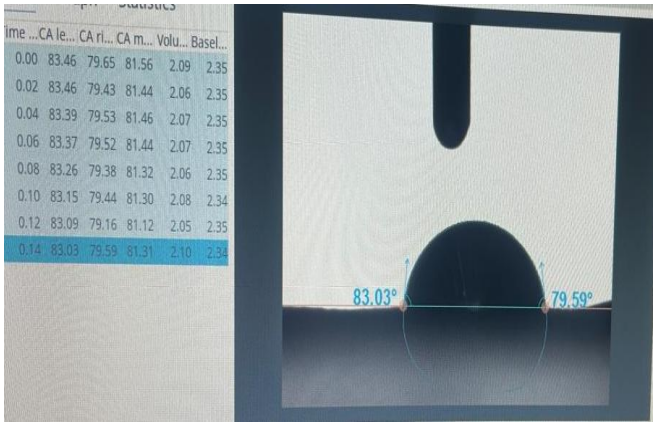


Fig 21. Surface tension measurement **Fig 22**. Contact angle measurement

3.85 Surface free Energy (SFE) Measurement-

SFE refers to the extra energy that is present at the surface of the material. Surface free energy is often expressed in joules per square meter. Surface free energy provides insights regarding the adhesion, wetting characterstics and surface-related processes. By monitoring SFE, one can measures and control quality control. It can be calculated by means of optical tensiometer by sessile drop method or pendent drop methods. By taking at least three readings of the surface tension can help in generating the value for surface free energy in joules per square meter.

3.9 Acoustical parameters-

After checking the above parameters, further acoustical parameters can were calculated using following acoustical parameter formula-

- **Adiabatic compressibility**-$\beta a = 1/U\rho$
  Where, βa *is adiabatic compressibility*
  U *is ultrasonic velocity.*
  ρ *is density.*

- **Acoustic impedance** - $Z = U\rho$
  Where Z *is acoustic impedence*
  U *is ultrasonic velocity*
  ρ *is density*

- **Relaxation time** -$\tau = \frac{4}{3}\eta\beta a$
  Where, τ *is relaxation time*
  η *is viscosity*
  βa *is adiabatic compressibility.*

CHAPTER 4

RESULTS

4.1 ***In- vitro* raised plants exposed to sound waves**:

The study shows the effects of acoustic waves on the growth of the plants. Plants exposed under sound system provide better result than the plants in the normal conditions i.e. better than control plants. 7-8 jars of plant as control samples without sound exposure and 7-8 plants as test samples exposed under different frequencies i.e. 500 Hz, 1Khz, 1.5Khz. Later, these were compared after 10-15 days. The plants under acoustical setup shows fast and better growth with increase number of shoots as compared to the plants under normal conditions (control plants). Shoot number as well as the leaf number was also increased in the test samples while it was remained almost same in the control samples. Although, growth as well as the development was fast in each of the frequency but more number of shoots, healthy leaf growth as well as better plant length was observed under the frequency 1Khz. On the other hand the test samples under 500 Hz showed normal to better growth while the test sample plants under the frequency 1.5Khz shows a stagnant kind of growth as well as in some samples leaf whitening or leaf bleaching were also observed.

4.1.1 Different growth parameters observed under different Hz of frequencies–

Plants	**Shoot length**		**No. of shoots**		**No. of leaves**	
	Before	After	Before	After	Before	After
C_1	2 cm	2 cm	Multi-shoot	Multi-shoot	14 leaves	14 leaves
C_2	1 cm	2.6 cm	Double shoot	Double shoot	11 leaves	15 leaves
C_3	1.5 cm	2.3 cm	Double shoot	Double shoot	4 leaves	8 leaves
C_4	2.9 cm	3 cm	Double shoot	Double shoot	10 leaves	11 leaves
C_5	0.9 cm	1.5 cm	Single shoot	Triple shoot	4 leaves	8 leaves

Table 2. Growth parameters observed as control samples at (500Hz)

C_6	2.4 cm	2.4 cm	Triple shoot	Triple shoot	7 leaves	7 leaves
C_7	2 cm	2.1 cm	Single shoot	Single shoot	4 leaves	4 leaves

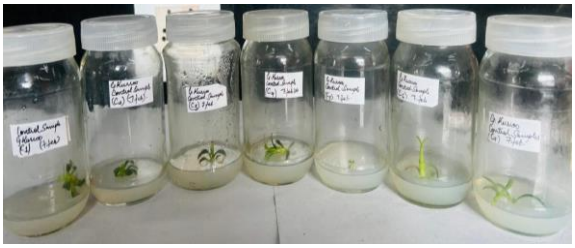


Fig 23. Control samples (Before)

Fig 24. Control samples(After)

