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# *In Vitro* Evaluation of Antibacterial Potential of *Coccinia grandis* Against Human Pathogens

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## Abstract

*Coccinia grandis*, the ivy gourd, also known as scarlet gourd a Cucurbitaceae family fruit. In tradition medicine fruits have been used to treat leprosy, fever, asthma, bronchitis and jaundice etc. Under in-vitro conditions antibacterial activity of *Coccinia grandis* fruits extracts on human pathogens was studied. *Coccinia grandis* fruits crude extracts were prepared by cold solvent extraction method using different organic solvents such as acetone, ethanol, methanol and chloroform. All solvent extracts were solubilized in DMSO solvent. Antibacterial activity of all solvent extracts was tested against five different bacterial strains of potential human pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus* and *Salmonella typhimurium* by agar well diffusion assay. Aqueous solvent fruits extracts showed significant antibacterial activity with broad spectrum (against to gram positive as well as gram negative bacteria) against selected test human pathogens. Among the all aqueous solvent extracts maximum and effective antibacterial activity was observed with ethanol extracts against *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* Whereas rest other pathogens were susceptible. Minimum Inhibitory Concentration (MIC) of these extracts against test pathogens was tested by broth dilution method and ranged between 500-600 µg/ml. All solvent extracts were studied for phytoconstituents determination by qualitative analysis. These indicate that the herbal preparations of *Coccinia grandis* may have potential for preventing and treat the diseases caused by various human pathogens

**Keywords**
*Coccinia grandis,* antibacterial activity, human pathogens, agar well diffusion assay, Minimum Inhibitory Concentration (MIC)

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**INTRODUCTION:**

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great economic value all over the world (Arshad Hussain et.al. 2010)[5]. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Herbal

medicine is still the mainstay of about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents (Fisseha Alemu, et. al. 2017)[4]. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in photochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines (Abha Verma et. al. 2013)[3].

*Coccinia grandis (Cucurbitaceae)* the ivy gourd otherwise called red gourd, tindora, and kowai fruit, is a tropical vine. It develops fundamentally in tropical atmospheres and is normally found in the southern Indian province of Kerala, where it frames a piece of the neighborhood food (L. Shilpa Santheesh & K Murugan et. al. 2011)[6]. The geography of India which is in the tropical belt with its changed climatic zones makes it a huge storage facility of therapeutic plants. *Coccinia grandis* is cooked as a vegetable. In Southeast Asia, it is developed for its palatable youthful shoots and eatable organic products. The plant is incidentally developed for its palatable products of the soil its restorative uses in tropical and sub-tropical zones, particularly in India, Malaysia and Indonesia (Pekamwar S.S, et.al.2013)[9]. The plant is sold in nearby markets and is likewise once in a while traded to forte markets. In traditional medicine, fruits have been used to treat leprosy, fever, asthma, bronchitis, and jaundice. The fruit possesses mast cell-stabilizing, antianaphylactic, and antihistaminic potential. In Bangladesh, the roots are used to treat osteoarthritis and joint pain. A paste made of leaves is applied to the skin to treat scabies. The juice of roots and leaves useful for the treatment of diabetes (Manisha Choudhari et.al. 2015)[12]. The present study focused on preparation of crude extract of *Coccinia grandis* fruits in different solvent such as ethanol, methanol, acetone and chloroform. Evaluated antibacterial activities of *Coccinia grandis* fruits extract selected human pathogens were having Multi Drug Resistance (MDR) ability (H.Giamarellou et.al. 2010)[20]. Further extracts were allowed to determination of minimum inhibitory concentration (MIC) against selected pathogens (V.A. Gargade et. al. 2015)[14]. Crude fruits extract was analyzed by various phytochemical estimation methods for alkaloids, tannins, glycosides, Saponins, Flavonoids, Terpenoids, carbohydrates and sulphuric acid (Swapna Gurrapu et.al. 2017)[8].

