IN-VITRO: MICROPROPAGATION OF MINT AND INVESTIGATE THE ANTIBACTERIAL ACTIVITY OF MINT EXTRACT.

Shubham M. Shende[1*], Pooja Bhandare[2], Nilam Nehare[3], Puja Durge[4], Mona Gajhbiye[5], Pooja E. Waware[6], Lalchand Devhare[7]

Abstract:
Micropropagation is a method to grow plants in a sterile environment from tissue culture. The objective of this study was to investigate the feasibility of micropropagation of mint (Mentha spp.) and evaluate the antibacterial activity of mint extracts. The in vitro regeneration of mint varieties was performed on Murashige and Skoog (MS) medium supplemented with various plant growth regulators. The established in vitro propagated plantlets were subjected to acclimatization, and the plantlets were transferred to greenhouse conditions. The antibacterial activity of the methanolic extract of mint was evaluated against Gram-positive Bacillus cereus and Gram-negative Escherichia coli using the Disc diffusion method. Mint extracts exhibited a considerable inhibitory effect against both the tested bacteria.

Keywords: Mint, MS media, BAP, NAA, Antibacterial Activity etc.

[1*,2,3,4,5,6,7] Department of Pharmacy, School of Pharmacy, G H Raisoni University, Saikheda. (MP) -480337.
ORCID: 0000-0002-6564-5745

***Corresponding Author*:** Shubham M. Shende
School of Pharmacy, G H Raisoni University Saikheda, MP, India, Email Id: Sshende35@gmail.com

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INTRODUCTION:

Tissue culture is the in vitro aseptic culture of cells, tissues, organs or on the other hand entire plant under controlled dietary and ecological conditions frequently to produce the clones of plants. The resultant clones are consistent with sort of the chose genotype. The controlled conditions give the way of life a domain helpful for their development and growth.In1902, In vitro culture is one of the keys of plant biotechnology that makes use of the totipotency nature of plant cells, a concept proposed by Haberlandt and he is referred as "Father of Tissue Culture" .[1]

Fig No. 01: Mint Leaves

Mint is aromatic herb, it is a member of the Lamiaceae family with a several medicinal and culinary uses. Essential oil extracted from mint contains an array of bioactive compounds like menthol, limonene, and menthone that exhibit antimicrobial properties. In vitro propagation is a cost-effective technique to increase the yield of plant species and can be employed for the production of large-scale mint planting material.[3] Current study were to develop a protocol for micropropagation of mint from tissue cultures and to investigate the antibacterial activity of mint extracts against pathogenic bacteria

Methods:

A) For In-Vitro Micropropagation Of Mint

1) Sample collection

1-Monthes old plant of Mentha was collected from Lohara nursery near Chandrapur. Shoot tip were collected from the plant and were use as explants for micropropagation.

2) Media Preparation and sterilization

Murashige and Skoog (MS) Medium:

MS medium contains a comprehensive range of nutrients required for plant growth and development. The ingredients used in MS medium and their concentrations are as follows:

Table No.1 Formula for Murashige and Skoog (MS) Medium

Sr.No.	Ingredients	Quantity Taken
	Macronutrients	
1	Ammonium nitrate	1650 mg/L
2	Potassium nitrate	1900 mg/L
3	Calcium chloride	440 mg/L
4	Magnesium sulfate	370 mg/L
5	Monopotassium phosphate	170 mg/L
	Micronutrients	
6	Iron(III) sodium ethylenediaminetetraacetate	27.8 mg/L
7	Manganese sulfate	22.3 mg/L
8	Zinc sulfate	8.6 mg/L
9	Copper sulfate	0.025 mg/L
10	Boric acid	6.2 mg/L
11	Sodium molybdate	0.25 mg/L
	Vitamins	
12	Myo-inositol	100 mg/L
13	Nicotinic acid	0.5 mg/L
14	Pyridoxine hydrochloride	0.5 mg/L
15	Thiamine hydrochloride	0.1 mg/L
	Carbon source	
16	Sucrose	30 g/L
	Gelling Agent	
17	Agar	8 g/L

The final pH of MS medium should be adjusted to 5.8 before sterilization using HCl or NaOH. Dissolve the macronutrients first in distilled water, followed by micronutrients, vitamins, and finally carbon source, before adding agar to solidify the medium. The MS medium can be sterilized by autoclaving at 121°C and 15 psi for 20-30 minutes.

