ANTIMICROBIAL ACTIVITY OF *SPHAERANTHUS INDICUS* LINN AGAINST SOME SELECTED HUMAN PATHOGENIC BACTERIA.

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Abstracts- *Sphaeranthus indicus* linn is a very useful medicinal plant which found near rice field throughout in India. Whole plant *Sphaeranthus indicus* 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 extracts and Ciprofloxacin 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 present study, our aim was to find out the antimicrobial activity of different extracts of whole plant *Sphaeranthus indicus* linn. Some different extracts such as methanol, petroleum ether, chloroform and aqueous extract exhibits comparable antimicrobial activity with the standard.

Keywards - Meceration method, antimicrobial activity, *Sphaeranthus indicus* linn, *Basillus subtilis, Klebsiella pneumonae, Candida albicans.*

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 pharmaceutical purpose has increased. The survey of world health organization was found that the medicinal plants would be best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Haidan Yuan, 2016). The plant extracts used as 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 incidence 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 metabolites 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, microbial resistance has developed. It has forced to scientist for search 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 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 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 metabolites but concentrations varies according to the plant parts, season climate and particular growth phase. The plant part leaf is one of the highest accumulations of such compounds. People are generally preferred this part of the plant used for therapeutic purposes. The growth of disease is inhibited by some active compounds (Dhia, 2006). The vast potentiality of plants as sources for antimicrobial drugs to antibacterial agents, a step wise investigation was undertaken to screen the local flora for antibacterial activity of *Sphaerantus indicus.* The plant *Sphaeranthus indicus* linn belonging to Asteraceae family used traditionally in Ayurveda for jaundice, diabetes, leprosy, hyperlipidemia, epilepsy, mental illness, AIDS, fever, cough, hernia, hemorrhoids, dyspepsia, helminthiasis, and skin

diseases and antimicrobial. The reports showed that it is also used for hypertensive, anxiolytic, neuroleptic, immune-modulatory, anti-oxidant, anti-inflammatory, bronchodilator, anti-hyperglycemic and hepatoprotective. It grows in tropical parts of India as rice fields, dry waste places and cultivated lands. It is distributed all over India, Africa, Sri Lanka, and Australia (Ambavade, 2006). The antimicrobial properties of certain Indian medicinal plants were reported based on their old litrature information (Dayal and Purohit, 1971; Hook and Thomas, 1995; Reddy, 1995; Suresh *et al*., 1995; Mehmood *et al*.,1999; Ahmad *et al*., 1998; Perumal Samy *et al*., 1998) and inhibitory activity against some pathogenic fungi and bacteria (Taylor *et al*., 1995).

MATERIALS AND METHODS

Plant material: The fresh leaves of *Sphaeranthus indicus* Linn 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 cutter mill. Powders of plant parts were stored in an airtight container at room temperature for further study.(Khandelwal,2005;Kokate,1995;Mukharji,2007).

Antimicrobial activities of whole plant material of *Sphaeranthus indicus* Linn

Pathogenic microbes used

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

Media preparation (broth and agar media)

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	-	100 ml.
pH	-	7

Composition of Potato dextrose agar media;

Potato infusion	-	20 gms
Dextrose	-	2gms.
Agar	-	1.5gms.
Distiled water	-	100 ml
PH	-	7

This agar medium was dissolved in distilled water and boiled conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely (Aneja, 2003).

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /$inch^2$ (121^0C) for 15 minutes.

Preparation of plates

The media after sterilization was poured (20 ml/ plate) into sterile Petri dishes. The poured plates were left at room temperature to solidify and incubate at 37^0C overnight to check the sterility of plates. The plates were dried at 50^0C for 30 minutes before use.

Revival of the microbial cultures

The microbial cultures which were used for study obtained in the lyophilized form from the laboratory. The lyophilized cultures inoculated in sterile nutrient with the help aseptic techniques. Potato dextrose broth plates are also incubated for 24 hours at 37^0C, after incubation period growth of microorganisms show turbidity. These broth cultures were further inoculated on the nutrient and potato dextrose agar plates with loop full of microbes and further incubated for next 24 hours at 37^0C.

