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An Insight into the Anti Cancer and Anti-Microbial Activity of Novel Plant Extracts From *Ziziphus Spina Christi L.* Against Breast Cancer Through *in Vitro* Approaches

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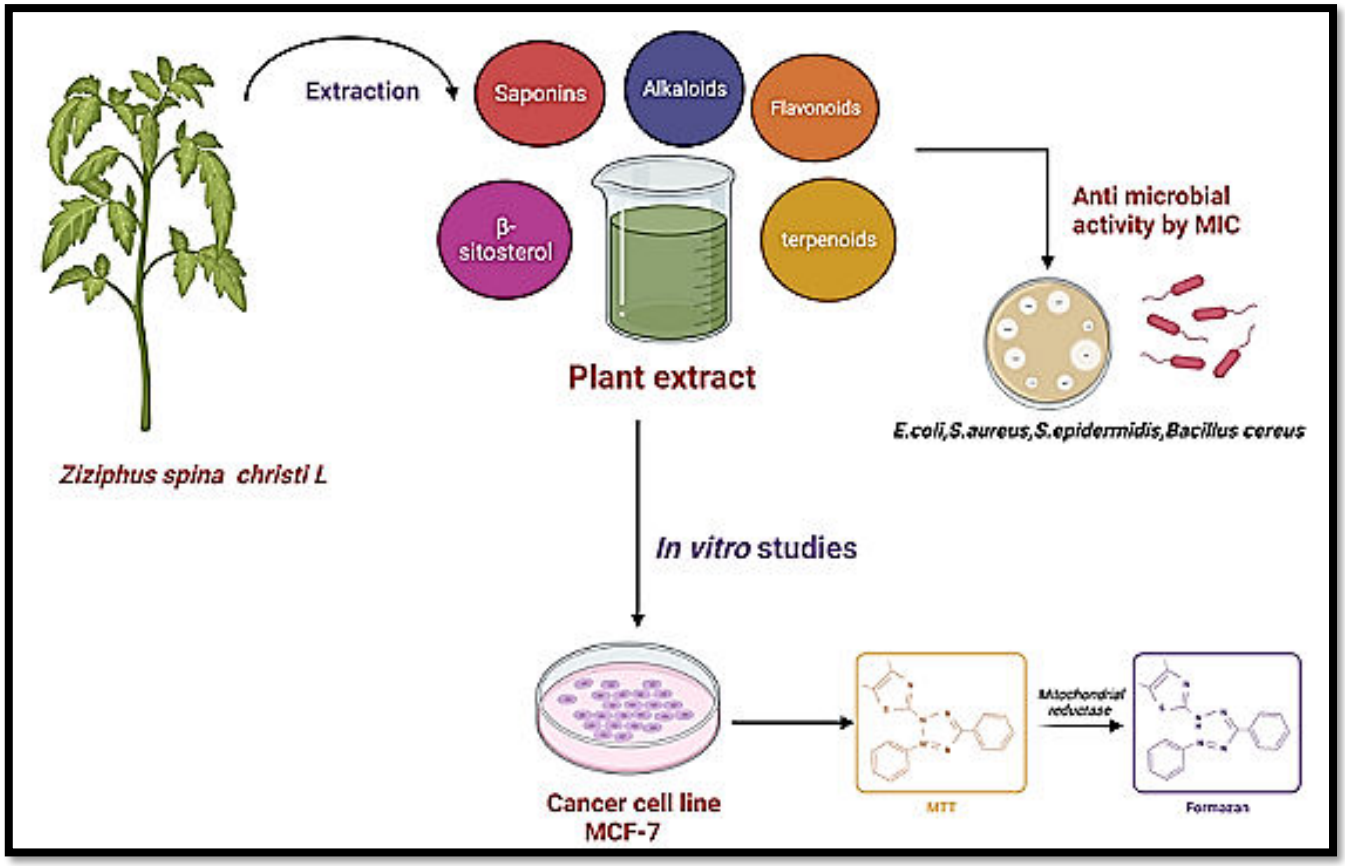
Abstract

Background: Evaluation of anti canceractivity of *Z. spina Christi* in breast cancer and antimicrobial activityagainst various microbial strains, including *Bacillus cereus, S. aureus, S. epidermidis,* and*E. coli*,which may play a pivitol role in BC progression, and phytochemical screening of secondary metabolites responsible for the antimicrobial activity of the methanol, water, and ethanol extracts of *Z. spina Christi*. **Method:** MTT assay were performed to determine the cytotoxic activity of*Z. spina Christi* leaves extracts (ethanol, methanol, water, acetone, and chloroform) *against MCF 7 cells.* The anti-microbial activity was conducted on all the extracts by cup plate method. Furthermore, a MIC study was conducted on the most effective extracts (methanol, water, and ethanol) of *Z. spina christi against* various microbial strains, including *Bacillus cereus, S. aureus, and S. epidermidis,* and*E. coli*. **Result:**In the MTT assay, we found that as the conc. increased from 10 μg/ml to 320 μg/ml of *Z.Spinachristi*varying extracts, the MCF-7 cells % cell viability were found to be decreased.The maximum ZOI was observed for methanol extract at 320 μg/ml in *Bacillus cereus* (19.3±0.3 mm), followed by *S. aureus* (16.5±0.2 mm), *S. epidermidis* (14.2±0.6 mm), and *E. coli* (12.5±0.4 mm).In phytochemical screening study, the methanol *Z. spina Christi leaf extracts* tested positive for alkaloids, anthraquinone glycosides, saponins, terpenoids, flavonoids, phenolic compounds, and tannin and negative for steroids, which are mainly responsible for anti-microbial activity. **Conclusion:** Findings of the study suggest that the chemical compounds present in *Z. spina Christi* extract can emerge as a potent anti-cancer agent against BC. Furthermore, it has shown significant antimicrobial activity,suggesting its effectiveness in inhibiting the growth of microbial strains and BC progression by certain microbial strains.