Table 3. Growth parameters observed at test samples (1Khz**).**

TEST SAMPLES						
Plants	**Shoot length**		**No. of shoots**		**No. of leaves**	
	Before	**After**	**Before**	**After**	**Before**	**After**
T_1	1 cm	2.6 cm	Single shoot	Double shoot	8 leaves	8 leaves
T_2	1.2 cm	2.1 cm	Single shoot	Single shoot	9 leaves	9 leaves
T_3	1.5 cm	2 cm	Triple shoot	Triple shoot	16 leaves	22 leaves
T_4	0.9 cm	2.4 cm	Single shoot	Double shoot	8 leaves	13 leaves
T_5	0.9 cm	2.4 cm	Single shoot	Triple shoot	10 leaves	16 leaves
T_6	1.2 cm	2.4 cm	Double shoot	Double shoot	8 leaves	11 leaves
T_7	0.5 cm	1.6 cm	Single shoot	Double shoot	2 leaves	7 leaves

Fig 25. Test samples **(Before**)

Fig 26. Test Samples (**After**)

4.1.2 **Observation of growth parameters at 1kHz**

Table 4. Growth parameters observed as control samples at (1kHz)

CONTROL SAMPLES						
Plants	Shoot length		No. of shoots		No. of leaves	
	Before	After	Before	After	Before	After
C_1	1.2 cm	1.5 cm	Double shoot	Double shoots	13 leaves	17 leaves
C_2	0.9 cm	1.3 cm	Multi shoots	Multi shoots	15 leaves	19 leaves
C_3	1 cm	1.3 cm	Single shoot	Single shoots	7 leaves	11 leaves
C_4	1 cm	1.4 cm	Single shoots	Single shoots	5 leaves	12 leaves
C_5	1.1 cm	1.6 cm	Single shoot	Single shoots	7 leaves	14 leaves
C_6	1.5 cm	1.9 cm	Single shoot	Single shoot	8 leaves	17 leaves
C_7	1.7 cm	2.1cm	Single shoot	Single shoot	4 leaves	10 leaves

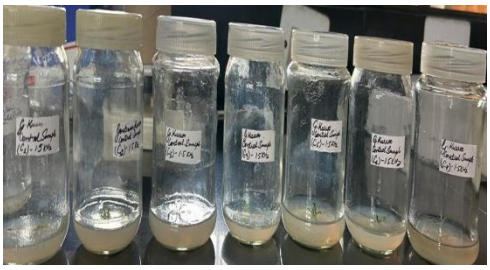


Fig 27. Control samples **(Before)**

fig. 28. Control samples **(After)**

Table 5. Growth parameters observed as test samples at (1kHz)

TEST SAMPLES						
Plants	Shoot length		No. of shoots		No. of leaves	
	Before	After	Before	After	Before	After
T_1	1.5 cm	2.3 cm	Single shoot	Double shoot	6 leaves	12 leaves
T_2	0.9 cm	1.7 cm	Single shoot	Triple shoot	4 leaves	9 leaves
T_3	1 cm	3.2 cm	Single shoot	Triple shoot	6 leaves	26 leaves
T_4	1.4 cm	2.6 cm	Single shoot	Double shoot	5 leaves	17 leaves
T_5	1.4cm	3.5 cm	Double shoot	Double shoot	9 leaves	20 leaves
T_6	0.7 cm	3.2 cm	Single shoot	Single shoot	4 leaves	6 leaves
T_7	1.5 cm	4.2 cm	Single shoot	Single shoot	9 leaves	15 leaves

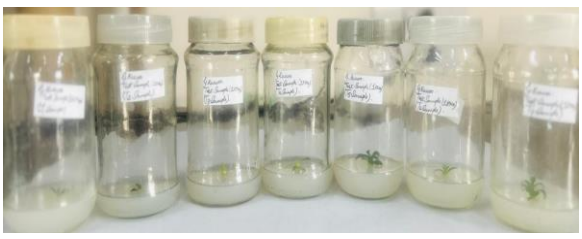


Fig. 29 Test samples **(Before)**

Fig. 30 Test samples **(After)**

4.13 Different growth parameters observed under different Hz of frequencies .

Table 6. Growth parameters observed as control samples at (1.5kHz)

CONTROL SAMPLES						
Plants	Shoot length		No. of shoots		No. of leaves	
	Before	After	Before	After	Before	After
C_1	1.5 cm	1.7 cm	Single shoot	Single shoot	6 leaves	7 leaves
C_2	0.6 cm	1.1 cm	Single shoot	Single shoot	2 leaves	6 leaves
C_3	1.8 cm	2.0 cm	Single shoot	Single shoot	6 leaves	6 leaves
C_4	2 cm	2.6 cm	Single shoot	Single shoot	6 leaves	8 leaves
C_5	1.6 cm	1.9 cm	Single shoot	Single shoot	8 leaves	9 leaves
C_6	1.2 cm	1.7 cm	Single shoot	Single shoot	4 leaves	6 leaves
C_7	1.9 cm	2.1 cm	Single shoot	Single shoot	4 leaves	6 leaves

