Sampreshan
UGC CARE GROUP 1
https://sampreshan.info/
Vol. 17, Issue No. 2, June 2024
ISSN: 2347-2979

ANTIMICROBIAL ACTIVITY OF HELIANTHUS ANNUS AGAINST SOME SELECTED HUMAN PATHOGENIC BACTERIA

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Abstracts: Helianthus annus is a very useful medicine plant which found near rice field throughout in India. Whole plant Helianthus annus there are six crude extracts were prepared using different solvents by cold maceration method. Antimicrobial activity against Basillus subtilis, Klebsiella pneumonae and candida albicans were detected with extacts and Ciprofloxacine and fluconazole use as standard by cup plat agar diffusion method. The extracts were subjected to screening to detect potential antimicrobial activity against Basillus subtilis, Klebsiella pneumonae and candida albicans, Ciprofloxacin and Fluconazole as standard by cup plate agar diffusion method. In presence study, our aim was to find out the antimicrobial activity of different extracts of whole plant Helianthus annus. Some different extracts such as methanol, petroleum ether, chloroform and aqueous extract exhibits comparable antimicrobial activity with standard.

Keywords: Antimicrobial, bacteriological, *Helianthus annus*, seed extract, water treatment, zone of inhibition

Introduction

Plants have been a valuable source of natural products since long period of time. They are maintaining human health, from the last decade with more intensive studies for natural therapies .Now-a-days, in many countries the use of phytochemicals for pharmaceuticals purpose has increased. They survey of word health organization was found that the medicinal plants (Haidan Yuan, 2016). The plant extracts used a crude, the known antimicrobial properties of their parts and phytochemicals it can be most significant in the therapeutic treatments. For antimicrobial activity the plant product screenings had shown a potential source of novel antibiotic. (Afolayan, 2003). It has been an increasing incident of multiple resistances created in human pathogenic microorganisms. Maximum plants have been used because of their antimicrobial traits or properties, which are due to the secondary metabolities synthesized by the plants. The active substances are known by their products as phenolic compounds which is the part of the essential oils, as well as in tannin. In current years, largely indiscriminate use of commercial antimicrobial drugs employed in the infectious diseases treatment, micobial resistance has developed. It has forced to scientist for searach for newer anti-microbial substances from various sources like the medicinal plants. Plant produces a large range of secondary metabolites it is used either directly as lead compounds or precursors in the chemical or pharmaceutical industries. Plant extracts shows target sites other than used by antibiotics. It will be active against drug resistant pathogenic

ISSN: 2347-2979

microorganisms . There is a little information is available on such activity of medicinal plants and very large number of plant species on earth. only a small number has been systematically investigated for their antimicrobial activities (Shyamala, 2012). In the plant cells bioactive compounds are normally accumulated secondary metabolities but concentrations varies according to the plant parts, season climate and particular growth phase. The plant part leaf is one of the used for therapeutic purposes. The growth of disease is inhibited by some active compounds (Dhia, 2006). The antimicrobial properties of certain Indian medicinal plants were reported based on their old literature information (Dayal and Purohit,1971; Hook and Thomas,1995; Reddy,1995; Suresh et al.,1995;Mehmood *et al* .,1999;Ahmed *et al*.,1998;Perumal Samy *et al* ., 1998) and inhibitory activity some pathogenic fungi and bacteria (Tayer *et al*., 1995)

Material and Method

Plant Material: The fresh leaves of Helianthus annus was collected from Betul in the month of march 2017(Latitude: 21.9672^0 N; Longitude: 77.7452^0; Magnitude: The area of district has 10043 km^2).

For the further study, microbial culture of pathogenic bacteria *Basillus subtilis*, *Klebsiella pneumonae* and fungal culture *Candida albicans* were obtained from "Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India". After collection of leaves it was dried in shade or dark area and powdered to coarse consistency in culture mill. Powders of plant parts were stored in an airtight container at room temperature for further study.(Khandelwal,2005;Kokate ,1995;Mukharji,2007).

Storage of microbial culture

Bacterial cultures were maintained on Nutrient agar plates grown continuously for 24-48 hrs and fungal culture was maintained on Potato dextrose agar plates grown on 7 days and sub cultured regularly.

Drying of Plant materials

Drying of fresh plant parts was carried out in sun but under the shade. Collected plant material was washed under running tap water and kept in shade for drying. Avoidance of any microbial growth on plant material was done by visual observation. Dried plant material was pulverized using mechanical grinder. Pulverized plant material was observed for colour, order, texture and was kept in air tight container and kept till further processing.

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1.2 Extraction procedure

Extraction involves the separation of medicinally active portion of plant tissue from the inactive or inert components by using selective solvents in standard extraction procedures. The products obtained from plants are relatively impure liquids, semisolids or powders. Following procedure was adopted for the preparation of extract from the shade dried and powdered aerial parts (Khandelwal, 2005; Kokate, 1994)

1.2.1 Defatting of Plant Material

The shade dried Whole plant materials of *Helianthus annuus* were extraction with petroleum ether using maceration method (Mukherjee, 2007). The extraction was continued till the defatting of the material had taken place.

1.2.2 Extraction by maceration Method

Plant material were extracted in two solvents of different polarity viz water, methanol and chloroform. Powdered plant material 100gm of *Helianthus annuus* were extracted by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis (Mukherjee, 2007).

1.8 Antimicrobial activity of extracts of *Helianthus annus*

Pathogenic microbes used

The pathogenic bacteria and fungus used in the current study obtained from Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India.

Media preparation (broth and agar media)

Materials – Agar -1.5 g (15%); Beef extract – 0.3g; Peptone 0.5g (1%)
Nuetrient Agar Media was prepared with above ingredients and suspend the ingredients complete to warm the media. The supernatant was filtered and used as NAM media. It was supplemented with 15 gm of Agar, 03 gm of Beef extract, 0.5 gm of Peptone and the volume was made to 1000 ml with DW and pH was adjusted to 7 with the help of NaCl. The medium was autoclaved for 20 min at 15 lbs.

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Composition of nutrient agar media;

Agar - 1.5 gms.

Beef extract - 0.3 gms.

Peptone - 0.5 gms.

Sodium chloride - 0.55 gms.

Distilled water - to make 100 ml.

pH – 7

Potato Dextrose Agar (PDA) Preparation

Materials – Potato -200 g; Dextrose -10 g (1%); Agar-agar -20 g (2%).