Keywords:-*Z.spina Christi*, breast cancer,anti-microbial,MCF-7 cells, secondary metabolites.

Graphical Abstract



1. Introduction

Z.*spina Christi*, also known as Christ's thorn, is a thorny shrub and evergreen tree belonging to the family Rhamnaceae. It is also referred to by Arabic names such as *Nabeq*and*Sidr*. This plant is a renowned indigenous tree that has a large prevalence in Africa and may also be found in tropical and subtropical locations around the globe. Among various Ziziphus plants, *Z. spina Christi* is known to contain multiple biologically active compounds, including triterpenoids, alkaloids, flavonoids, sterols like β-sitosterol, saponins, and sapogenins(Hussein, 2019),(Iqbal et al., 2022) A previous study on *Z. spina-christi* has shown that the plant's leaf, fruits, and barks have antioxidant, analgesic, hypoglycemic, antidiabetic, antidiarrheal, antihyperlipidemic, and antihyperlipidemic effects. (Elghaffar et al., 2022),(Dhanalekshmi et al., 2022), (Belayneh et al., 2022) Cancer is a leading global cause of mortality, and in recent years, several investigations have concentrated on developing therapies that have fewer adverse effects in comparison to conventional therapy. (Belayneh et al., 2022)BC is the predominant form of cancer among women, impacting around 1 in 20 individuals worldwide. Despite decades of research in laboratories, epidemiology, and clinical settings, the prevalence of BC continues to rise. It continues to be the most prevalent form of cancer among women, impacting one in twenty individuals worldwide and as

many as one in eight in high-income countries.. (Britt et al.,2020),(Burguin et al., 2021), (Lima et al., 2021)*Z. spina Christi* stemmethanol extract contains phenolic compounds, including quercetin, rutin, syringic acid, coumaric acid, apigenin, and chlorogenic acid. Betulin is a naturally occurring pentacyclictriterpene alcohol that is extracted from the Z. *spina Christi* stem bark. In a recent investigation, betulin ($C_{30}H_{50}O_2$) and betulinic acid ($C_{30}H_{48}O_3$) isolated from the *Z. spina Christi*stem bark showed significant anticancer and antimicrobial activity in the MCF-7 breast cancer cell line (IC50 1.74 to 89.44 μM). (Ads et al., 2022c)Betulinic acid is also known to possess cytotoxic effects on melanoma, neuroectodermal, and malignant brain tumors. Traditional practices included the use of *Z. spina Christi* honey as an alternative cytotoxic treatment to patients with liver, breast, and colon tumors. Some researchers have also found thatsaponin-rich fraction and saponinosomes from *Z. spina-christi* exhibit high toxicity against B16F10 melanoma cells, making them a potential treatment for malignant melanoma. . (Soliman et al., 2018) (Nazemoroaya et al., 2022)

Another investigation showed that the ethanolic extract fractions derived from the bark of*Z. spinachristi*had cytotoxic properties against various human cancerous cell lines, such as MCF-7 and A-549. (Rajendrasozhan et al., 2021) There are additional risk factors associated with the progression of BC, and one significant factor that has gained attention in recent years is the 'Human Microbiome.' It has recently been discovered that the human microbiome plays a significant role in the the formation and progression of several cancers. According to recent research, BC development, progression, and overall prognosis may be influenced by both distant and local microbiota. An imbalanced microbiota enhances the probability of cancer development by triggering genetic instability, DNA, triggering a favourable immune response, disrupting metabolic control, and altering the response to treatment.(Fernández et al., 2018), (Tekle& Garrett, 2023) Previous research has shown that BC patients have an abundance of microorganisms such as*Bacillus cereus, Staplylococcusaureus (S.aureus),Staplylococcusepidemidis (S.epidermidis),Escherichia coli(E. coli)and Corynebacterium.* BC samples also exhibited a higher abundance of the *Enterobacteriaceae* family. (Parida&Sharma, 2019), (Chadha et al., 2020), (Qi et al., 2020)Prior research indicated that *Z. spina Christi* has antimicrobial properties against Gram-positive *Bacillus subtilis*and *Streptococcus pyogenes*, as well as against Gram-negative bateria E. *coli.* The fatty acid fraction of *Z. spina Christi* fruit was shown to possess significant antimicrobial effects against *B. subtilis*and*E. coli* [19]. Additionally, the methanol extract of *Z. spina christi*leaf showed higher antimicrobial activity towards the two strains of Gram-positive bacteria (*B. subtilisand*S.*aureus)* as compared to that of Gram-negative bacteria (P.*aeruginosaand E. coli)* [20] .

Moreover, *Z. spina christi* ethyl acetate extract has shown antibacterial activity against E. coli*, Bacillus subtilis, and Streptococcus pneumoniae and* cytotoxic

effects on colon and breast cancer cells [21].Furthermore, it has been documented that *Z. spina Christi* leaf ethanolic extract inhibits the growth of *Bacillus subtilis* and were found to exhibit anti-inflammatory and antipyretic effects.Elghaffar et al., 2022)*Z. spina Christi* leaf and fruit methanolic extracts were found to possess antifungal property against the development of *Alternariaradicina, A. citri, and A. alternata*. (El-Shahir et al., 2022)Another study has shown that 28-O-(N-acetylanthraniloyl) betulin is the most effective chemical constituent present in *Z. spina Christi* against *E. faecalis*and*S. aureus*, with MIC values of 6.25 μM. (Ads et al., 2022c)In our current investigation, we evaluated different *Z. spina Christi* leaf extracts (water, chloroform, ethanol, methanol, and acetone) for their anti-cancer activity in the MCF-7 BC cell line. We also investigated *Z. spina Christi*antimicrobial activity against various microbial strains, including *Bacillus cereus, S. aureus, and S. epidermidis,* and*E. coli*, which may play a pivitol role in BC progression, and furthermore, we performed phytochemical screening of secondary metabolites responsible for the antimicrobial activity of the methanol, water, and ethanol extracts of *Z. spina Christi*.