Antimicrobial Studies

The pure broth culture isolates of test microorganisms which are sensitive for the plant extracts used were prepared a loop of culture is transferring into sterile nutrient and potato dextrose broth and incubated at 37^0C for 24-48 hours. From these broths a loop full culture was taken and inoculated onto sterile nutrient agar and potato dextrose agar plates to develop diffused heavy lawn culture. The well diffusion method was used to determine the antimicrobial activity of the extract prepared from whole plant material of *Sphaeranthus indicus* linn using standard procedure (Bauer, 1966). There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antimicrobial studies. Antibiotics placing in wells is a essential feature on the surfaces of agar after inoculation with the tested organism. Inoculums should be used as fresh diluted broth culture. The inoculated plates were incubated at 37^0C for 24 hours and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drugs.

Result

The various crude extracts of *Sphaeranthus indicus* linn showed significant activity against both the bacteria which are tested. The antibacterial activity of the *Sphaeranthus indicus* linn 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 chloroform, methanol and aqueous showed high activity (18-23 mm of zone of inhibition) on all organisms. Methanol showed a moderate activity (14 - 19 mm). Aqueous extract of *Sphaeranthus indicus* linn showed minimum activity (15-21 mm).The obtained results of the crude extracts are comparable with the standard antibiotics such as Ciprofloxacin and fluconazole. All the tested organisms are highly sensitive to the organic solvents of *Sphaeranthus indicus* linn (14-23 mm) than the standard ciprofloxacin and fluconazole antibiotics.

Table No. 15: Results of antimicrobial activity of *Sphaeranthus indicus* extracts against selected microbes on different temperature

Microbes	**Chloroform Extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at 25ºC		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	14±0	18±0.47	23±0.47
Candida albicans	11±0.47	14±0.81	18±0.47
	at 37ºC		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	16±0.47	20±0.47	22±0.47
Candida albicans	10±0.47	12±0.47	16±0.47
	at 50ºC		
Bacillus subtilis	11±0.47	16±0	18±0.47
Klebsiella pneumoniae	14±0.47	16±0.47	21±0.47
Candida albicans	13±0.47	15±0.47	20±0

Microbes	**Methanolic Extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at 25ºC		
Bacillus subtilis	-	-	-
Klebsiella pneumoniae	11±1.24	13±0.47	14±0.47
Candida albicans	-	-	-
	at 37ºC		

Bacillus subtilis	7±0	10±0.47	13±0.47
Klebsiella pneumoniae	7±0.47	12±0	13±0.47
Candida albicans	9±0.47	10±0.47	15±0.47
	at 50ºC		
Bacillus subtilis	8±0.47	13±4.1	19±0.47
Klebsiella pneumoniae	8±0.47	11±0	13±0.47
Candida albicans	10±0	12±0	17±0

Microbes	**Aqueous extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at 25ºC		
Bacillus subtilis	6±0	11±2.05	12±0.94
Klebsiella pneumoniae	8±0.47	12±0.47	14±0.47
Candida albicans	7±0	8±0.47	9±0.47
	at 37ºC		
Bacillus subtilis	8±0.47	9±0.47	11±0.47
Klebsiella pneumoniae	14±0	18±0.47	21±0
Candida albicans	-	-	-
	at 50ºC		
Bacillus subtilis	10±0.47	15±0	18±0.47
Klebsiella pneumoniae	8±0.47	10±0	12±0.47
Candida albicans	9±0.81	11±0	15±0.47

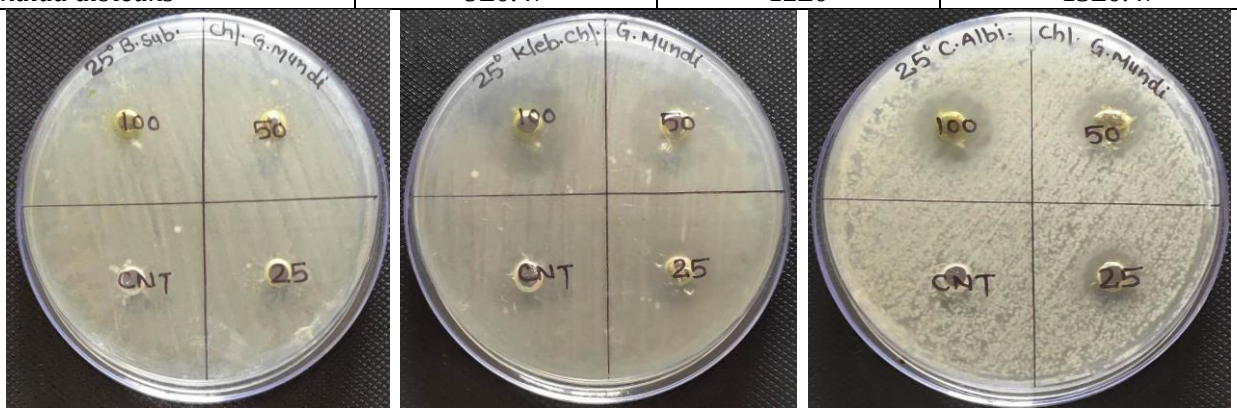
Table No. 16: Results of antimicrobial activity of *Sphaeranthus indicus* extracts against selected microbes on different pH