Procedure: 200 gm potatoes were peeled, grated and soaked in DW. It was kept in refrigerator overnight. The supernatant was filtered and used as potato extract. 200 ml of this potato extract was supplemented with 10 gm of dextrose and 20 gm of agar powder and the volume was made to 1000 ml with DW and pH was adjusted to 6. The medium was autoclaved for 20 min. at 15 lbs. Cholramphenicol powder was added to the above medium in molten state to the final concentration of 2 mg ml^{-1}.

Potato infusion - 20 gms.

Dextrose - 2 gms.

Agar - 1.5 gms.

Distilled water - to make 100 ml.

pH – 7

Method of preparation

This ingredient with agar medium was dissolved and boiled in distilled water in conical flask of sufficient capacity. These dry ingredients are move to flask containing required quantity of distilled water and heat the medium to dissolved completely.

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Sterilization of culture media

The flask was cotton plugged which containing medium and it placed in autoclave for sterilization at 121ºC temperature & 15 lbs /inch^2 for 15 minutes.

Preparation of inoculums

Spores of the selected fungus were harvested from 7 days slant cultures by suspension in sterile distilled water containing 0.01% Tween-80. The spore suspension containing about 10^6 - 10^7 spores/ml was diluted approximately and used as the inoculums.

Preparation of plates

The media after sterilization, from flask was immediately poured (20 ml/ plate) into sterile Petri dishes. For solidify the media poured plates were left at room temperature and to check the sterility of plates incubate at 37ºC overnight. The plates were dried at 50ºC for 30 minutes before use.

Revival of the microbial cultures

In lyophilized form microbial cultures used in the study were obtained from the laboratory. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient and potato dextrose broth than incubated for 24 hours at 37ºC. After the incubation growth is observed in the form of turbidity and it can be checked by spectrophotometrycally. These broth cultures were further inoculated on to the nutrient and potato dextrose agar plates with loop full of microbes and further incubated for next 24 hours at 37ºC obtain the pure culture and stored as stocks culture that to be used in future for further research work.

Antimicrobial Studies

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the plant extracts used in present study were prepared by transferring a loop of culture into sterile nutrient and potato dextrose broth and incubated at 37ºC for 24-48 hours. From these broths a loop full culture was taken and seeded onto sterile nutrient agar media plates and potato dextrose agar plates through sterile cotton swab to develop diffused heavy lawn culture.

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The well diffusion method was used to determine the antimicrobial activity of the extract prepared from *Helianthus annus* using standard procedure (Bauer *et al.*, 1966). There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antimicrobial studies. It's essential feature that the placing antibiotics of wells with the surfaces of agar after inoculation with the organism tested. It should never be used undiluted over night broth cultures as inoculums. After these preparations the plates were incubated at 37ºC for 24 hours and examined for clear zones of inhibition around the wells which become particular concentration of drug.

Effect of temperature and pH on microbial growth

The effect of temperature was performed to take various aliquots of the temperature range. Microbes are classified according to temperature range at which they can grow. The rates of growth are highest at the optimum temperature for the organisms. The lowest temperature at which the organism can replicate and survive is its minimum growth temperature. The temperature highest at which growth can occur is its maximum growth temperature. There are few ranges of permissible growth temperatures are only and can vary according to other environmental conditions. Organisms categorized as mesophiles, are adapted to moderate temperature, with optimal growth temperatures ranging from room temperature about 20^0C to 45^0C. As would be expected from the core temperature of the human body, 37^0C, normal human microbiota and pathogen like *E.coli, Salmonella* spp and *Lactobacillus* spp they are mesophiles.

Result

The various crude extracts of helianthus annus showed significant activity against both the bacteria which are tested. The antibacterial activity of the helianthus annus was assessed using the agar well diffusion method by measuring the diameter of growth inhibition zones and its subsequent concentration was tabulated and represented in table and figure. In the extracts choloroform, methanol and aqueous showed high activity (18-23 mm zone of inhibition) on all organisms. Methanol showed a moderate activity (14-19mm)Aqueous extract of Helianthus annus showed minimum activity (15-21mm). The obtained results of the crude extracts are comparable with the

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standard antibiotic such as Ciprofloxacin and fluconazole. All the tested organisms are highly sensitive to the organic solvents of helianthus annus (14-23mm) than the standard ciprofloxacine and fluconazole antibiotics.

Table No. 01 : Results of antimicrobial activity of *Helianthus annus* extracts against selected microbes on different temperature

Microbes	**Chloroform extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at 25ºC		
Bacillus subtilis	6±0	8±0.47	14±0.81
Klebsiella pneumoniae	-	-	-
Candida albicans	6±0	7±0.47	8±0.47
		at 37ºC	
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	-	-	-
Candida albicans	-	-	-
		at 50ºC	
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	6±0	8±0.47	10±0.47
Candida albicans	-	-	-

Microbes	**Methanolic extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at 25ºC		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	-	-	-
Candida albicans	8±0.47	11±0.47	12±0.47
		at 37ºC	
Bacillus subtilis	-	-	-

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Klebsiella pneumoniae	6±0	7±0	8±0.47
Candida albicans	-	-	-
		at 50ºC	
Bacillus subtilis	6±0	6±0	13±0.47
Klebsiella pneumoniae	6±0	8±0.47	11±0.47
Candida albicans	6±0	6±0	10±0.47

Microbes	**Aqueous extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at 25ºC		
Bacillus subtilis	8±0.47	9±0.47	10±0.47
Klebsiella pneumoniae	6±0.47	7±0.47	8±0.47
Candida albicans	6±0.47	7±0.47	8±0.47
		at 37ºC	
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	-	-	-
Candida albicans	-	-	-
		at 50ºC	
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	9±0.47	11±0.47	13±0.47
Candida albicans	-	-	-

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Table No. 18: Results of antimicrobial activity of *Helianthus annus* extracts against selected microbes on different pH

Microbes	**Chloroform extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at pH-5		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	-	-	-
Candida albicans	-	-	-
	at pH-7		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	-	-	-
Candida albicans	-	-	-
	at pH-9		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	-	-	-
Candida albicans	-	-	-

Microbes	**Methanolic extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at pH-5		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	-	-	-
Candida albicans	6±0.47	7±0.47	9±0.47
		at pH-7	
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	6±0	7±0	8±0.47
Candida albicans	-	-	-
		at pH-9	
Bacillus subtilis	6±0	10±0.47	11±0.94

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Klebsiella pneumoniae	7±0.47	11±0.47	13±0
Candida albicans	-	-	-