2. Materials and methods

2.1 Materials

Ethanol, Methanol, Chloroform, Acetone, MEM Media, Antibiotic-Antimycotic Solution,), HEPES, Foetal Bovine Serum (FBS),MTT dye (Sigma-Aldrich), DMSO (dimethylsulfoxide), PBS (Phosphate Buffer), MCF-7 cell line (NCCS Pune), Muller-Hinton agar (Hi media).

2.1.1 Test micro-oraganism

The bacterial strains, American-type cell culture (ATCC) of *E.coli*(ATCC 25922), *Bacillus cereus (ATCC 10876), Staphylococcus aureus* (ATCC 25923) *and Staphylococcus epidermidis,* were procured from the microbiology laboratory of SantGadge Baba University, India. . (Owayss et al., 2019)

2.1.2 Plant material extraction method

*Z. spina Christi*leaves were collected from the medicinal garden of Dr.RajendraGode Institute ofPharmacy, Maharashtra, India.The leaves were dried in an oven at 40 °C for 84 hours. The Soxhlet apparatus was employed for the extraction of *Z. spina Christi*leaves, which was performed by the Soxhlet extraction method, and crushed *Z. spina Christi*leaves were placed in the soxhlet thimble chamber.Methanol, water, ethanol, chloroform and acetone were used as extraction solvents. We used approximately 100 grams of dried *Z. spina Christi*leaf powder and conducted the extraction using 250 ml of solvent (water, chloroform, ethanol, methanol, and acetone) in a Soxhlet apparatus for 48

hours. The extract was concentrated by evaporating it at 70 °C for 8 hours. The concentrated extract was kept at room temperature for subsequent phytochemical analysis.

2.1.3 Assessment of cytotoxic activity of *Z. Spinachristi* leaf extract by MTT Assay

MTT serves as a reliable marker for assessing cell viability. This assay relies on the ability of viable mitochondrial cells to reduce MTT, resulting in the formation of insoluble formazan crystals.Briefly, the MCF-7 cell line was cultured in a 96-well plate at a density of 1×10^4 cells per well. The MCF-7 cells were then incubated for 24 hours at a temperature (temp.) 37 °C in 5% CO2. After 24 hours, the media were removed, and the treatments were performed in triplicate using concentration (conc.) of 10, 20, 40, 80, 160, and 320 μg/ml for Z. *Spinachristi*leaf ethanol, methanol, water, acetone, and chloroform extract separately, and the volume of each well was make up to 300 μl with media. Three wells were kept untreated as controls. Subsequently, the treated MCF-7 cells were incubated for 24 hours at 37 °C in 5% CO2. Following a 24-hour treatment period, 25 μL (5 mg/mL) of MTT solution were added to the cells. The cells were then thereafter put in an incubator at 37 °C with a 5% CO2 conc. for 2-3 hours. Subsequently, the media were taken out of each well, and 200 μL of DMSO were added to solubilize the formazan crystals. Absorbance were measured at a specific wavelength of 570 nm using a microplate reader (Bio-Tek Inc. (USA), Gen 5). (Khan et al., 2022)

2.1.4 Assessment of antibacterial activity of the*Z. Spinachristi*leaf extract by cup plate method

The antibacterial activity of various *Z. spina Christi* extracts, such as ethanol, methanol, chloroform, water, and acetone, was performed by the cup-plate method. (Srinivasan et al., 2001),(Abdel-Aziz et al., 2013)

]. Sterilized sabourd dextrose agar or sterile nutrient agar were poured into sterilized petri plates and kept in aseptic conditions. About 100 μl of the bacterial strains *Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, and E. coli* were spread on the defined sterile plates. Holes were made in the agar plates using a sterile corkborer (5 mm in diameter). The tetracycline antibiotic (30 μg) and plant extracts (40 and 80 μg/ml) were added to separate holes in the plates. The plates were then stored at a temp. of 4°C for at least one hour to allow diffusion into the agar medium, followed by incubation at 37°C for 24-48 hours. The zones of inhibition (ZOI) were measured using a vernier calliper.

2.1.5 Assessment of minimum inhibitory concentrationof the *Z. Spinachristi*leaf extract

Minimum inhibitory concentration (MIC) stands for the minimum inhibitory conc. of the antimicrobial agent, which refers to the lowest conc. that effectively prevents the growth of microorganisms following a 24-hour incubation period. MIC was performed on an effective extract of *Z. spina christi* (methanol, water, and ethanol extract) against the bacterial strains *Bacillus cereus, S. aureus, S. epidermidis, and E. coli.*100 µl of each standardised broth culture was cultured on the surface of plates.Plates were kept in aseptic conditions for solidification. A sterile cork borer was used to make holes(5mm in diameter) in each agar plate.*Z. spina* christi extracts with varying conc.of 20, 40, 80, 160, and 320 µg/ml and tetracycline were added to the separate holes,followed by incubation at 37°C for 24-48 hours. ZOI were measured using a vernier calliper.(Ismael et al., 2021)

2.1.6 Phytochemical screening of secondary metabolites in *Z.Spinachristi* extracts

a) Test for alkaloids (Mayer's test)

Plant extract (500 mg) was boiled with methanol (20ml) and then filtered. Then, 1.5 ml of 2% HCL and two drops of Mayer's reagent were added. Creamy white precipitate formation and turbity indicate the presence of free alkaloids. Furthermore, a test for alkaloids was conducted on a small number of plant materials. This included diluting 2.5 mg of the extract with 2.5 ml of 1% HCL in a tube, which was further heated. Subsequently, filtered solution (1ml) was mixed with diluted ammonia (1ml). Ultimately, a volume of 1 ml of chloroform ($CHCl_3$) was added to determine the alkaloidal base.(Kebede et al., 2021)

b) Test for Flavonoids

A quantity of 7.5 mg of dry extract were mixed with diluted ammonia solution (1ml). The presence of flavonoids is indicated by the formation of a yellow colour.

c) Test for anthraquinones (Borntrager's Test)