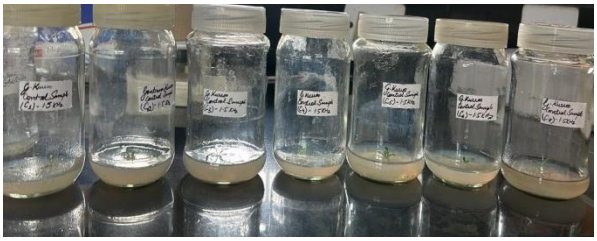


Fig 31. Control samples **(Before)**

Fig.32 Control samples **(After)**

Table 7. Growth parameters observed as test samples at (1.5Khz)

TEST SAMPLES						
Plants	Shoot length		No. of shoots		No. of leaves	
	Before	After	Before	After	Before	After
T_1	1.6 cm	2 cm	Single shoot	Single shoot	6 leaves	8 leaves
T_2	1.3 cm	1.9 cm	Double shoot	double shoot	11 leaves	12 leaves
T_3	0.8 cm	0.9 cm	Single shoot	Single shoot	5 leaves	7 leaves
T_4	1.2 cm	1.6 cm	Single shoot	Single shoot	7 leaves	8 leaves
T_5	1 cm	1.5 cm	Single shoot	Single shoot	5 leaves	5 leaves
T_6	1.5 cm	2.2 cm	Single shoot	Single shoot	5 leaves	7 leaves
T_7	1 cm	1.4 cm	Single shoot	Double shoot	3 leaves	7 leaves

Fig 33. Test samples **(After)**

4.2 Phytochemical Analysis of *in-vitro* raised *G.kurroo*

4.21 Qualitative test-

Various phytochemical analysis were done to check the presence of different phytochemicals as well as the secondary metabolites in control as well as different test samples under different frequencies under (500 Hz, 1 Khz, 1.5 Khz.). Test for tannins, alkaloids, cardiac glycosides, flavanoids, phenolics, carbohydrates as well as terpene test were done in order to check them in different test as well as control samples.

Table 8. Qualitative analysis of control as well as plants incubated under different Hz of frequency.

Phytochemicals test	Control Plants	Sound exposed plant(500hz)	Sound exposed plant (1Khz)	Sound exposed plants (1.5 Khz)
Alkaloids	+	+	+	+
Flavanoids	+	+	+	+
Tannins	+	+	+	+
Cardiac glycosides	+	+	+	+
Carbohydrates	-	-	-	-
Phenolics	+	+	+	+

Terpenes	-	-	-	-
Saponins	+	+	+	+

4.2.2 Quantitative tests of control and plants incubated under different Hz of frequency

4.2.2.1 Total phenolic content-

Total phenolic content was compared between the control samples and the test samples (plants under acoustic setup under different frequencies i.e. 500 Hz, 1Khz and 1.5Khz). Maximum accumulation of total phenolic content was observed in 1kHz and the minimum accumulation of total phenolic content was observed in 500 Hz. As shown in fig.