## MATERIALS AND METHODS

### Collection and extraction of plant material of *Coccinia grandis*

Fruits of *Coccinia grandis* were collected from crop field of solapur region and stored in sterile plastic bag. Collected plant material was confirmed by botanical authentic person from D.B.F. Dayanand College of Arts and Science, Solapur. The collected samples were washed by sterile distilled water and cut into fine pieces. Then dried under sun for one week. Dried *Coccinia grandis* fruits were ground into a coarse powder with the use of a mortar-pestle (Abha Verma et. al. 2013)[3]. 35 gm of powdered material of both extracts were taken in sterile, clean, flat- bottomed glass containers and soaked in 200 ml of 95% ethanol (Fisseha Alemu et.al.2017)[4]. Prepared the same set for other solvents such as acetone, methanol and chloroform. The containers of various solvents were sealed and kept for a period of 10 days accompanying occasional shaking and stirring (A.Sivaraj et.al.2011)[1]. After the proper extraction period the whole mixture then underwent a coarse filtration by sterile clean white cotton material. Then mixtures of various solvents were filtered through Whatman's filter paper (Bibby RE200, Sterilin Ltd., UK).

### Evaporation of the solvent

Collected filtrate was used and evaporated by exposure to air dry and rotator evaporator to the concentrate. Final filtrate was obtained as powdered form and stored in refrigerator until further investigation (Abha Verma et. al. 2013).

### Test organisms

Bacterial cultures were used for determination of antibacterial activity of *Coccinia grandis* fruits extracts. Primarily standard and drug resistant bacteria were used for the evaluation or test *Coccinia grandis* fruits extract. Standard as well as drug resistance gram positive and gram-negative strains: *Staphylococcus areus* (MCC 2408), *Escherichia coli* (MCC 2412), *Pseudomonas aeruginosa* (MCC 3458), *Klebsiella pneumoniae* (MCC 2570) *and Salmonella typhimurium* obtained from Department of Microbiology, V. M. Medical College, Solapur and Yashodhara Hospital, Solapur. Obtained cultures were also confirmed by morphologial, cultural and biochemical characterization. (G.H.Talbot *et.al.* 2006)[15]

### Qualitative estimation of phytochemicals from *Coccinia grandis*

Analyzed of various chemical constituents of *Coccinia grandis* fruits extracts. Prepared solvent extracts as powdered form and then solubilized in DMSO solvent. ( Abha Verma et. al. 2013). This form of solution was used for the chemical analysis of extract. The Preliminary qualitative phytochemical screening was carried out with the following methods (Swapna Gurrapu et.al.2017)[8].

### Test for Alkaloids

In this test 2 ml of plant extract solution was taken in a test tube and added 0.2 ml of dilute hydrochloric acid (HCL). Then few drops of Dragendorrf's reagent were added and observed the reaction.

**Test for Tannins**

3 ml of plant extract solution was taken in a test tube and added 2-3 drops 5% ferric chloride ($FeCl_3$) solution. Observed the development of brownish green to dark blue coloration indicates the positive reaction.

**Tests for Glycosides**

In this test 2 ml glacial acetic acid was added to the 2 ml plant extract solution. Then 1 ml of dil. Sulphuric acid was added to the reaction mixture. Observed the brown ring coloration indicates the positive test for glycosides.

**Test for Terpenoids**

1 ml of plant extract was taken in a test tube and added 2 ml of Chloroform. Then 2-3 ml of conc. Sulphuric acid ($H_2SO_4$) solution was added to the reaction mixture by side wall of tube to form two different layers. Observed the reddish violet coloration indicates the presence of terpenoides.

**Test for Flavonoids**

5ml of plant extract was taken in a test tube and was mixed with 1 ml of diluted ammonia ($NH_4OH$). Observed the yellow coloration indicates the positive test for Flavonoids.

**Test for Steroids by Sulphuric acid test**

1 ml of aqueous solvent extract was taken in a test tube and then added 1 ml of sulphuric acid. Observed colour development in the reaction.