Extract (1 mg) was mixed with benzene (2 ml) and then filtered with Whatman's No. 1 filter paper. Next, the filtrates were mixed with a 10% ammonia solution (2.5 ml) and well agitated. The presence of free anthraquinones was indicated by a pink, violet, or red colour in the ammoniacal layer.(Kebede et al., 2021)

d) Test for steroids

10 ml of chloroform was added to 2 ml of each of the three plant extracts. Additionally, 1 ml of acetic anhydride was added to the extracts, and 2 ml of strong sulfuric acid was added around the test tube's edges. The presence of steroids is shown by the formation of a blue-green colour at the junction.(Nigussie et al., 2021)

e) Test for Terpenoids (Salkowski test)

2 ml of chloroform were mixed with 7.25 mg of extract, and 3 ml of strong H_2SO_4 were added with caution.The presence of terpenoids is indicated by the formation of a reddish-brown layer at the contact.

f) Test for Saponins (Frothing test)

A quantity of 2.5 mg of the plant extract was mixed with 5 ml of water in a test tube. The froth samples were heated. The presence of saponin is indicated by the persistent development of foam.

3. Result

3.1 Cytotoxic activity of *Z.Spinachristi* extract

The cytotoxic activity of *Z. spina christi* ethanol, methanol, water, acetone, and chloroform extracts were determined *in vitro* against the MCF-7 cell line. Following the conc. increase from 10 μg/ml to 320 μg/ml of *Z. spina christi extracts*, the MCF-7 cell viability was found to be decreased from 94% to 57% for ethanol extract, 83% to 59% for methanol extract, 86% to 28% for chloroform extract, 96% to 71% for water extract, and 94% to 50% for acetone extract significantly at conc.of 10, 20, 40, 80, 160, and 320 μg/ml, respectively.

As shown in figure 1& 2, MCF-7 cells % cell viability was recorded as 70%, 64%, and 57% for ethanol extract, 78%, 72%, and 59% for methanol extract, 65%, 49%, and 28% for chloroform extract, 79%, 76%, and 71% for water extract, 72%, 64%, and 50% for acetone extract at conc.of 80, 160, and 320 μg/ml, respectively, by the MTT assay. The decline in % cell viability was shown to be dependent on the conc. and was statistically significant at $p < 0.05$. The IC_{50} values obtained by the MTT assay for ethanol, methanol, chloroform, water, and acetone extract were 44.90, 31.70, 66.64, 38.30, and 58.21 μg/ml, respectively.