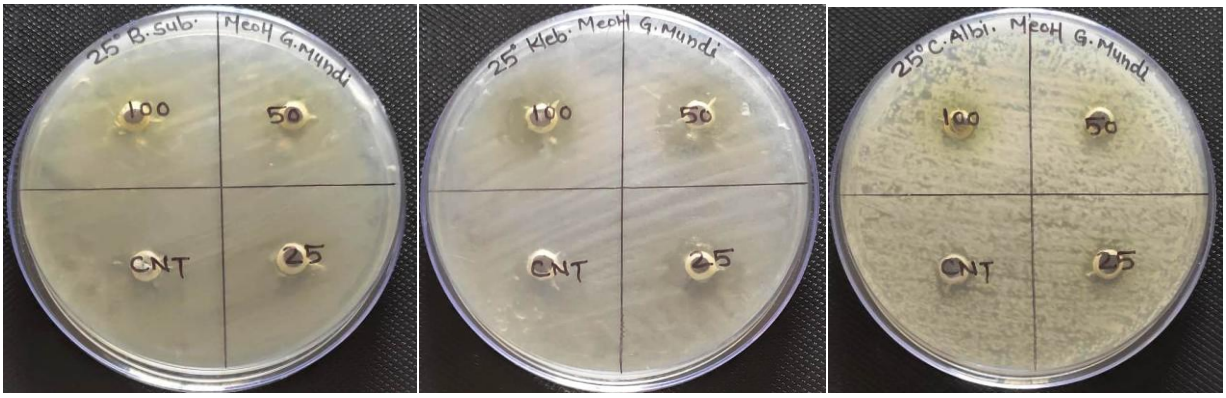
Microbes	**Chloroform Extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at pH-5		
Bacillus subtilis	16±0.47	22±0.47	25±0.47
Klebsiella pneumoniae	15±0.47	20±0.47	22±0
Candida albicans	15±0.47	19±0.47	20±0
	at pH-7		
Bacillus subtilis	-	-	-
Klebsiellapneumoniae	16±0.47	20±0.47	22±0.47
Candida albicans	11±0.47	12±0.47	16±0.47
	at pH-9		
Bacillus subtilis	9±0.47	13±0.47	15±0
Klebsiella pneumoniae	15±0.47	18±0.47	21±0
Candida albicans	14±0.47	16±0.47	18±0.47

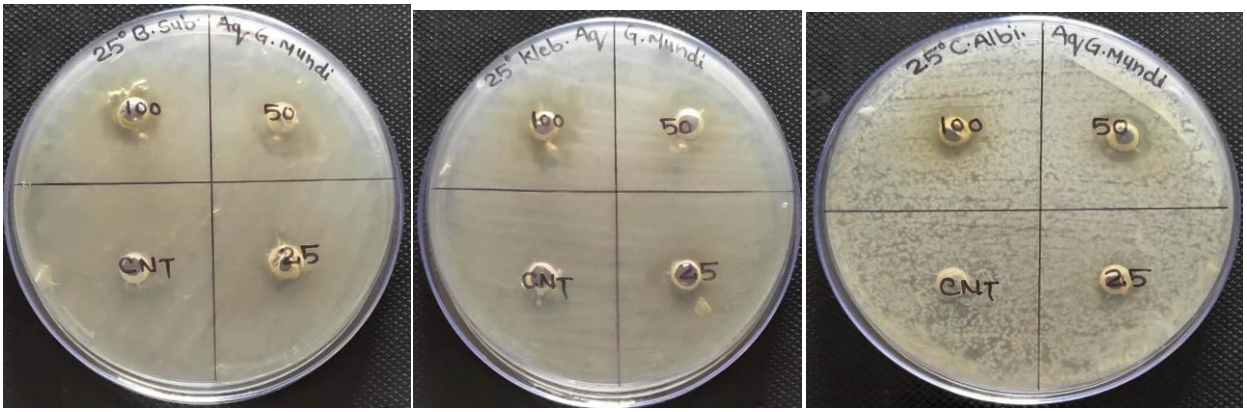
Microbes	**Methanolic extract**		
	25mg/ml	**50 mg/ml**	**100mg/ml**
	at pH-5		
Bacillus subtilis	8±0.47	10±0.47	12±0.94
Klebsiellapneumoniae	10±0	14±0.57	17±0.47
Candida albicans	11±0.47	12±0.47	14±0.47
	at pH-7		
Bacillus subtilis	10±0.47	11±0.47	14±0.47
Klebsiellapneumoniae	9±0.47	10±0.47	12±0
Candida albicans	8±0	9±0	11±0.47
	at pH-9		
Bacillus subtilis	8±0.47	10±0.47	11±0.94
Klebsiellapneumoniae	12±0.47	14±0.47	18±0.47
Candida albicans	13±0.47	16±0.47	19±0