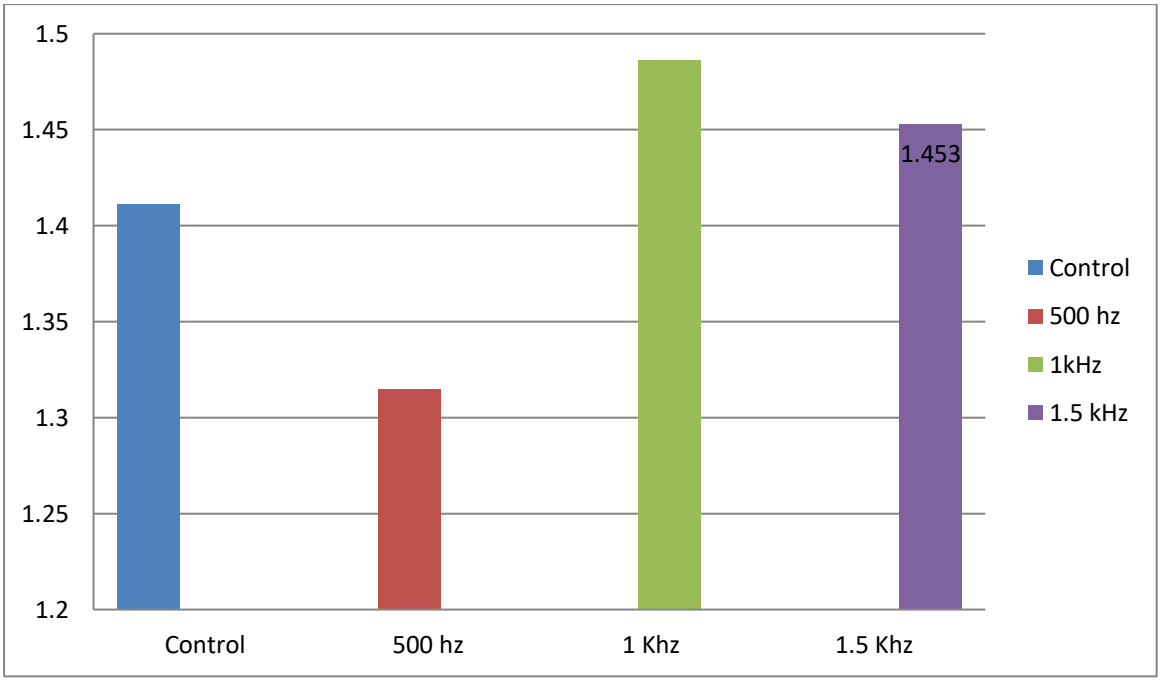


Fig 34. Impact of sound waves at different frequencies on the total TPC content of ***G.kurroo*** after 10 days.

4.2.2.2 Total flavanoid content (TFC)

Total phenolic content increased in sound exposed plants. It was checked and confirm by comparing flavanoids content of test as well as control samples. Control plants has less flavanoid content than test samples plants.

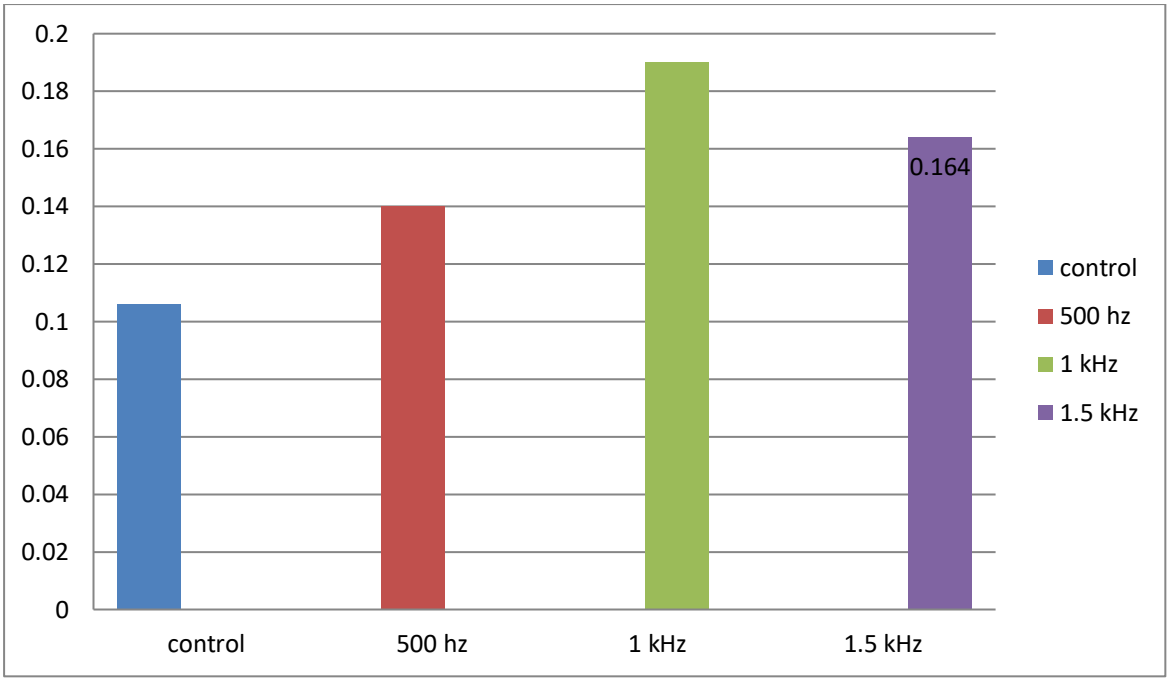


Fig 35. Impact of sound waves at different frequencies on the total TFC content of ***G.kurroo*** after 10 days

4.3 DPPH assay.

The anti-oxidant activity is usually checked by DPPH assay in each sample. The amount of anti-oxidants is in more percentage in test samples than in control samples.