3) Procedure for in vitro propagation

The procedure for in vitro propagation of the mint varieties were as follows Fresh, healthy plant materials were collected and sterilized using 1% sodium hypochlorite for 10 minutes followed by three washes with sterile distilled water. The sterilized explants were then placed onto the sterile MS medium containing various concentrations of plant growth regulators, including BAP (6-(benzylamino purine), IAA (indole-3 acetic acid), and NAA (naphthalene acetic acid). The cultures were then placed under controlled conditions of light (16-hour photoperiod) at 22°C ± 2°C. The obtained shoots were then used as donor materials for subsequent proliferation.[2,6,8]

B) For Antibacterial activity of mint

1) Method Of Extraction:

Obtain fresh mint leaves and wash them thoroughly to remove any foreign particles or debris. Chop the mint leaves into small pieces and place them in a clean glass container. Pour enough methanol over the chopped mint leaves to cover them completely. Seal the glass container tightly and allow it to stand at room temperature for about 24 hours. After 24 hours, strain the liquid into a clean glass container using a muslin cloth or filter paper. Repeat the process of adding fresh methanol to the mint leaves and allowing it to stand for 24 hours, followed by straining, at least two more times. Combine the extracted liquids in a clean container and evaporate the methanol using a rotary evaporator or a vacuum evaporator at low temperature, until a dry powder is obtained. Store the dry methanolic extract of mint in a clean, airtight container at room temperature for future use.

Note: Ensure that proper safety precautions are taken when dealing with methanol, as it is highly flammable and toxic.

2) In-Vivo Study on Pathogenic Bacteria:

For the antibacterial activity study[7], the methanolic extract of mint was prepared from the leaves of the in vitro regenerated plants. The extract's antibacterial activity was assessed using the disc diffusion method against Gram-positive bacteria Bacillus cereus and Gram-negative bacteria Escherichia coli.

3) Method of disc diffusion method

The disc diffusion method is a microbiological technique used to determine the antimicrobial activity of various substances against specific microorganisms. The method involves the following steps are First, prepare the appropriate culture media for the microorganism you want to test. This may involve sterilizing the media using an autoclave. Using a sterile swab, inoculate the culture media with a standardized amount of the test microorganism. Spread the culture evenly over the surface of the media. Soak sterile filter paper discs into the substance to be tested. Ensure that the discs are saturated with the substance. Place the saturated filter paper discs on the surface of the inoculated culture media. Use a sterile forceps to apply gentle pressure to ensure the discs are in full contact with the surface of the media. Incubate the plates for a specified period of 24 hours according to the optimal growth conditions at 37°C ± 0.5°C and maintain uniformity ±1°C under 3tube illumination of lights for the microorganism. After the completion 24 hours of incubation period, observe the plates for the presence of a circular zone of inhibition. This zone indicates the amount of antimicrobial activity exerted by the extract against the microorganism being tested.[5] (Refer Fig.No.03)

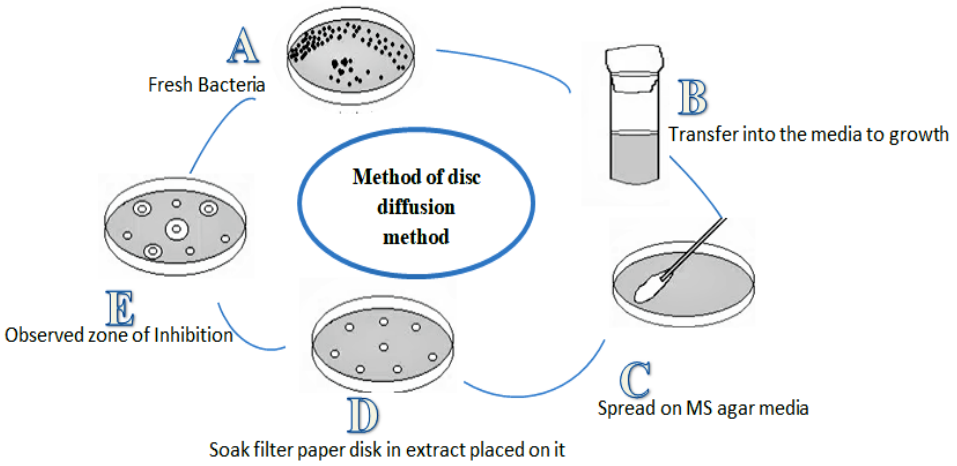


Fig.No.02: Method of disc diffusion method

RESULTS:

The results indicated that the MS medium supplemented with BAP (150µl/50ml) and NAA (20µl/50ml) resulted in the highest shoot multiplication rate 82.6% and 88.8% Respectively. The established in vitro propagated plantlets were acclimatized and transferred to greenhouse conditions where they grew healthily.