Microbes	**Aqueous extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at pH-5		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	-	-	-
Candida albicans	-	-	-
	at pH-7		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	-	-	-
Candida albicans	-	-	-
	at pH-9		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	10±0.81	10±0.94	11±0.47
Candida albicans	-	-	-

Photoplates of antimicrobial activity of *Helianthus annuus*extracts against selected microbes on different temperature

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ISSN: 2347-2979
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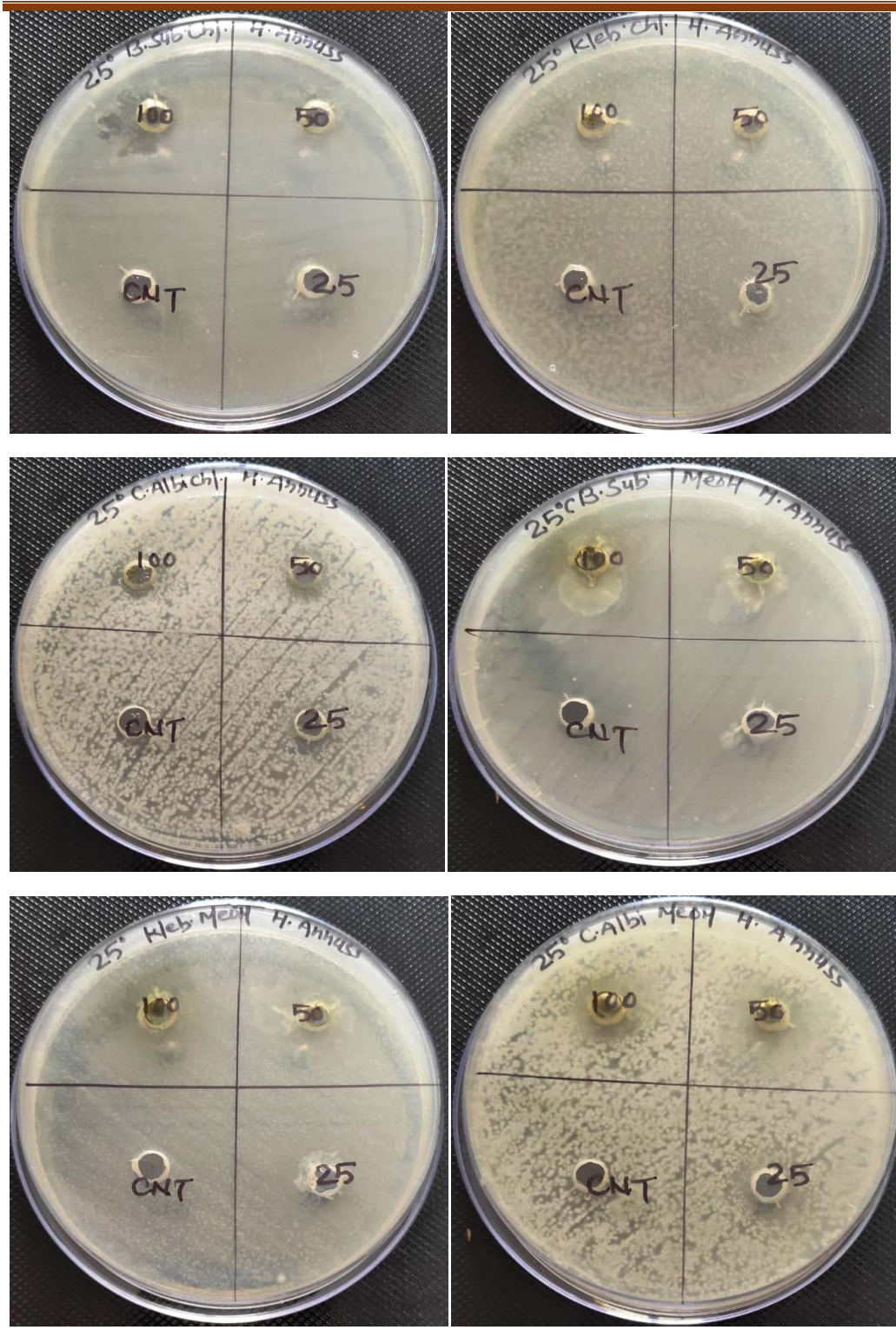

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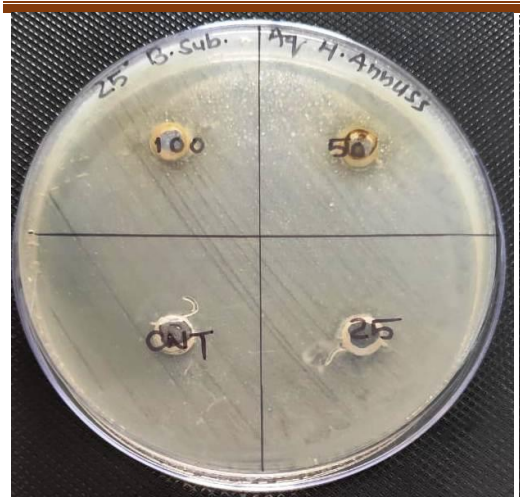