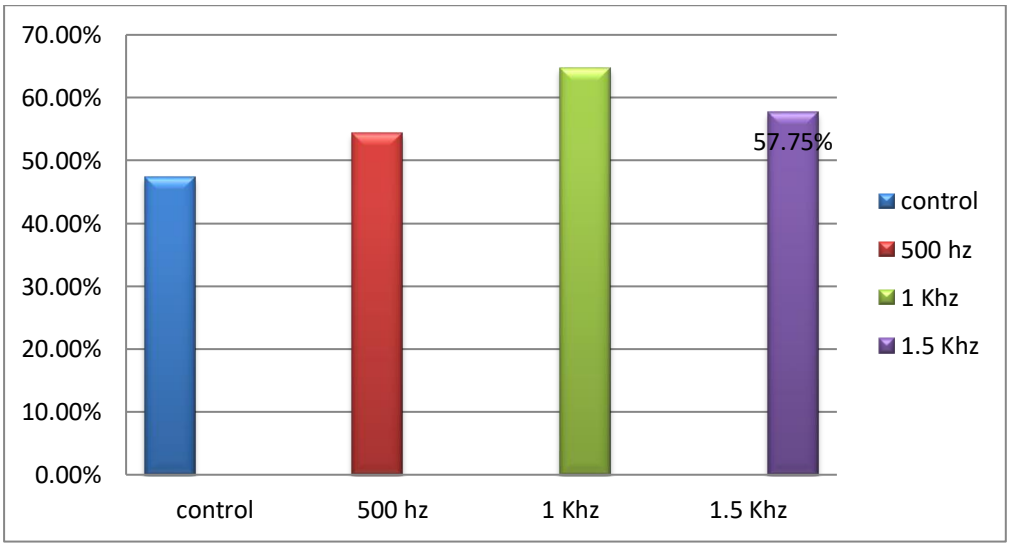


Fig 36.Impact of sound waves at different frequencies on the % radical scavenging activity of ***G.kurroo*** after 10 days

4.4 PHYSICO-CHEMICAL CHARACTERIZATION-

For calculating surface tension as well as the surface free energy on both the surface of the leaf (on adaxial and abaxial regions of the leaf) with the help different solvents such as

water, ethanol, toluene,0.1N Hcl, liq.NH_3 and by means of different surfactants SDS(anionic), CTAB(cationic), Tween—20 (non-ionic). These surfactants plays a crucial role as they reduce surface tension and surface free energy on leaf surfaces, thus enhance the wetting behaviour of the leaf.

Table 9. Different types of surfactants are taken for calculating contact angle and SFE

Solvents	Contact angle	Surface free energy
Water	48.321, 53.832, 47.698	53.760
Ethanol	35.315, 29.998, 26.146	64.145
Toluene	6.712, 10.189, 6.206	72.122
0.1 N Hcl	81.370, 77.586, 75.726	36.584
Liq.NH_3	80.633, 76.928, 75.467	36.929

4.5 Measurement of Contact angle (for control sample and test sample)

With increase in contact angle, the surface wettability of the leaf hinders as more the contact angle, lesser will the surface free energy as well the surface wettability characterstics.

Thus, contact angle is inversely proportional to SFE (surface free energy)