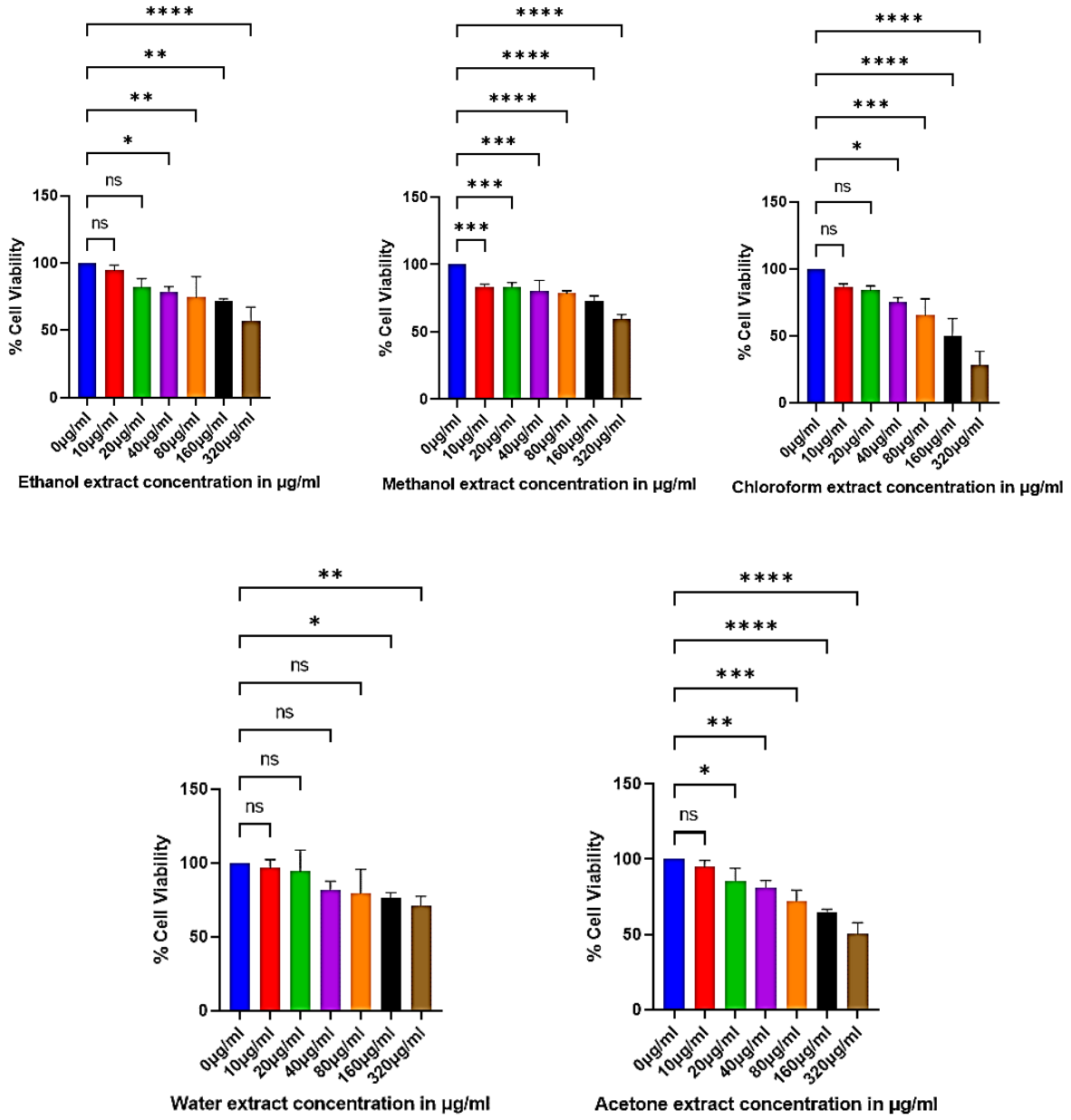


Fig 1:- Anti-cancer effect of *zizipuschristi* extracts on BC cell line. MTT assay in MCF-7 cell line tretaed with (a) Methanol extract (b) water extract (c) Ethanol (d) Chloroform (e) Acetone at conentration 10, 20, 40, 60, 80, 160 and 320 µg/ml for 72hrs. The results are indicated as mean ± SD of triplicate wells (n=3), p-value ≤ 0.05. (* p ≤ 0.05,p <0.01 *** p < 0.001, and **** p < 0.0001) (ns: not significant).**

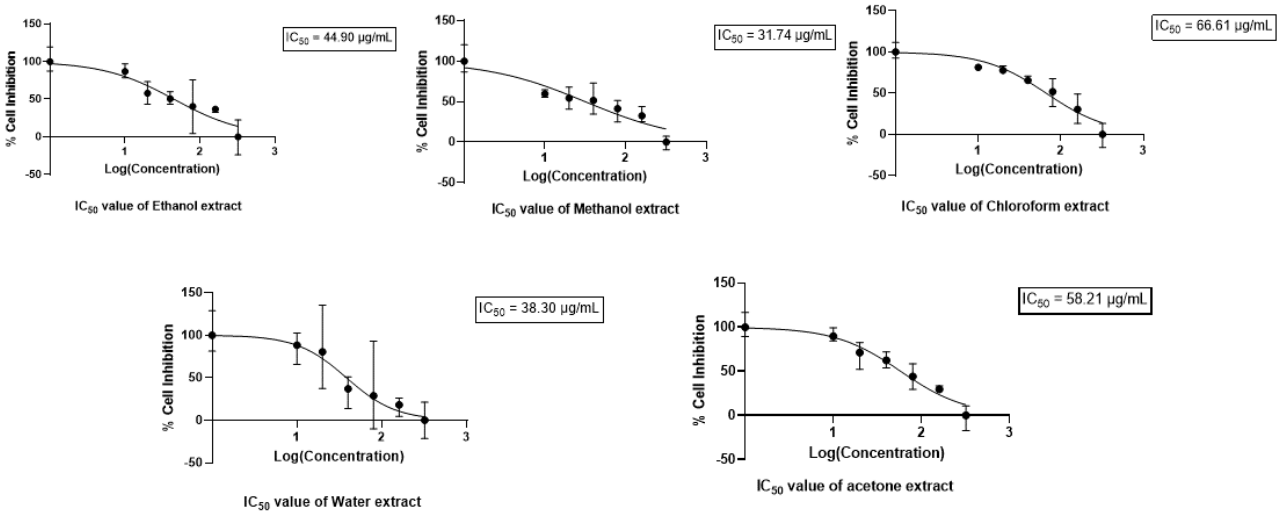


Fig 2:- IC_{50} values of *Z.Spinachristi* extracts (a) Ethanol extract (b) Methanol extract (c) Chloroform (d) Water (e) Acetone on MC7F-7 cell line.

3.2 Antibacterial activity of *Z.Spinachristi* extracts

*Z. spina*christileaf extracts (methanol, water, ethanol, acetone, and chloroform) were evaluated for antibacterial activity against microbial strains (*Bacillus cereus, S. aureus, S. epidermidis, and E. coli)* by the cup-plate method at conc. of 40 and 80 µg/ml. The evaluation of the antibacterial activity of *Z. spina* christi leaf extracts is mentioned in Table1. The findings demonstrated that all plant extracts exhibited efficacy in inhibiting the growth offour of the pathogenic strains (*Bacillus cereus, S. aureus, S. epidermidis,* and *E. coli*) with varying degrees of efficacy. The methanol extract exhibited the highest level of potency.

The findings from the study of antimicrobial activity of the five extracts indicate that *E. coli* exhibited the highest level of resistance to plant extracts, followed by *S. epidermidis*. On the other hand, *Bacillus cereus*and *S. aureus*were shown to be the most sensitive strains to the extracted plants, respectively. The methanolic extract of *Z. spinachristi* leaf had shown highestanti bacterial activity, followed by water and ethanol extracts.

Table 1:- Antibacterial activity of *Z. Spinachristi* extracts most effective conc. (40, 80 µg/ml) against different strains of bacteria *Bacillus cereus, S.aureus, S.epidermidis and E.coli.*

Extracts (µg/ml)	Zone of Inhibition (mm)			
Methanolic extract	***Bacillus cereus***	***S.aureus***	***S.epidermidis***	***E.coli***
40	7.2 ±0.5	6.4±0.7	5.3±0.4	4.2±2
80	13.7±0.5	11.4±0.7	10.2±0.4	9.2±2
Water extract	***Bacillus cereus***	***S.aureus***	***S.epidermidis***	***E.coli***
40	5.2±0.5	4.3±0.2	3.1±0.6	3±0.5
80	12±0.2	11±0.8	8±0.5	7.6±0.2
Ethanol extract	***Bacillus cereus***	***S.aureus***	***S.epidermidis***	***E.coli***
40	5.6±0.4	4.3±0.5	3.3±0.7	3.8±0.3
80	10.2±0.2	9.2±0.8	8.2±0.5	6.2±0.2
Chloroform extract	***Bacillus cereus***	***S.aureus***	***S.epidermidis***	***E.coli***
40	5.3±1	5.1±0.5	4.2±1	3.4±1
80	9.3±0.6	8.4±1	6.7±0.6	5.3±0.5
Acetone extract	***Bacillus cereus***	***S.aureus***	***S.epidermidis***	***E.coli***
40	5.2±0.2	4.3±0.7	2.1±1	1±0.2
80	8.3±0.6	6.4±1	4.2±0.6	3.4±0.5
Tetracycline (30µg)	***Bacillus cereus***	***S.aureus***	***S.epidermidis***	***E.coli***
16.3±0.37	14.2±0.6	13.4±0.2	11.5±0.5	