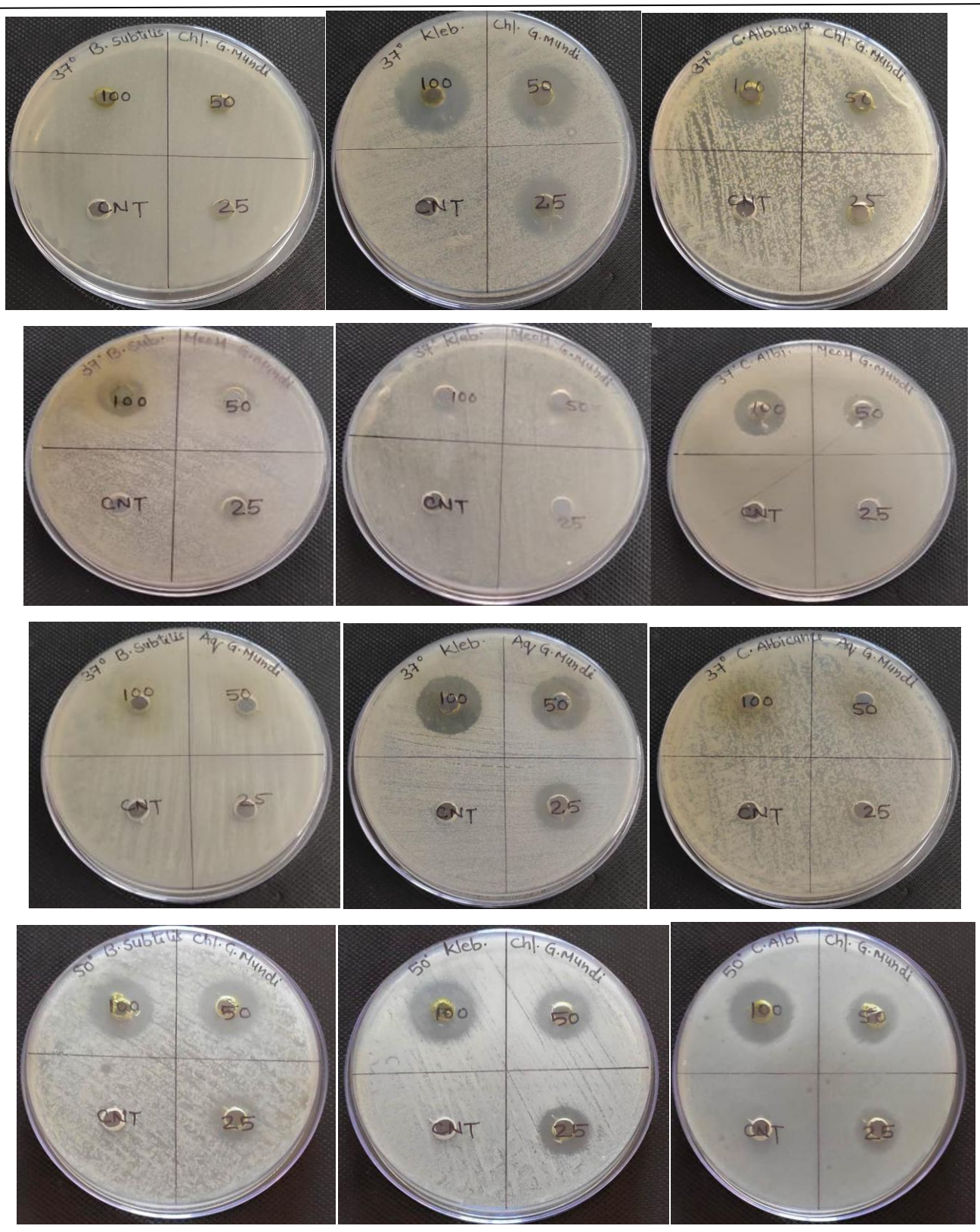
Microbes	**Aqueous extract**

	25mg/ml	50 mg/ml	100mg/ml
	at pH-5		
Bacillus subtilis	10±0.47	12±0.47	13±0.47
Klebsiella pneumoniae	-	-	-
Candida albicans	6±0	11±0	12±0.47
	at pH-7		
Bacillus subtilis	8±0.47	9±0.47	12±0.47
Klebsiella pneumoniae	14±0	18±0.47	21±0
Candida albicans	-	-	-
	at pH-9		
Bacillus subtilis	10±0.47	17±0.47	18±0.47
Klebsiella pneumoniae	8±0	9±0	11±0.47
Candida albicans	9±0.47	12±0	13±0.47








37° B. Subtilis Chl. G. Mundi
100
50
CNT
25
37° Kleb. Chl. G. Mundi
100
50
CNT
25
37° C. Albicans Chl. G. Mundi
100
50
CNT
25
37° B. Subtilis Aq. G. Mundi
100
50
CNT
25
37° Kleb. Aq. G. Mundi
100
50
CNT
25
37° C. Albicans Aq. G. Mundi
100
50
CNT
25
50° B. Subtilis Chl. G. Mundi
100
50
CNT
25
50° Kleb. Chl. G. Mundi
100
50
CNT
25
50° C. Albi Chl. G. Mundi
100
50
CNT
25