4.51. For SDS (Sodium Dodecyl sulphate)

Fig 36. SDS at different concentration for measuring the contact angle

(for control sample) **(for test samples)**

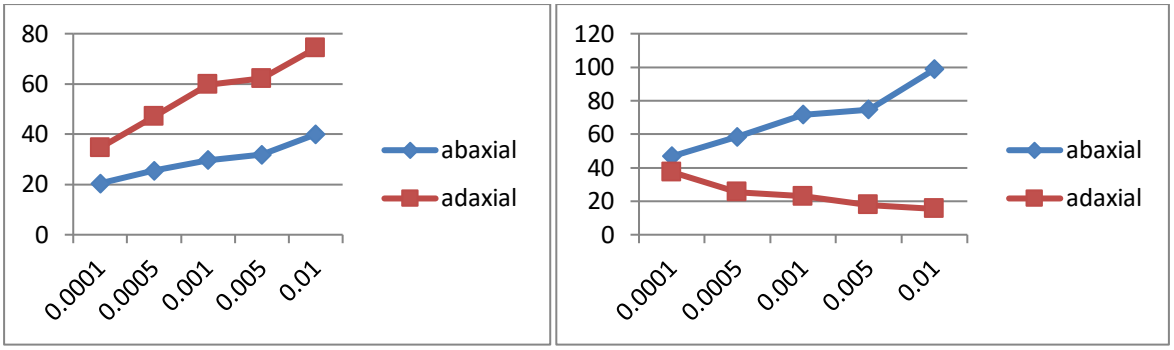


Fig. 36(a) and 36 (b). Contact angle measurements with different concentrations

4.52 **For CTAB(Cetyltrimethylammonia bromide)-**

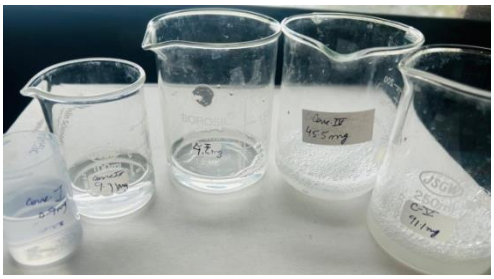
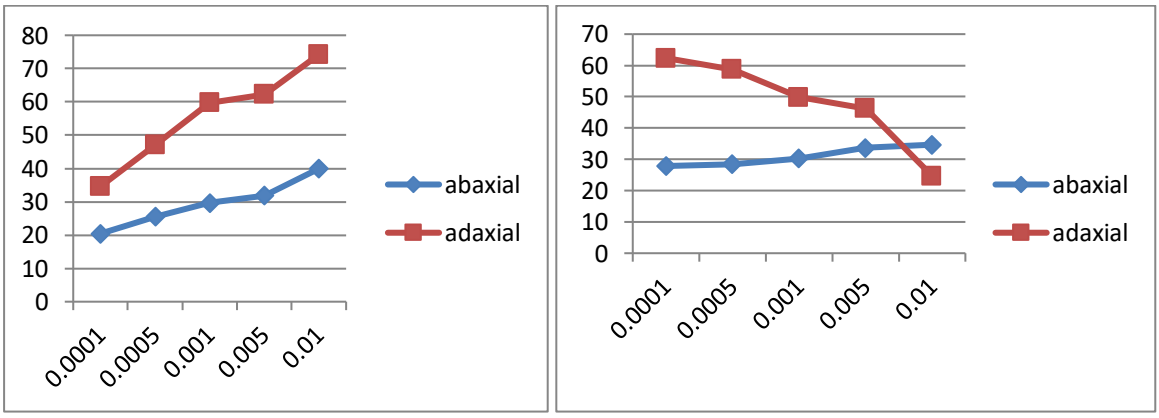


Fig 37.

Fig 37(a) and (b). CTAB at different concentration for measuring the contact angle

(For control sample) **(for test samples)**



4.53 **<u>For Tween – 20</u>**

Fig 38. Tween-20 at different concentration

(For control sample) **(for test sample)**

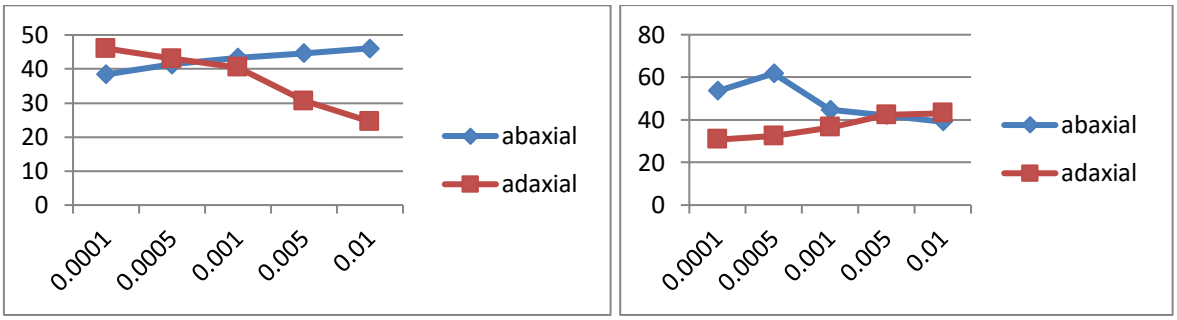
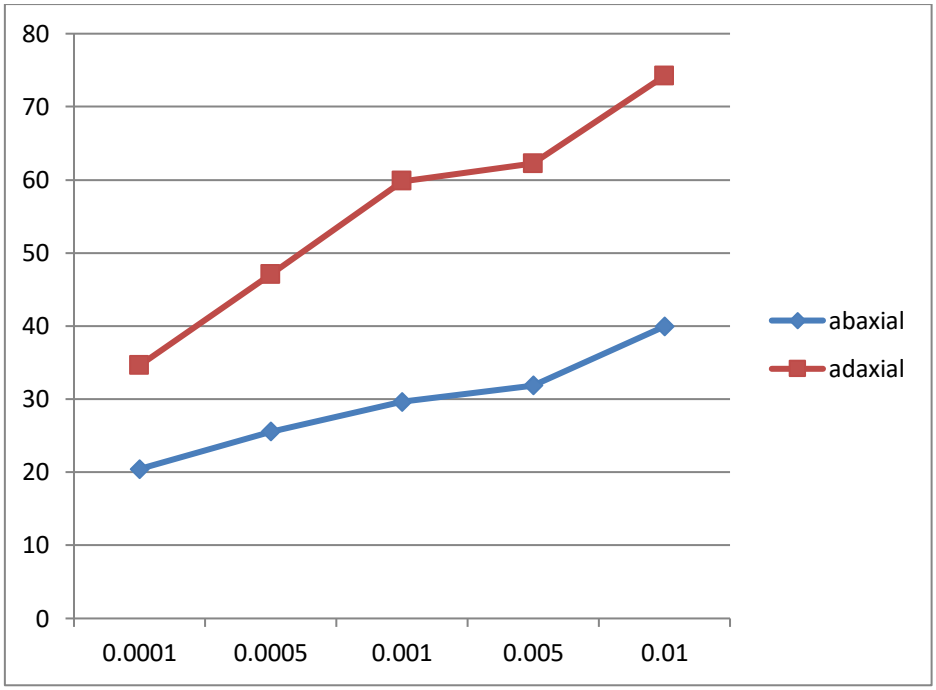