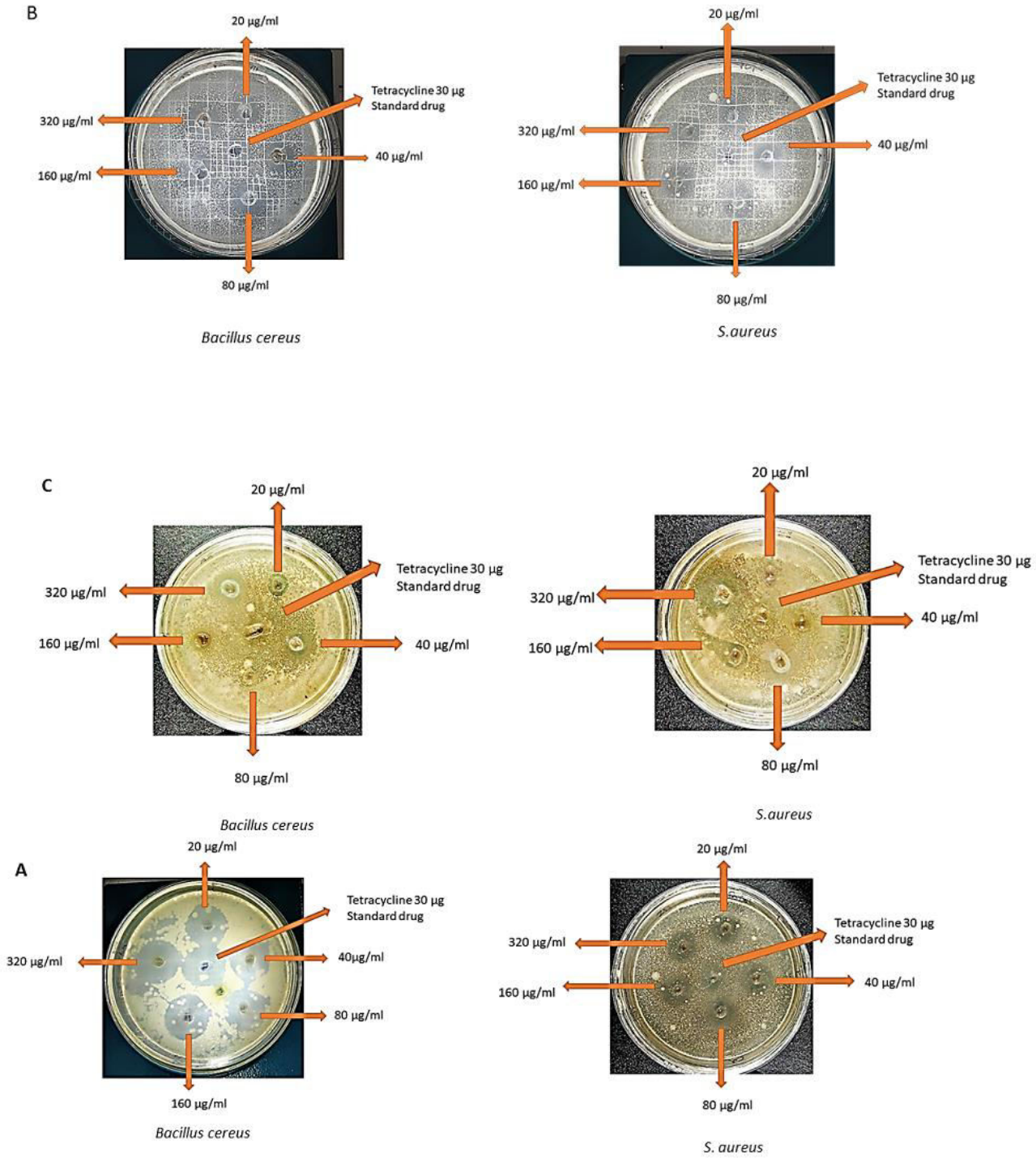


Fig 3:- Antibacterial activity of *Z.spina christi*extracts by cup plate method A) Methanol B) Water C) Ethanol against most suceptible bacterial strains *Bacillus cereus* and *S.aureus.*

3.3 MIC of effective plant extractof *Z.Spinachristi*against *Bacillus cereus, S.aureus, S.epidermidis and E.coli.*

*Z.Spinachristi*leaf extracts antibacterial activity were assessed by MIC for the most effective plant extracts (methanol, water, and ethanol) at conc. of 10, 20, 40, 80, 160, and 320 µg/ml, respectively (refer table 2). *Z. spina christi*leaf extracts exhibited their maximal level of activity at a conc. of 320 µg/ml. The maximum ZOI for methanol extract was detected in *Bacillus cereus* (19.3±0.3 mm), followed by *S. aureus* (16.5±0.2 mm), *S. epidermidis* (14.2±0.6 mm), and *E. coli* (12.5±0.4 mm). The maximum ZOI for water extract was detected in *Bacillus cereus* (17±0.6 mm), followed by *S. aureus* (15±0.1 mm), *S. epidermidis* (13±0.5 mm), and *E. coli* (11±1 mm). The maximum ZOI for ethanol extract was detected in *Bacillus cereus* (13.2±0.9 mm), followed by *S. aureus* (12.3±0.2 mm), *S. epidermidis* (11.1±1 mm), and *E. coli* (8.6±0.4 mm). The *Z. spina christi*leaf extracts exhibited dose-dependent antibacterial action against both Gram-positive and Grammeme-negative bacteria. The antibacterial efficacy of the *Z.spina Christi*leaf extracts was completely dependent on the conc., with the highest level of activity seen at a conc. of 320 µg/ml.(refer table 2)