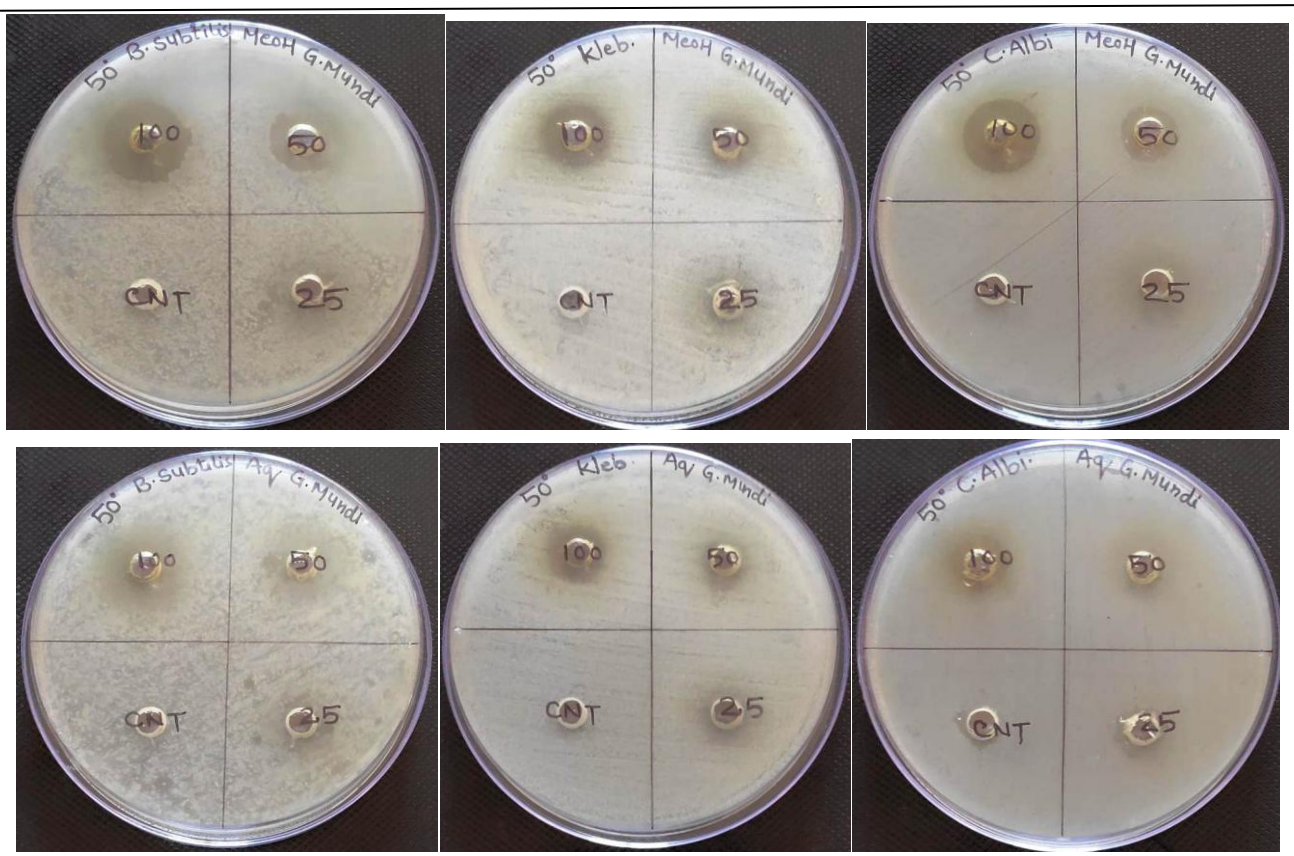
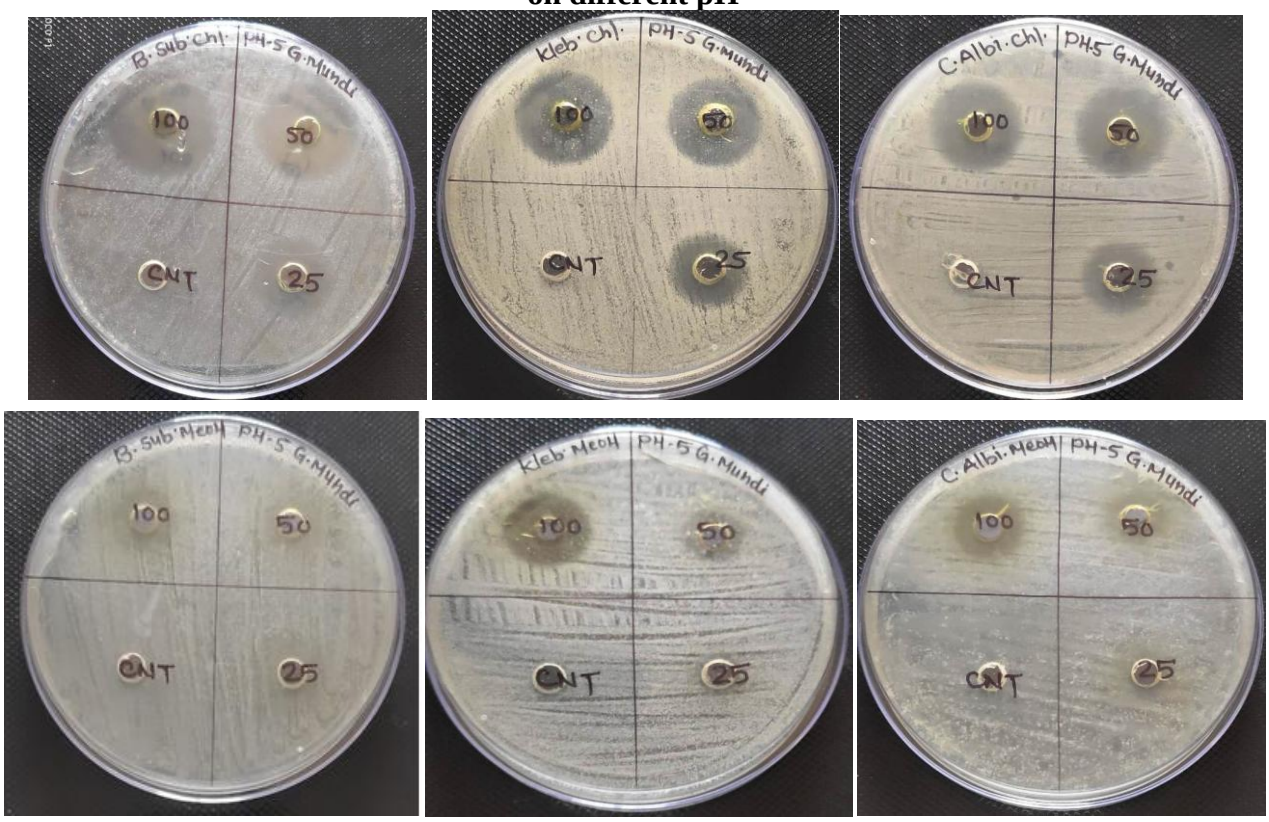


Figure 12: Photoplates of antimicrobial activity of *Sphaeranthus indicus* extracts against selected microbes on different temperature