Fig 38 (a) and Fig 38 (b). Contact angle measurement with different concentrations

Amongst, the surfactants that are used Tween-20 is the mildest of all due to its non-ionic nature. As the surfactants SDS as well as CTAB induces some changes in the leaf surface like- disruption of cuticular wax, phytotoxicity, alteration in ion exchange, genotoxicity etc. Whereas, on comparing the three surfactants, polysorbate- 20 is preferred because of its mildness, biocompatibility, versatility and stabilizing properties. As SDS and CTAB caused leaf whitening as well as bleaching by disrupting their leaf surfaces.

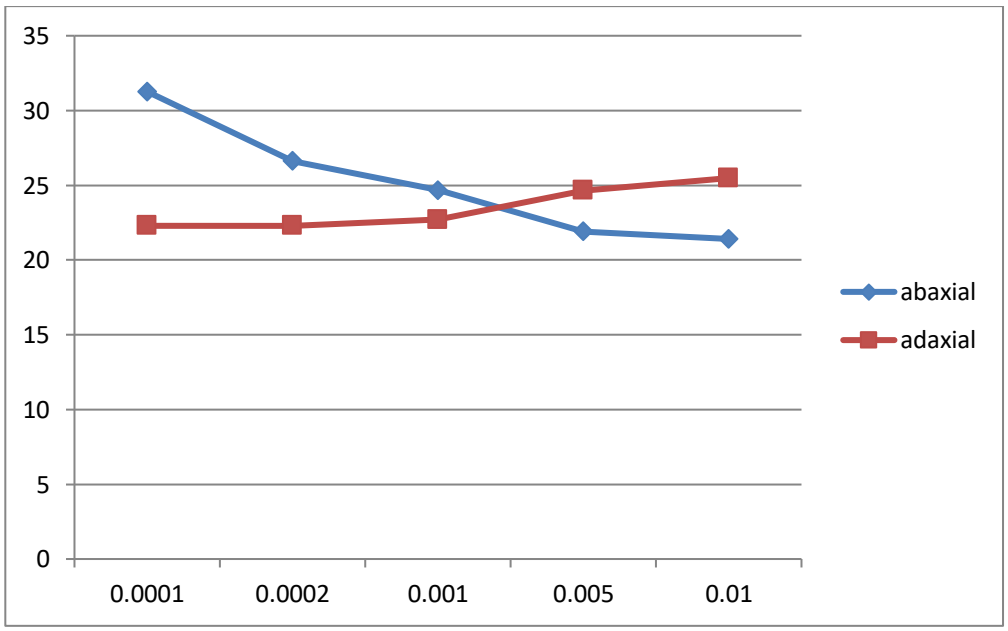
4.6 **Selection of Tween 20 for (1kHz and 1.5 kHz)-**

For test samples



4.7 **For 1.5 kHz**

For test sample



4.8 Investigation of thermo-acoustic parameters:

Table 10. Thermo-acoustic parameters of ***G.kurroo*** extracts.

Acoustical Parameters	Temperature K	Control	500 Hz	1kHz	1.5 kHz
Density ρ Kg/m^3	20℃ 25℃ 30℃ 35℃ 40℃	1.174g/cm^3 1.143g/cm^3 1.154 g/cm^3 1.1540g/cm^3 1.159 g/cm^3	1.132 g/cm^3 1.081g/cm^3 1.075g/cm^3 1.0725/cm^3 1.072g/cm^3	0.99g/cm^3 0.963g/cm^3 0.96 g/cm^3 0.95g/cm^3 0.93 g/cm^3	0.952g/cm^3 0.95 g/cm^3 0.941g/cm^3 0.94g/cm^3 0.88 g/cm^3
Viscosity,η Nsm^2	20℃ 25℃ 30℃ 35℃ 40℃	0.72cp 0.66 cp 0.60 cp 0.42 cp 0.30 cp	2.22 cp 2.15 cp 2.10 cp 1.56 cp 1.32 cp	3.60 cp 3.00 cp 2.76 cp 2.64 cp 2.34 cp	3.12 cp 2.94 cp 1.50 cp 0.72 cp 0.66 cp
Ultrasonic velocity, *U* m/s	20℃ 25℃ 30℃ 35℃ 40℃	1330.43m/s 1318.49m/s 1305.70m/s 1290.58m/s 1276.31m/s	1483.26 m/s 1476.09 m/s 1462.92 m/s 1453.05 m/s 1443.97 m/s	1507.27m/s 1502.37m/s 1493.34m/s 1483.74m/s 1474.27m/s	1477.8m/s 1465.5 m/s 1456.9m/s 1440.7m/s 1432.4m/s
Surface tension, *o* n/m	20℃ 25℃ 30℃ 35℃ 40℃	32.877n/m 31.958n/m 32.203n/m 37.606n/m 31.930n/m	30.814 n/m 30.024 n/m 29.985 n/m 29.613 n/m 27.795 n/m	29.768 n/m 29.673 n/m 27.362 n/m 27.551 n/m 25.417 n/m	28.620n/m 26.656n/m 26.325n/m 24.477n/m 24.226n/m
Surface energy, γ Joule/sqm	20℃ 25℃ 30℃ 35℃ 40℃	32.877mJ/m^3 31.958mJ/m^3 32.203 mJ/m^3 36.022 mJ/m^3 31.930 mJ/m^3	28.550 mJ/m^3 36.750 mJ/m^3 26.420 mJ/m^3 26.823 mJ/m^3 25.556 mJ/m^3	29.767 mJ/m^2 29.673 mJ/m^2 27.551 mJ/m^2 27.362mJ/m^2 25.417mJ/m^2	33.364mJ/m^2 40.470mJ/m^2 45.757mJ/m^2 49.345mJ/m^2 54.790mJ/m^2

