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Showcasing inhibition zone of homoeopathic culture media in fungus and bacteria culture assay

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Abstract
Through this research work showcase no inhibition of homoeopathic medicated culture media towards growth of fungus and bacteria assay by cup plate method. After in vitro bacterial and fungal assay, there was no such growth inhibition seen in Fungus and bacterial growth by using Homoeopathic Medicated Culture Media.

Keywords: Fungus, bacteria, cup plate, culture media, bacteria culture assay

Introduction
A pure culture serves as the basis for all infectious disease research, as suggested by Robert Koch [1, 2]. A connection between microorganisms and infectious diseases is established when a bacterium is isolated for the first time, allowing for the creation of experimental models to assess virulence and satisfy Koch's criteria [3]. The first stage in developing suggestions for efficient therapy is bacterial culture, which also makes it possible to investigate the antibiotic susceptibility of bacteria [4, 5]. Getting a pure bacterial culture also makes it possible to sequence the strains' genomes [6, 7] and perform proteomic studies that highlight particular proteins and use immunoproteomic techniques to assess their antigenicity. This ultimately makes it easier to produce the proteins that act as antigens in serologic testing [8]. Lastly, modification and transformation are made possible by pure bacterial culture, involving altering genes to examine the source of bacterial invasiveness, antibiotic resistance, and pathogenicity. But in clinical microbiology, culture has not made the same strides as molecular biology over the past three decades [9]. Compared to molecular approaches, bacterial culture is usually more challenging and requires more expertise. As a result, there are now fewer microbiologists who specialize in anaerobic bacteria than there were in the 1970s, and the number has been continuously declining over the past 30 years. Clinical microbiologists [10-12] who specialize in intracellular microorganisms were largely responsible for rekindling interest in bacterial culture. They have created axenic media, which are sterile media that only contain the organism being grown, in order to cultivate very picky microorganisms [11, 13, 14]. Following a brief overview of early culture strategies, we offer here a thorough analysis of both historical and contemporary culture methods for the cultivation of picky bacteria. In a follow-up review, we go into further detail on the advancements made possible by new identification techniques and how all of these advancements can be used, using the example of culturomics research on the human gut microbiota [15].

Materials and Methodology
Type of Study: Invitro study
Site of study: PIPR (Parul Institute of Pharmacy & Research), PIHR (Parul Institute of Homeopathy & Research), Parul University, Vadodara, Gujarat
Duration of study: 1 month

Preparation of Homoeopathic Culture Media
1. The first step is to sterilize every piece of equipment used in the lab by immersing them in a hot water bath for fifteen minutes.

2. The second step is to make a homoeopathic tincture of Echinacea angustifolia Q with 20 millilitres of distilled water and 1 gram of potassium Ferricynaide as a biocatalyst.
   After that, combine it in a 100 ml sterile beaker to create an Echinacea Nano solvent.
3. The third phase involves filling a 50 ml sterile bottle with 20 ml of Echinacea Angustifolia solvent. The bottle is then potentized using an electric potentizer machine for 100 strokes, giving the medication a strength of 1/100, this process is now known as Echinacea Nano solvent.
4. Use 1 millilitre of Echinacea angustifolia Nano solvent in nine parts olive oil as a basis, then heat it indirectly with hot water bath for 15 minutes, which produced the mother base Nano solvent 1X for Echinacea olive oil (3 ml Echinacea Nano solvent: 27 ml olive oil) in a 1:9 ratio according to HPI.

The fifth step is to prepare Echinacea olive oil Mother base Nano solvent 2X (3 ml Echinacea Nano solvent: 27 ml olive oil) as 1:9 according to HPI by taking 1 ml Echinacea Angustifolia olive oil Mother base Nano solvent in 9 parts olive oil as a base and indirect heating it by a hot water bath for another 15 minutes.
Step 5: A SEM scanning electrochemical analysis should be performed using Echinacea angustifolia Nano solvent and Echinacea olive oil mother base Nano solvent 2X.[16].