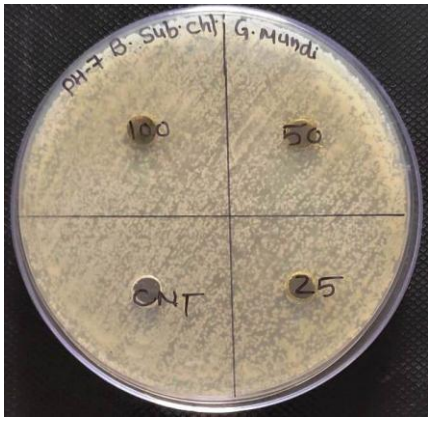
Photoplates of antimicrobial activity of *Sphaeranthus indicus* extracts against selected microbes on different pH




B. Sub Aq PH-5 G. Mundi
100
50
CNT
25


Kleb Aq PH-5 G. Mundi
100
50
CNT
25


C. Albi. Aq PH-5 G. Mundi
100
50
CNT
25


PH-7 B. Sub Chl G. Mundi
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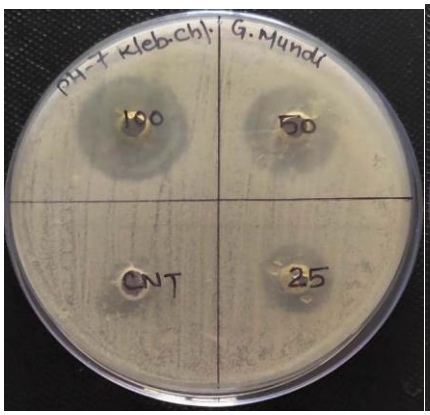
50
CNT
25


PH-7 Kleb Chl G. Mundi
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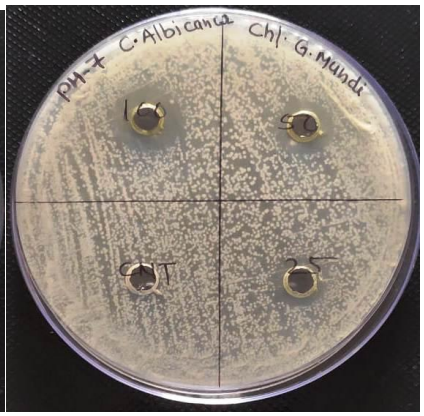
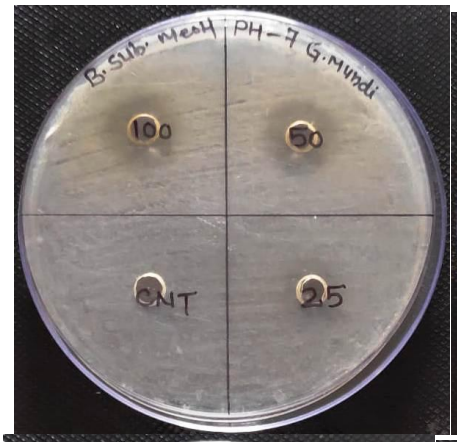
50
CNT
25