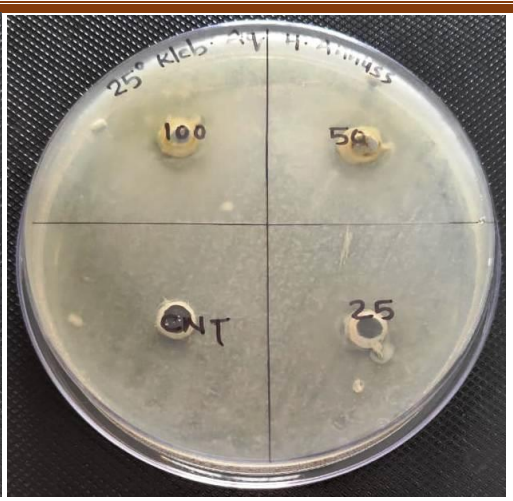

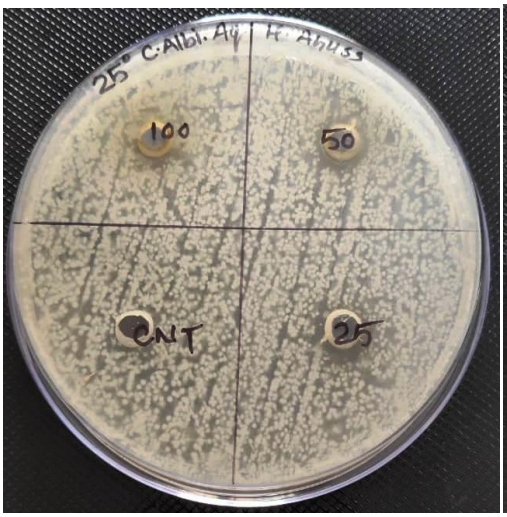

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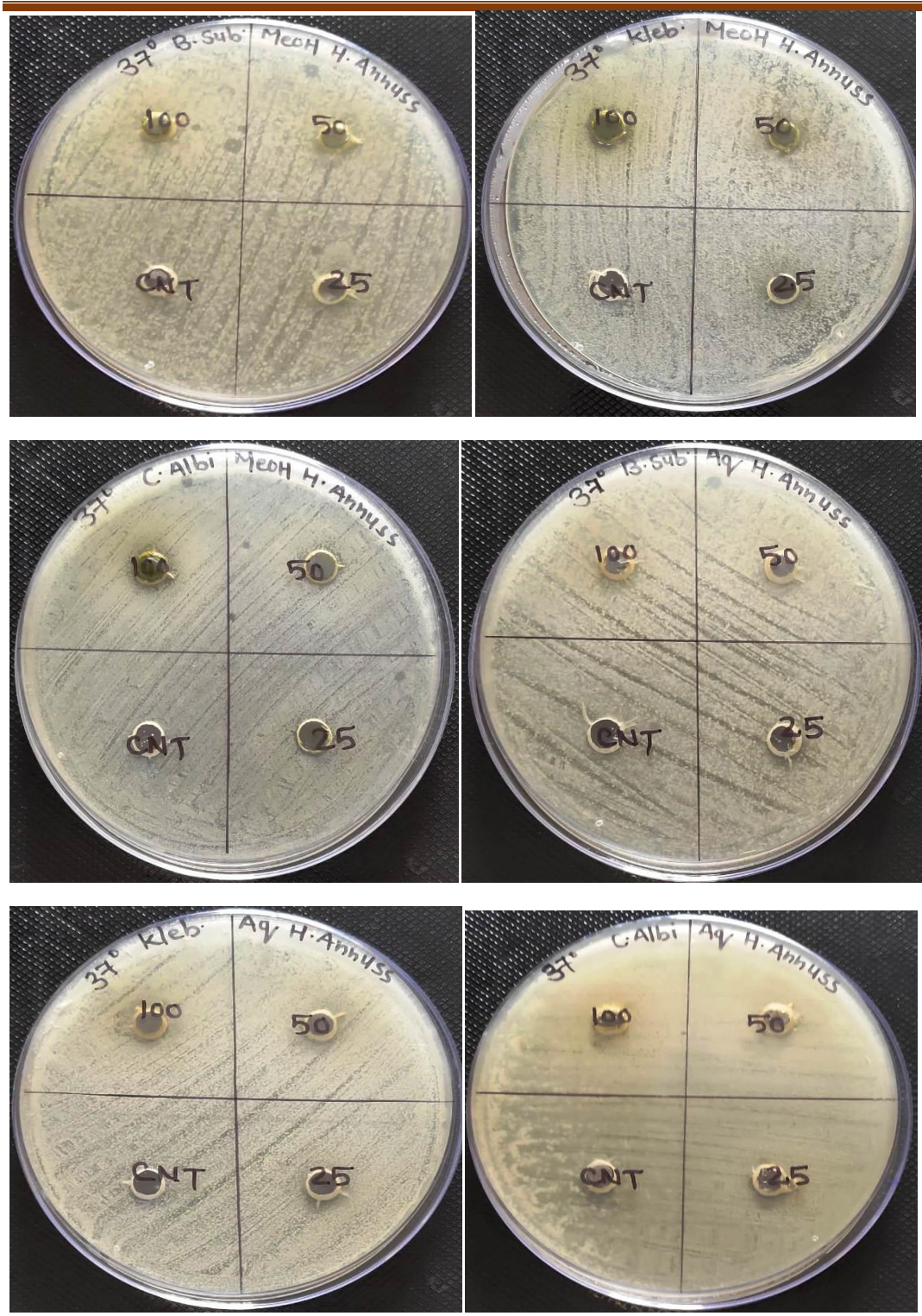

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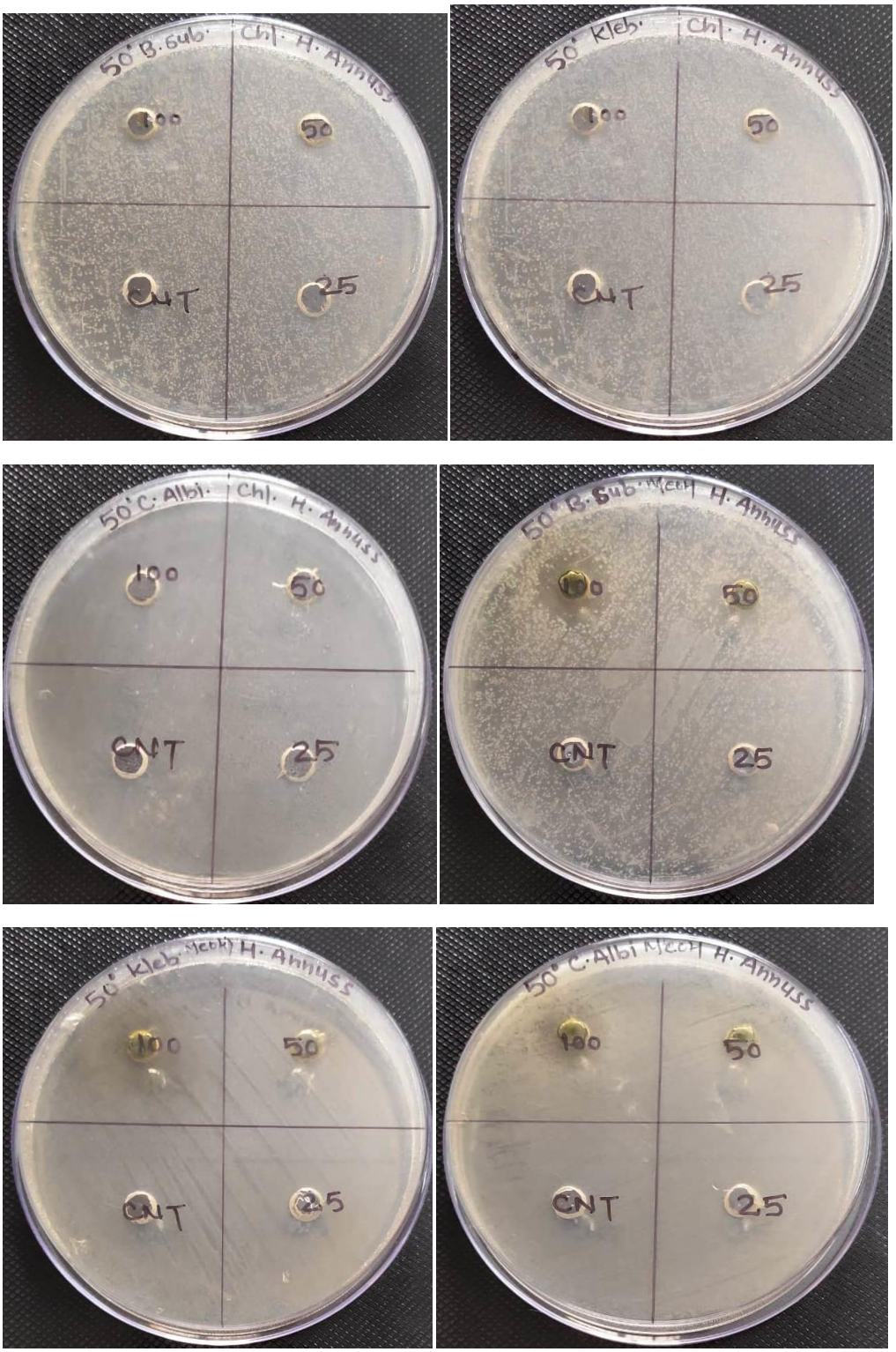