Table 2:- MIC value of most effective *Z.Spinachristi* extracts against *Bacillus cereus, S.aureus, S.epidermidis and E.coli*

Extracts (µg/ml)Zone of Inhibition (mm)			
Methanol extract	***Bacillus cereus***	***S.aureus***	***S.epidermidis***
E.coli			
20	10±0.2	8±0.5	6.3±0.5
2.1±0.7			
40	11±0.6	10.5±1	8.3±1
5.2±1			
80	13.2±0.5	11±0.7	10.6±0.4
9.2±0.4			
160	16.4±0.7	14.3±0.8	11.5±0.7
10.7±0.8			
320	19.3±0.3	16.5±0.2	14.2±0.6
12.5±0.4			
Water extract	***Bacillus cereus***	***S.aureus***	***S.epidermidis***
E.coli			
20	8.3±0.4	7.3±0.6	3.4±0.2
0.0 ±0.0			
40	10.6±0.3	9.5±0.6	6.5±0.6
4.3±0.8			
80	12.8±0.2	11.2±0.8	8.3±0.5
7.2±0.2			

160	15.4±0.8	13.8±2	11.5±0.9
9.2±0.2			
320	17±0.6	15±0.1	13±0.5
11±1			
Ethanol extract	***Bacillus cereus***	***S.aureusS.epidermidisE.coli***	
20	7.8±0.6	6.4±1	0.0 ±0.0
0.0 ±0.0			
40	9.3±0.5	7.4±1	5.6±1
4.2±0.5			
80	10±0.2	9.5±0.8	8.2±0.5
6.7±0.2			
160	11.4±0.4	10.3±0.3	9.4±1
7.4±0.5			
320	13.2±0.9	12.3±0.2	11.1±1 8.6
±0.4			

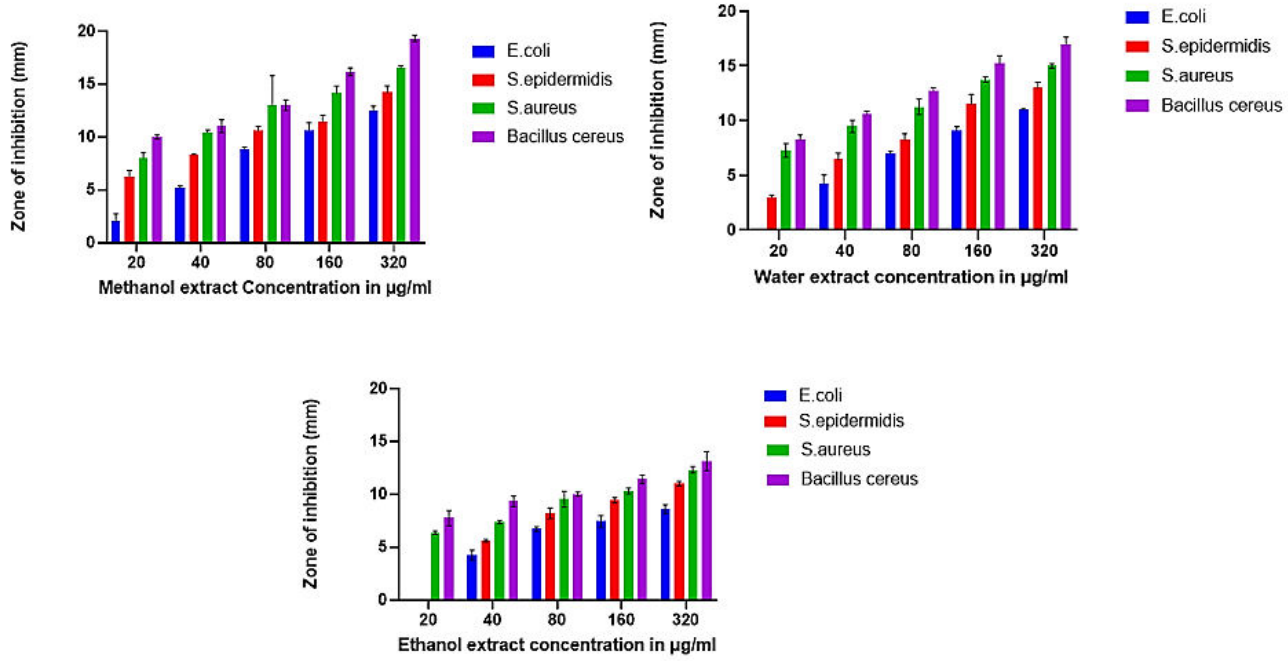


Figure 4:- MIC values of *Z.spina Christi* extract (a) Methanol (b) Water (c) Ethanol at conc.10, 20, 40,80,160 and 320 µg/ml against microbial strains *Bacillus cereus, S.aureus, S.epidermidis and E.coli.*

3.4 Phytochemical screening of secondary metabolites

The qualitative phytochemical examination of the medicinal plants revealed the presence of many phytochemical components, including alkaloids, anthraquinones, steroids, saponins, terpenoids, tannins, and flavonoids (Table 3). The bioactive compounds found in most extracts have been naturally occurring and have been shown to exhibit anti-fungal and anti-bacterial activities against the studied human pathogens,(Kebede et al., 2021), (Ibrahim & Ghareeb, 2019)

In our study, we performed the phytochemical screening of the most effective extract of *Z.spina Christi* (methanol, water, and ethanol) that had shown considerable antimicrobial activity against microbial strains *(Bacillus cereus, S. aureus, S. epidermidis, and E. coli).* The methanolic extract of *Z.spina Christi leaf*tested positive for alkaloids, anthraquinoneglycosides, saponins, terpenoids, flavonoids, phenolic compounds, tannins, and negative for steroids. The ethanol extract tested positive for alkaloids, saponins, terpenoids, flavonoids, and tannins and negative for anthraquinone glycosides, steroids, and phenols, whereas the water extract tested positive for alkaloids, anthraquinone glycosides, saponins, terpenoids, phenolic compounds, flavonoids, and negative for steroids and tannins.