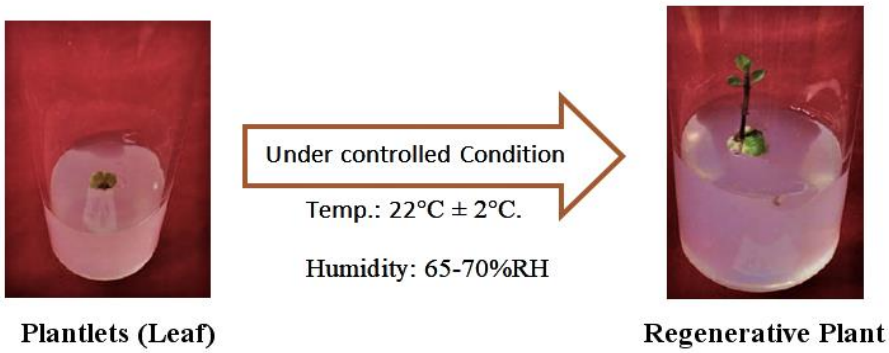


Fig.No.03: In-vitro Micro propagation /regeneration of Mint leaf

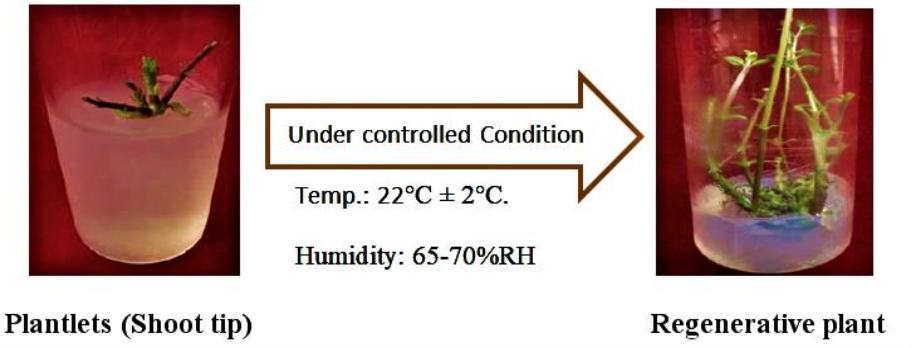


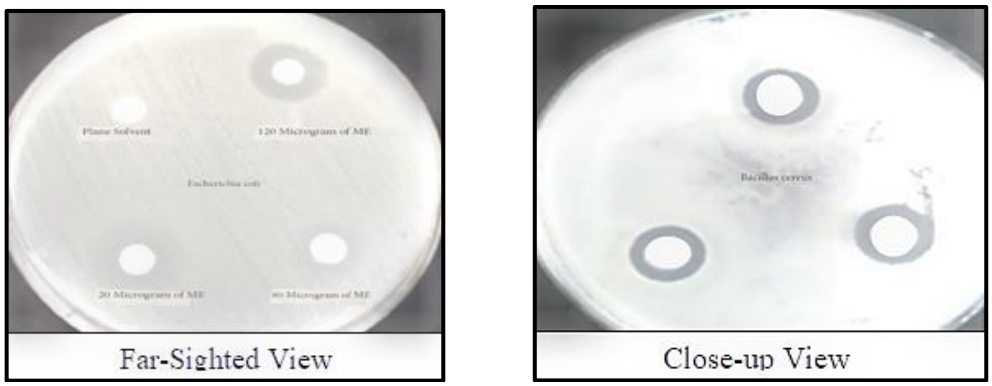
Fig.No.4: In-vitro Micro propagation /regeneration of Mint

Table No.2: Shoot regeneration was observed in media contains BAP and NAA

Sr. No.	Media	BAP	NAA	Intensity
1	MS	100µl	00µl	-
2	MS	150µl	20µl	++
3	MS	200µl	10µl	-

Table No.3: Shoot regeneration was observed in media contains BAP and IAA

Sr. No.	Media	BAP	IAA	Intensity
1	MS	100µl	00µl	-
2	MS	150µl	20µl	++
3	MS	200µl	10µl	-



A) Far-Sighted View (E.Coli) B) Closed Up(Bacillus Cereus)

Fig No.05: Zone of inhibition of Pathogenic Bacteria against Methanolic extract of Mints

The methanolic extract of mint exhibited considerable inhibitory effects against both Bacillus cereus and Escherichia coli with a zone of inhibition ranging from 10.5 mm to 13.7 mm.

CONCLUSION:

The micropropagation protocol developed for mint was found to be successful in producing in vitro propagated plantlets. The antibacterial activity results of the methanolic extract of mint indicated that the plant contains compounds possessing potential antibacterial (antimicrobial) activity. Further studies are needed to identify and isolate the bioactive compounds responsible for the observed antibacterial activity of mint.

Conflict of Interest- Nil

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