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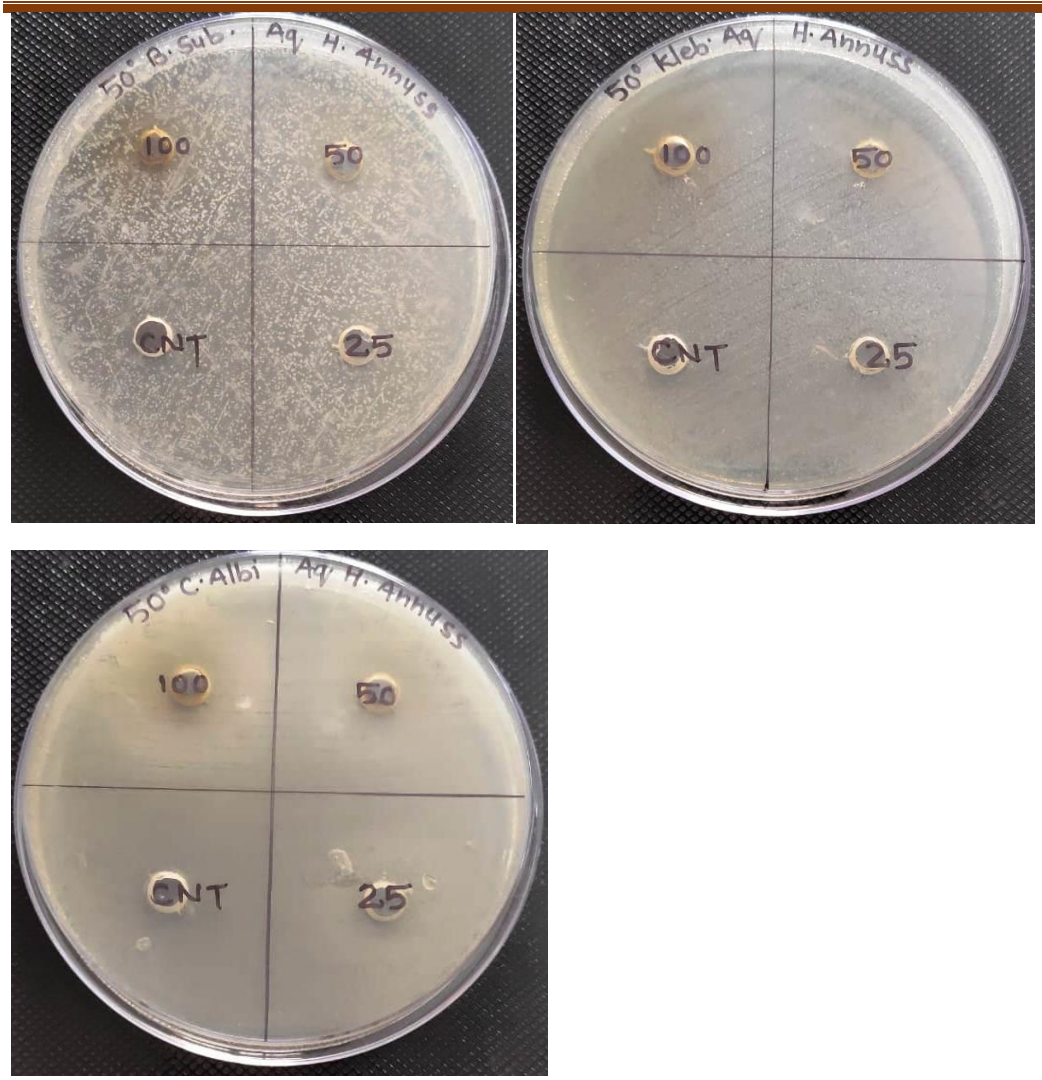

Figure 14: Photoplates of antimicrobial activity of *Helianthus annus* extracts against selected microbes on different temperature