Table 1: SEM & EDS Analysis of Homoeopathic Medicated culture Media at 50 micron [16]

Element	Signal Type	Wt%	Wt% Sigma
O	EDS	22.88	1.35
Na	EDS	13.67	0.58
Mg	EDS	2.55	0.37
Al	EDS	1.71	0.3
Si	EDS	1.07	0.25
Cl	EDS	9.98	0.42
K	EDS	29.17	0.78
Ca	EDS	1.91	0.36
Fe	EDS	17.05	0.87
Total	100		

Table 2: SEM & EDS Analysis of Homoeopathic Medicated culture Media at 50 micron [16]

Element	Signal Type	Wt%	Wt% Sigma
O	EDS	13.86	0.97
Na	EDS	9.42	0.39
Cl	EDS	5.29	0.26
K	EDS	50.54	0.81
Fe	EDS	20.9	0.74
Total	100		

Table 3: SEM & EDS Analysis of Homoeopathic Medicated culture Media at 50 micron [16]

Element	Signal Type	Atomic%
O	EDS	28.01
Na	EDS	13.25
Cl	EDS	4.83
K	EDS	41.81
Fe	EDS	12.1
Total	100	

Table 4: SEM & EDS Analysis of Homoeopathic Medicated culture Media at 100 micron [16]

Element	Signal Type	Wt%	Wt% Sigma
C	EDS	82.7	0.63
O	EDS	16.74	0.63
K	EDS	0.57	0.09
Total	100		

Table 5: SEM & EDS Analysis of Homoeopathic Medicated culture Media at 100 micron [16]

Element	Signal Type	Atomic%
C	EDS	86.65
O	EDS	13.17
K	EDS	0.18
Total	100	

Procedure

1. Procedure for In-vitro antifungal activity determination

In-vitro antifungal activity of the sample formulations were determined by cup-plate method/Agar well diffusion assay. Stock cultures of fungi was be maintained at 4 0 C on slant of Sabouraud dextrose agar. Active cultures for experiment was prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for fungi/ sterile distilled water and incubated for 24 hours at 37 0 C. The assay was performed by cup-plate technique. The required quantity of Sabouraud dextrose agar dissolve in 100 ml of distilled water with continuous heating and agitation, maintain the pH 5.5. The prepared media was autoclaved at a temperature of 121°C for 15 min for sterilization. Take sterile glass Petri plates pour the media into the plates aseptically. When the media solidified, pierce the surface of the agar plate using a sterile cork borer to make cups of 6 mm diameter. Inoculate the fungi species by streaking on the surface of the media. The prepared hall was then filled with the sample formulation and allowed to rest for at least 30 min and incubated for 24 to 48 hrs at a temperature of 25±1°C. After incubation, the most uniform outer diameter of the zone of inhibition was recorded in millimetres. Each assay was carried out in triplicate

Antibacterial assay using agar well diffusion method

The antibacterial activity, of the test formulations, was determined by agar well diffusion method. To perform antibacterial assay, initially the stock cultures of bacteria were revived by inoculating in broth media and incubated at 37°C for 24 hr. The agar plates of the Nutrient Agar/ Muller Hilton agar media were prepared. Each plate was inoculated with an aliquot (0.1 ml) of the bacterial suspension (105-106 CFU/ml) *Staphylococcus aureus & E.coli*, in the respective media, which was spread evenly in the plate under aseptic condition. Suspension in each plate was allowed to dry for 20-25 min. Then, wells having 6 mm diameter were made with the help of sterile cork borer in the solid medium and filled with test samples of different volume/concentration. All the plates were incubated at 37°C for 24 hr. The antibacterial activity of each sample was assessed by measuring the diameter of the zone of inhibition (in mm) around each well. Three replications were carried out for each test formulations against each of the test organism. The results were compared with alcohol as control.

Table 6: Zone of Inhibition of Test formulations against *Candida albicans*

Sample	Volume [ml]/ Concentration[µg/ml]	Diameter [cm]			Average [cm]
ENPOS	50 µl	-	-	-	-
ENPOS	100 µl	-	-	-	-

ENPSO-Homoeopathic Medicated Culture Media

Table 7: Zone of Inhibition of Test formulations against *B. subtilis*

Sample	Volume [ml]/ Concentration[µg/ml]	Diameter [cm]			Average [cm]
ENPOS	50 µl	-	-	-	-
ENPOS	100 µl	-	-	-	-

ENPSO-Homoeopathic Medicated Culture Media

Table 8: Zone of Inhibition of Test formulations against *E. coli*

Sample	Volume [ml]/ Concentration[µg/ml]	Diameter [cm]			Average [cm]
ENPOS	50 µl	-	-	-	-
ENPOS	100 µl	-	-	-	-