Test	**Water**	**Methanol**	**Ethanol**
Steroids	-	-	-
A-Glycosides	+	+	-
Saponin	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+
Phenols	+	+	-
Tannins	-	+	+

4. Dicussion

BC is the predominant form of cancer among women, impacting around 1 in 20 individuals worldwide. Prior research has indicated that betulin present in *Z. spinachristi* showed anti-cancer activity against MCF-7 BC cells. Additionally, saponinosomes from *Z. spinachristi* have demonstrated high toxicity against B16F10 melanoma cells and may be used to treat malignant melanoma [12].Another study revealed that butanol, chloroform, methanol-water, and aqueous extracts of *Z. spinachristi* had a cytotoxic impact on Hela cells. Furthermore, the chloroform-methanol extract of *Z. spinachristi* shows the presence of cyclopeptide alkaloids such as spinanine A and flavonoids like

quercetin, rutine, and triterpenoidsaponin glycosides, which may be responsible for its anticancer activity.(Jafarian et al., 2014b)

Prior research found that sidrin isolated from the chloroform and aqueous methanol fractions of *Z. spinachristi*leaf possesses cytotoxic activity against breast cancer (MDA-MB-231/ATCC,BT-549,), human leukaemia (RPMI-8226, Hl-60), and lung cancer (A549) cell lines [13](Mustafa et al., 2023). Previous studies have shown that BC patients exhibit an abundance of bacterial strains such as *E. coli, Bacillus cereus, Bacillus epidermidis,Corynebacterium, and Thermus*, which can contribute to cancer progression and worsen the patient's condition[16].Additionally, *Z.spina Christi* was found to exhibit antimicrobial properties against Gram-positive *Bacillus subtilis*and *Streptococcus pyogenes*, as well as against *E.coli*. The fatty acid fraction shown significant efficacy against *B. subtilis and E. coli*.(Nazif, 2002)The methanol-ethyl acetate extract of *Z. spinachristi*leafwere found to exhibit higher activity towards the two Gram positive bacteria (*S.aureus, B.subtilis*) than the two Gram negative (*P.aeruginosa, E.coli*). (Al-Bayatti et ai., 2011), (Ads et al., 2017) Hence, in our study, we evaluated different *Z. spina Christi* leaf extracts (water, chloroform, ethanol, methanol, and acetone) for their anti-cancer activity in the MCF-7 BC cell line. We also investigated *Z. spina Christi*antimicrobial activity against various microbial strains, including *E. coli, Bacillus cereus, S. aureus, and S. epidermidis*, which may play a pivotal role in BC progression. In the MTT assay, we found that as the conc. increased from 10 μg/ml to 320 μg/ml of *Z.Spinachristi*varying extracts, the MCF-7 cells % cell viability were found to be decreased from 94% to 57% for ethanol extract, 83% to 59% for methanol extract, 86% to 28% for chloroform extract, 96% to 71% for water extract, and 94% to 50% for acetone extract significantly at conc. 10, 20, 40, 80, 160, and 320 μg/ml, respectively. The anti-microbial activity was conducted on all the extracts at conc. of 40 and 80 μg/ml, among which methanolic, water, and ethanol extracts showed the maximum ZOI. Furthermore, a MIC study was conducted on the most effective extracts (methanol, water, and ethanol) of *Z. spina christi*at conc. of 20, 40, 80, 160, and 320 μg/mL, respectively. The maximum ZOI for methanol extract was observed at 320 μg/ml in *Bacillus cereus* (19.3±0.3 mm), followed by *S. aureus* (16.5±0.2 mm), *S. epidermidis* (14.2±0.6 mm), and *E. coli* (12.5±0.4 mm). A previous study stated that medicinal plants containing alkaloids, flavonoids,terpenoids, tannins, and phenolicspossess potential bacteriostatic, bactericidal, and fungicidal effects against selected human pathogens.(Kebede et al., 2021)

In our phytochemical screening study, the methanol *Z. spina Christi leaf extracts* tested positive for alkaloids, anthraquinone glycosides, saponins, terpenoids, flavonoids, phenolic compounds, and tannin and negative for steroids, which are mainly responsible for anti-microbial activity. The ethanol extract tested positive for alkaloids, saponins, terpenoids, flavonoids, and tannins and negative for anthraquinone glycosides, steroids, and phenols, whereas the water extract

tested positive for alkaloids, anthraquinone glycosides, saponins, terpenoids, flavonoids, and phenolic compounds and negative for steroids and tannins. The present research aims to conduct extensive investigations on plant extracts that exhibit significant anti-cancer and antibacterial properties. Furthermore, it indicates that the methanol, water, and ethanol extracts exhibited significant antibacterial properties compared to the acetone and chloroform extracts. Ultimately, these findings suggest that the chemical compounds present in *Z. spina Christi* extract can emerge as a potent anti-cancer agent against BC. Furthermore, it has shown significant antimicrobial activity, suggesting its effectiveness in inhibiting the growth of microbial strains and BC progression by certain microbial strains.

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Authors' contributions: -All authors have read and approved the manuscript. Anam.N.Khan:- Conceptualization ,Writing, Original draft, methodology; Ekanttaywade:-Writing, Investigation; Rahul.D.Jawarkar:- Conceptualization, data curation, formal analysis, software; Umang Shah:-Methodology, software.

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Abbreviation

1) Zizipus Spina Christi-*Z.*spina Christi
2) BC-Breast cancer
3) Concentration-Conc.
4) Temperature-temp.
5) MIC-Minimum Inhibitory concentration
6) ZOI-Zone of inhibition
7) MTT-3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide
8) DMSO-Dimethyl sulphoxide
9) mg-milligrams
10) IC_{50}-Half-maximal inhibitory concentration
11) HCL-Hydrochloric acid

12) *Staphylococcus aureus-S. aureus*
13) *Staphylococcus epidermidis-S.epidermidis*
14) Escherichia coli-*E.coli*