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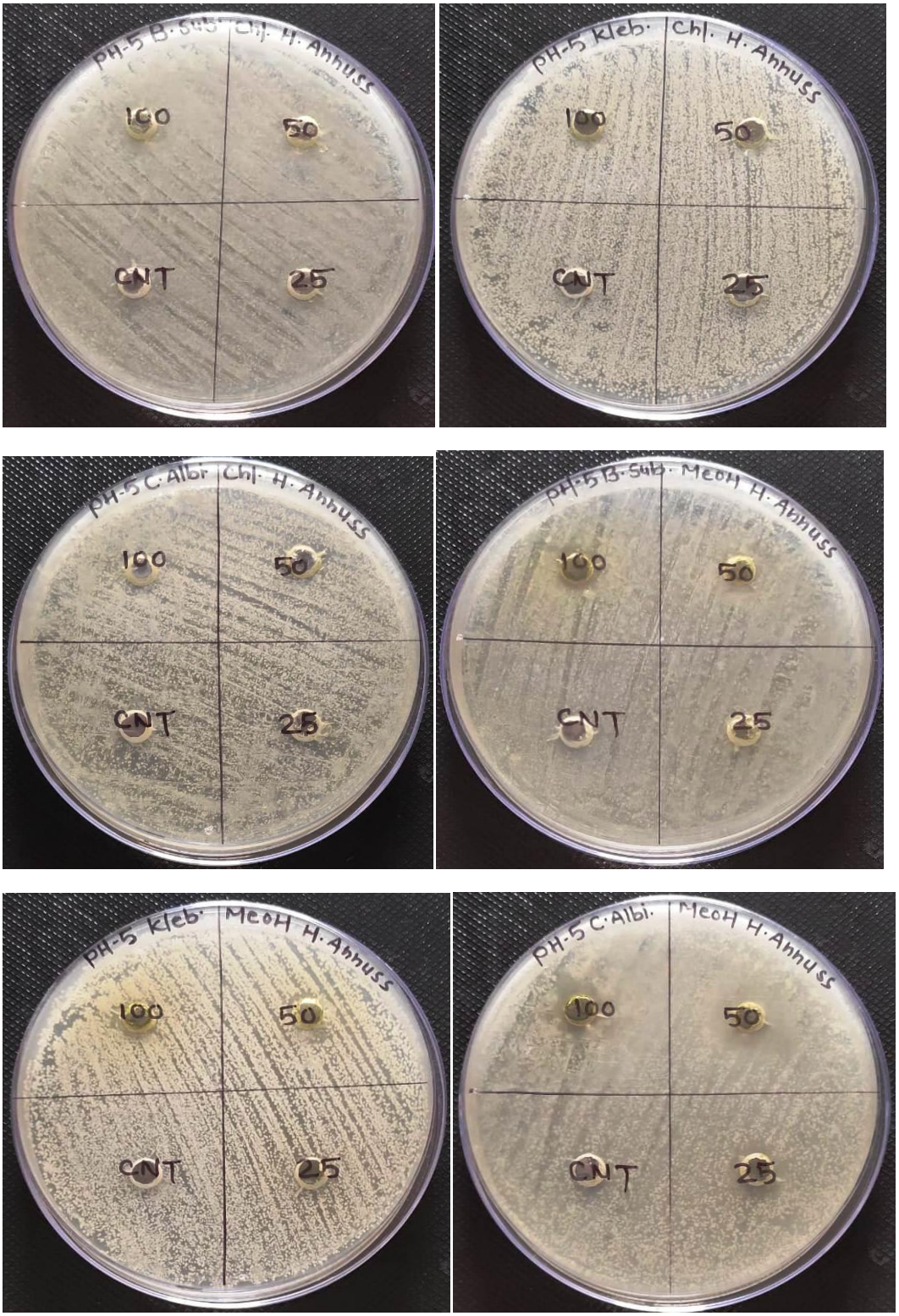
Photoplates of antimicrobial activity of *Helianthus annus* extracts against selected microbes on different pH



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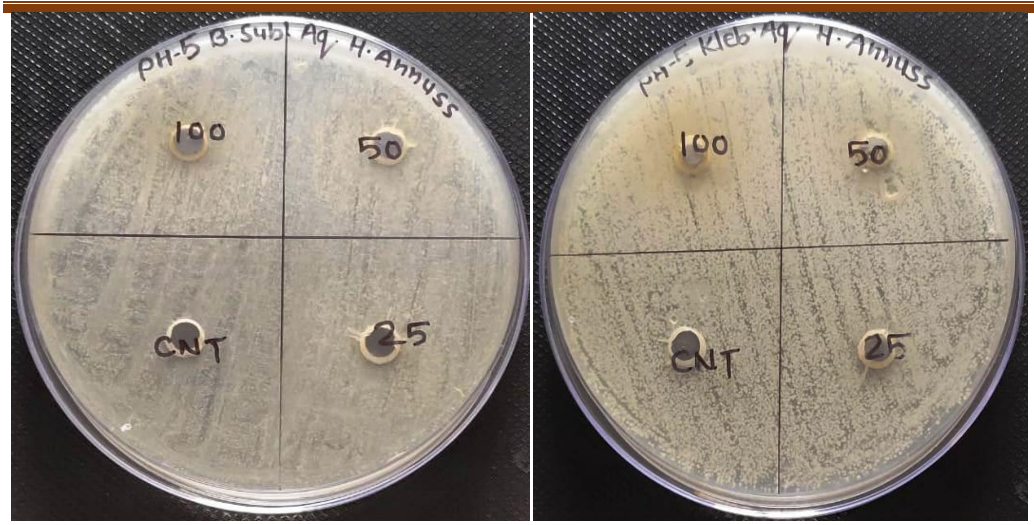

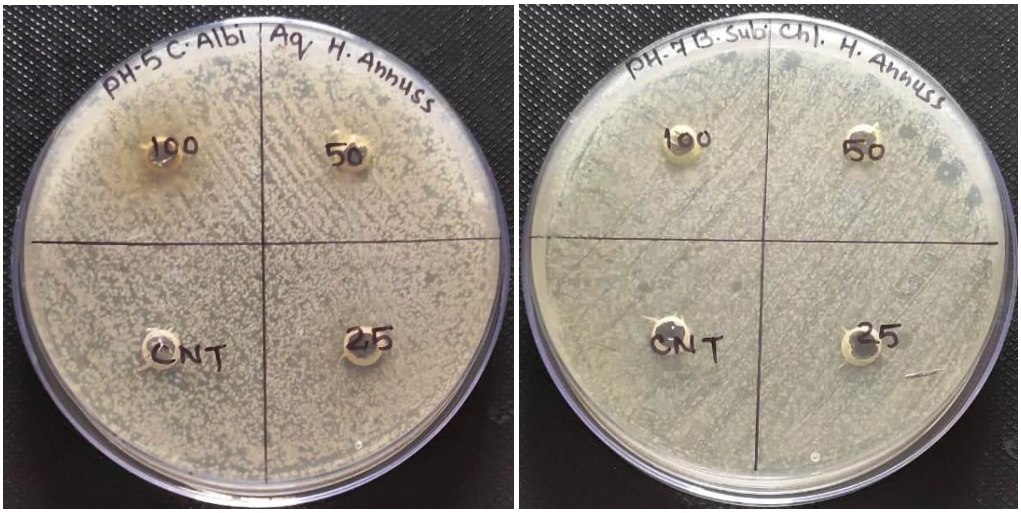

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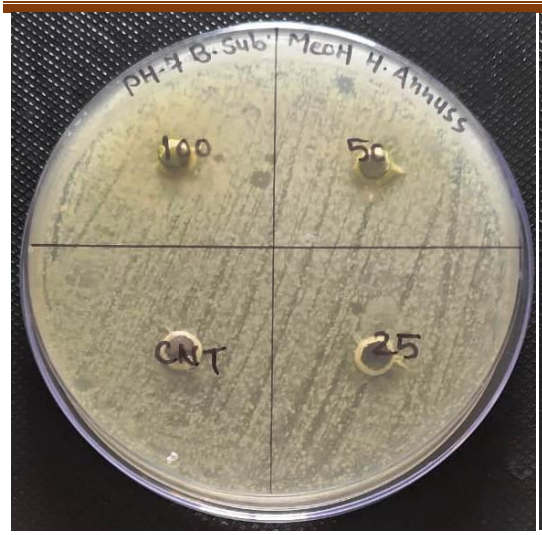