ENPSO-Homoeopathic Medicated Culture Media

Table 9: Zone of Inhibition of Test formulations against *Staphylococcus aureus*

Sample	Volume [ml]/ Concentration[µg/ml]	Diameter [cm]			Average [cm]
ENPOS	50 µl	-	-	-	-
ENPOS	100 µl	-	-	-	-

ENPSO-Homoeopathic Medicated Culture Media

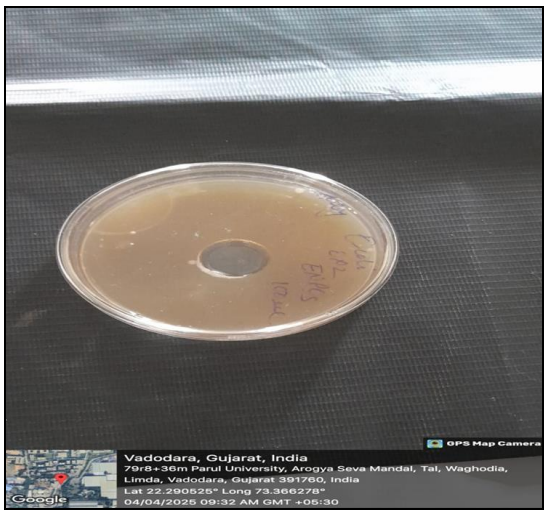

Fig 1: Homoeopathic Culture media on *E. coli* at 100 microliter

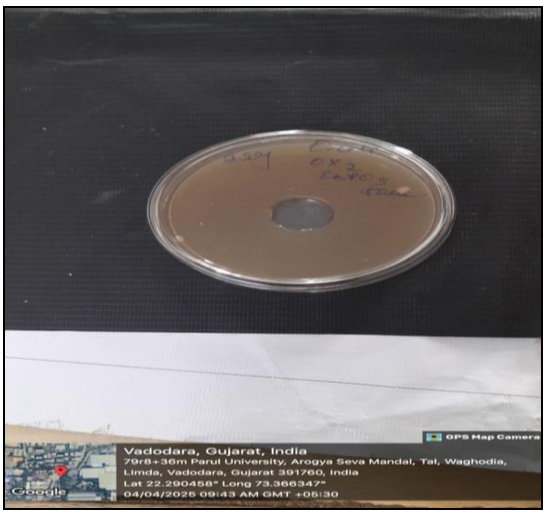

Fig 2: Homoeopathic Culture media on *E. coli* at 50 microliter

Fig 3: Homoeopathic Culture media on *Staphylococcus aureus* at 50 microliter

Fig 4: Homoeopathic Culture media on *Staphylococcus aureus* at 100 microliter

Fig 5: Homoeopathic Culture media on *B. subtilis* at 50 microliter

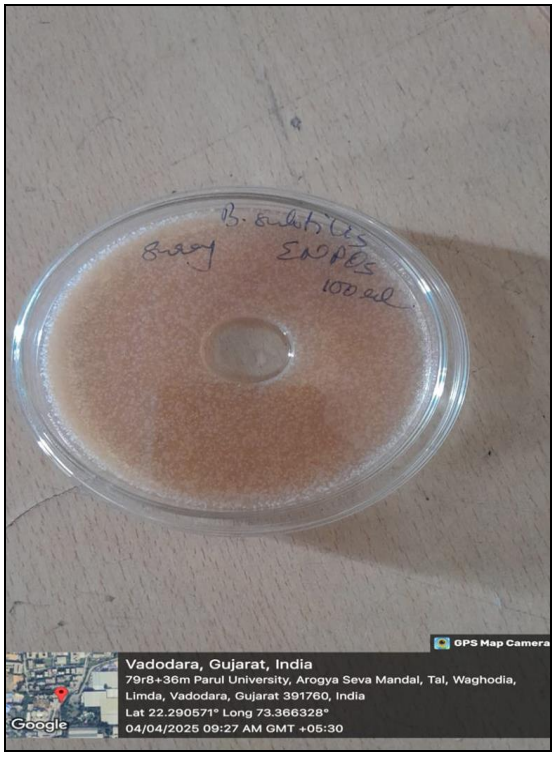

Fig 6: Homoeopathic Culture media on *B. subtilis* at 100 microliter

Fig 7: Homoeopathic Culture media on *Candida albicans* at 50 & 100 microliter

Conclusion

There was no such growth inhibition seen in Fungus and bacterial growth by using Homoeopathic Medicated Culture Media.

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Conflict of Interest

Not available

Financial Support

Not available

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