CHAPTER 5

(DISCUSSION)

4.9 DISCUSSION-

G.kurroo, a medicinal herb contains a large number of therapeutic as well as pharmaceutical importance that make it more valuable. Because, it is critically endangered plant there are different researches going on to preserve this plant as this specie of **Gentianaceae** contains different secondary metabolites that can be use in the field of Ayurveda as well as its extract can be further use to treat chronic diseases. According to researches, acoustic or the sound waves help the plant in growing faster than the plant under normal conditions. Presently, researchers shows some positive impact of the sound waves on the plant especially on its shoots as well as roots. These waves help the plants to grow fast as when it enters the plant it affects its transcriptional processes producing two most important genes that are mainly involved in the process of photosynthesis i.e. rubisco -small and fructose aldolase. As [1] from the above reference plamt that are exposed to frequencies tends to grow at a much better and faster rate in comparision to the plants that are under normal conditions. This kind of wave treatment is also known as Sonication in which plants basically exposed to different frequencies. By means of these sound waves their occurs the enhancement in the growth of plants as well as the permeability of cell membrane is also affected. Thus ***G.kurroo*** exposed to different frequencies i.e. 500 Hz, 1Khz, 1.5Khz among which the plants under 1Khz shows the best results than others. The test samples that were under the impact of acoustic waves grew faster than the other plants under normal conditions. After this, investigation of normal plants (under normal condition) as well as the test samples (under various frequencies) helps in providing some good changes towards the composition of culture medium, which help in ensuring good conditions of the plants. Acoustic setup can influence gas exchange in tissue-cultured plants which shows great understanding regarding the diffusion rates as well as the gas solubility . By means of physic chemical characterisation it helps in providing adequate aeration and prevents hypoxia or hypercarbia , which can adversely affect plant growth. Physic chemical characterisation can be done by means of different parameter i.e. density, viscosity, velocity, surface tension and surface free energy. Overall, combining the acoustic studies with physic chemical characterisation help in monitoring the plant growth and helps in understanding the effect of acoustic waves on plant physiology. From[14] on combining the acoustical studies with the physico-chemical characterisation helps in the drug formulation because of the intramolecular force of attraction in *in-vitro* plant extract, prepared from the in-vitro grown plants. In-vitro plants can be used as one of the alternative resources for the development of herbal drugs.

CHAPTER 6

CONCLUSION

5.0 CONCLUSION-

Gentiana kurroo, commonly known as Kutki or Trayamana. It is a critically endangered plant native to the regions of India, Pakistan and Nepal. *G.kurroo* is basically known for its medicinal properties in ancient times. The effect of acoustic waves shows tremendous effect on the growth and development of the plant. By means of acoustic sound waves plant (especially in vitro plants) tends to grow with proper nutrient uptake, more amount of phenolic and flavanoids contents than control. By means of different assay, it is found that increase in the anti-oxidants can also be seen in the plants under acoustic setup than the control samples (plants under normal conditions). Different thermo-acoustical parameters also checked in the plants to know about the interaction of solute with the solvent in the liquid extract of *G.kurroo*. With the help of optical tensiometer, hydrophobicity and hydrophilicity can also be checked depends upon the surface of leaves. Optical tensiometer can also be used to optimize the contact angle due to which surface tension as well as the surface free energy can be calculated. More the contact angle, lesser will be the surface tension as contact angle is inversely proportional to surface tension. With the increase in temperature, there shows a decrease in the velocity, viscosity, density, acoustic impedance . Thus, on combining the acoustical studies with the physico-chemical characterisation helps in the drug formulation because of the intramolecular force of attraction in *in-vitro* plant extract, prepared from the in-vitro grown plants. In-vitro plants can be used as one of the alternative resources for the development of herbal drugs.

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