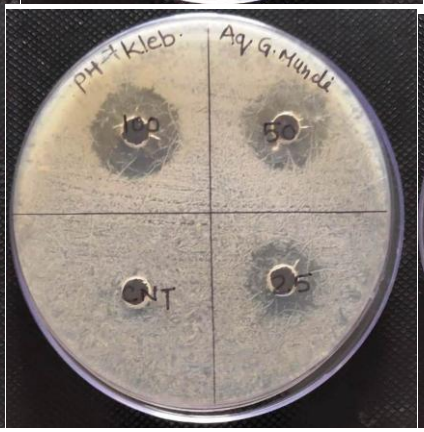

PH-7 C. Albicans Chl G. Mundi
100
50
CNT
25

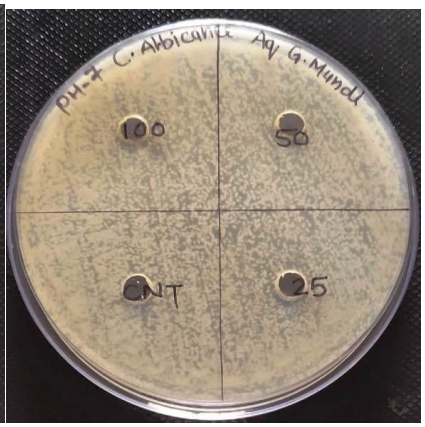

B. Sub MeoH PH-7 G. Mundi
100
50
CNT
25


Kleb MeoH PH-7 G. Mundi
100
50
CNT
25


PH-7 C. Albi. MeoH G. Mundi
100
50
CNT
25


PH-7 B. Subtilis Aq G. Mundi
100
50
CNT
25


PH-7 Kleb. Aq G. Mundi
100
50
CNT
25


PH-7 C. Albicans Aq G. Mundi
100
50
CNT
25

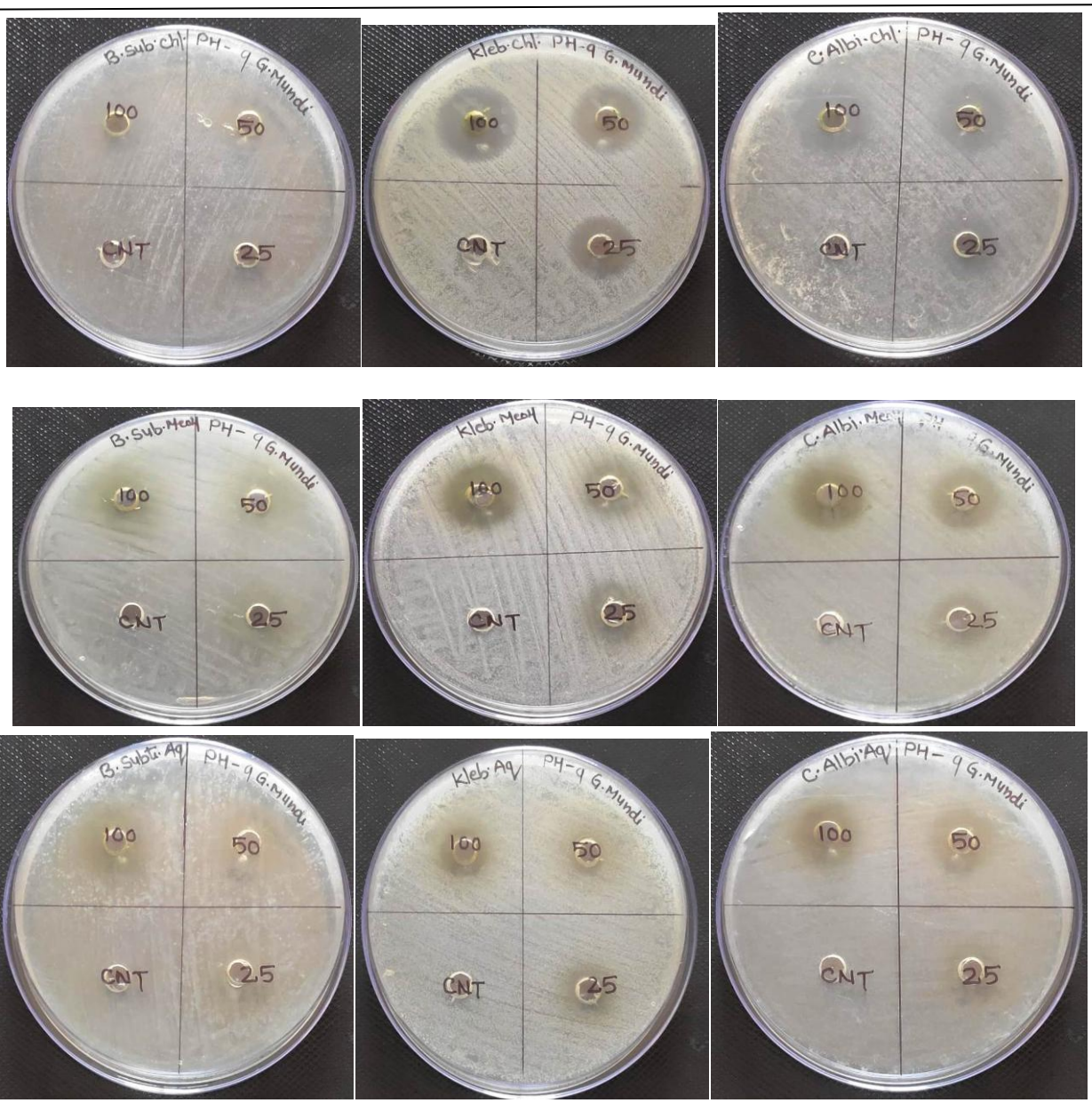


Figure 13: Photoplates of antimicrobial activity of *Sphaeranthus indicus* extracts against selected microbes on different pH

Discussion- Nowadays conventional methods are very useful to prevent or treatment of pathogenic disorders with the help of medicinal 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 extract 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 secondary metabolites. 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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