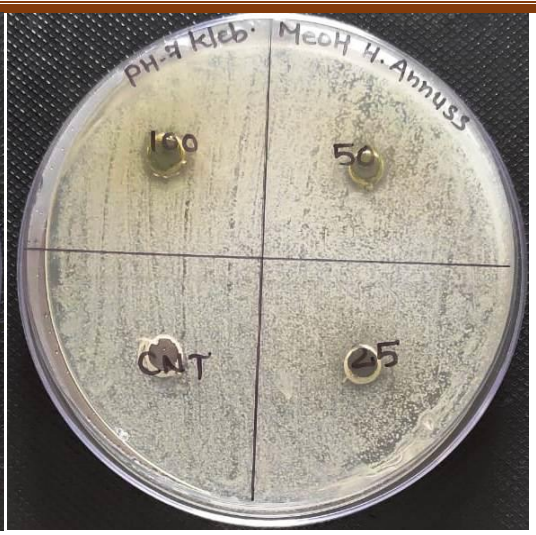

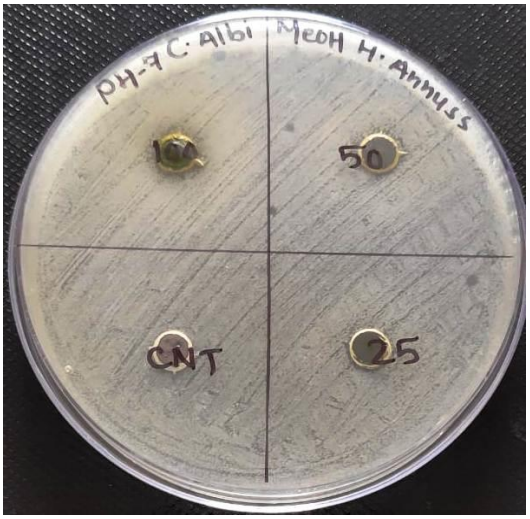

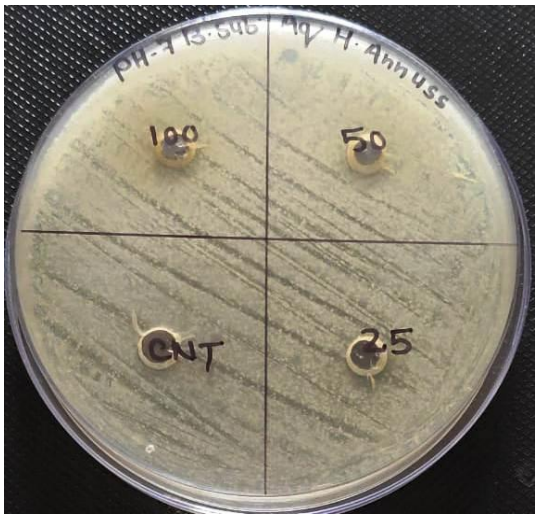

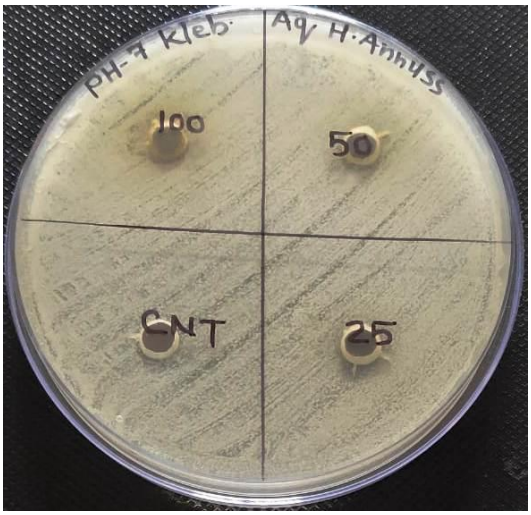

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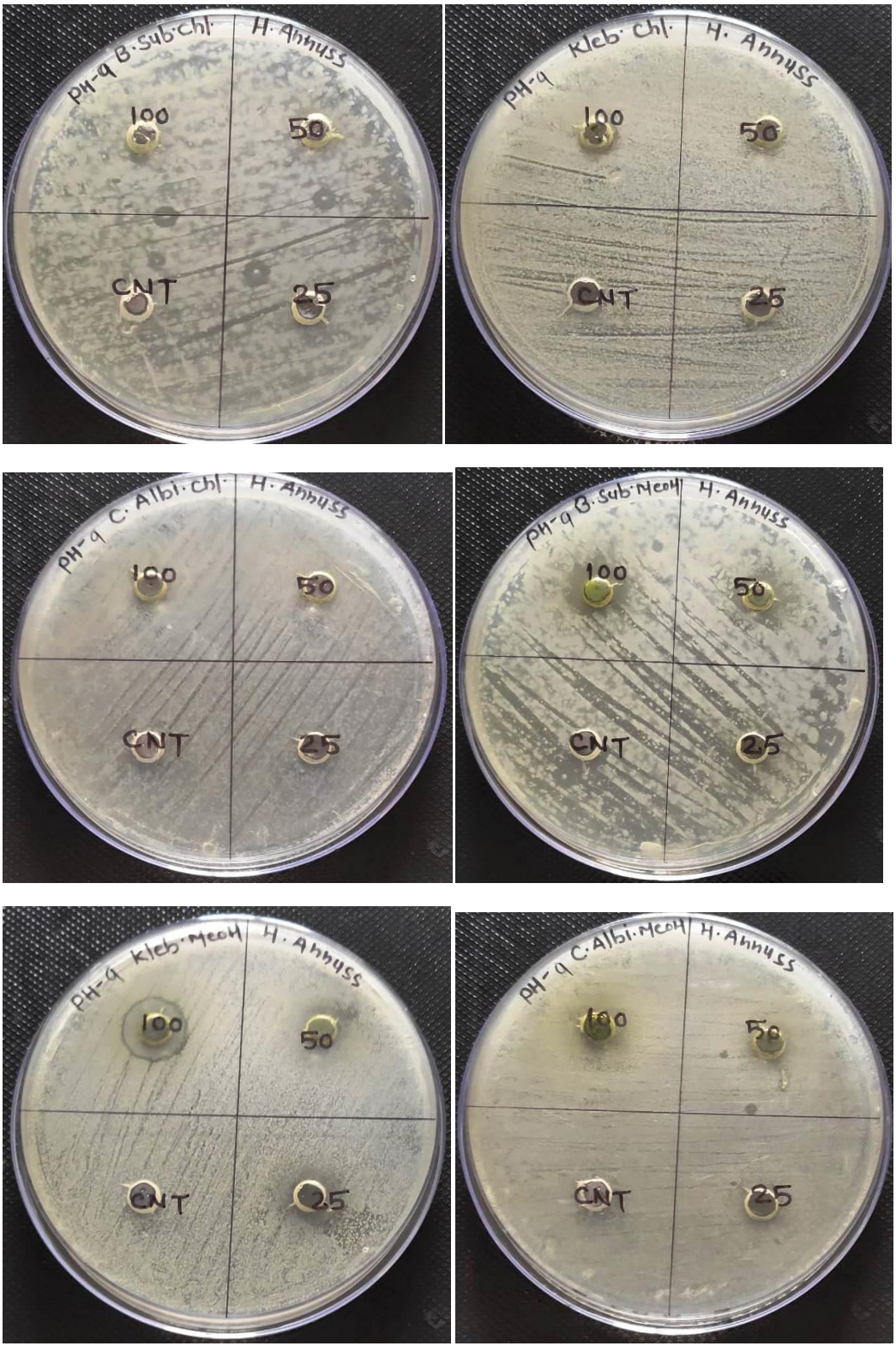

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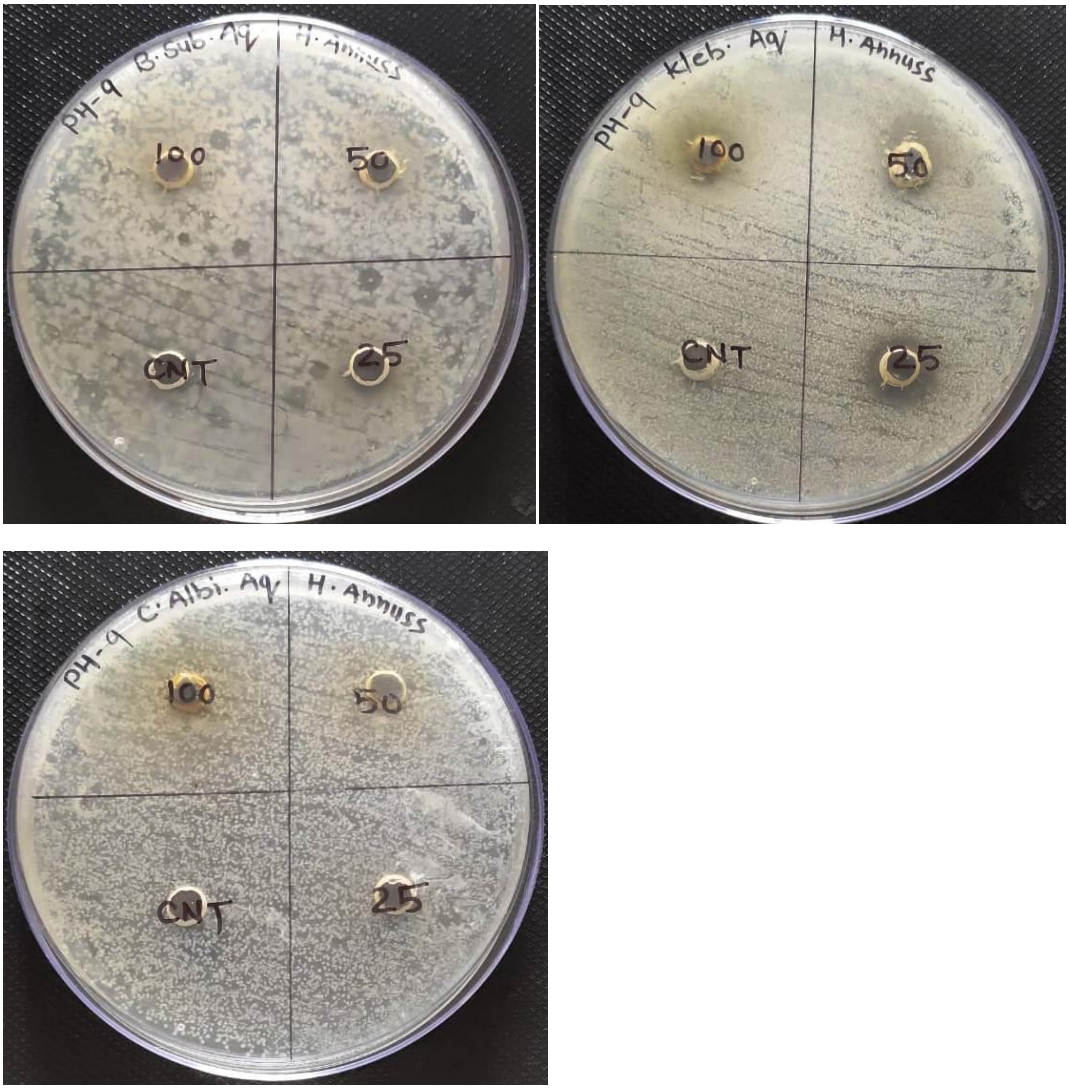

Figure 15: Photoplates of antimicrobial activity of *Helianthus annus* extracts against selected microbes on different pH

Discussion- Nowadays conventional methods are very useful to prevent or treatment of pathogenic disorders with the help of medicine plants.There are many components are useful to target specific pathogenic microorganisms (Aqil et al,2005;Nostro et al,2006).Our study reveals the stem etracts of some microorganisms show antimicrobial activity with methanol extract.Bacillus subtilis and klebsiella pneumonia shows broad zone of inhibition against ciprofloxacin. The significance of the plants due to their phytochemicals and

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secondary metabolities.One another micro organism fungi Candida albicans shows moderate zone of inhibition compare to bacteria ,in the presence of fluconazole antifungal antibiotic.

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