**Test for Saponins**

In this test 5ml of aqueous plant extract solution was filtered by Whatman's filter paper. Then 0.5 ml of filtrate was diluted with 5ml of distilled water and shaken vigorously for 2-3 minutes. Observed the formation of stable foam indicates the positive test for Saponins.

**Test for carbohydrate by Benedict's Test**

In this test 1 ml of aqueous extract was taken in a test tube and then added 5ml benedicts test reagent. Boiled this mixture for 5 minutes and allowed to cool spontaneously. Observed the reaction for the presence of the carbohydrate.

**Antibacterial assay**

Antibacterial activity of *Coccinia grandis* extracts against isolated human pathogens was studied by agar well diffusion method. (A. Sivaraj *et.al*.2011)[1]. Prepared sterile Muller Hinton Agar Butt for higher sensitivity and form confluent growth throughout the media. Pure isolates of various bacterial pathogens were subcultured in sterile nutrient broth and incubated at $37^0$C for 24 hours for incubation. Prepared inoculums approximately $10^8$ CFU/ml were inoculated into 20 ml of sterile Muller Hinton Agar butt and poured into sterile empty Petri plates. Poured plates were allowed to dry and prepared wells by sterile cork borer of diameter 6 mm. *Coccinia grandis* fruits extracts concentrate was solubilized in sterile DMSO, its universal solvent for many compounds for solubilization. Aseptically 50 µl of fruits extracts were added into agar wells of inoculated Muller Hinton Agar Plates. Sterile DMSO was added into wells and kept as negative control. After addition of extracts plates were allowed to stand for 40 minutes or more at $4^0$C for diffusion and then incubated at $37^0$ C for 24 hours. After incubation observed the zone of inhibition and measured the diameter of zone in mm.

**Determination of Minimum Inhibitory Concentration (MIC)**

The Minimum Inhibitory Concentration (MIC) of *Coccinia grandis* extracts were determined by broth dilution method (Arshad Hussain et.al. 2010)[5]. Prepared various concentrations of *Coccinia grandis* extracts were diluted into fresh sterile nutrient broth. Dilutions were ranging from 100 µg/ml to 1000 µg/ml. Diluted various concentrations of extract were serially increasing from first and added into test tubes containing the fixed bacterial cells ($10^6$ CFU/ml). All tubes were kept for incubation at $37^0$C for 24 hours. After incubation all tubes were observed for visible turbidity. Sterile DMSO was kept a negative control. Tubes were examined that the lowest concentration of extract inhibited visible growth of the tested organisms and recorded as MIC.

## RESULTS AND DISCUSSION

*Coccinia grandis* fruits were dried and ground into powder by the use of mechanical crushing in mortar-pestle device. Prepared fruits extract was soaked or macerated (35 gm in 200 ml) in various solvents such as ethanol, methanol, acetone and chloroform by cold extraction method. Aqueous solution of fruits extracts were extracted in solvent within 10 days by occasional shaking and stirring of container. Extracted material was filtered through sterile clean white cotton filter for coarse filtration followed by whatman filter paper and obtained final filtrate. Filtrate was evaporated under exposure to air and through rotary evaporator to the concentrate. Obtained final filtrate as powdered form and stored in refrigerator at $4^0$ until further investigation.

Preliminary phytochemical analysis was done for the estimation of possible chemical constituents in the *Coccinia grandis* fruits extracts by various standard protocols. Observed the reactions were carried out by standard protocol of qualitative estimation of phytochemicals of *Coccinia grandis* fruits extracts. All solvent extracts were used and revealed the presence of Alkaloids, carbohydrate, Saponins, Flavonoids, and glycosides whereas the other phytoconstituents were absent Swapna Gurrapu et.al.2017)[8]. Results were given in table no. 1.

Test organisms were confirmed and standardized by Morphological, cultural and biochemical characterization in laboratory. Evaluated sensitivity

towards the *Coccinia grandis* fruits extracts solution under control conditions (G.H.Talbot *et.al.* 2006)[15].

Antibacterial activity of *Coccinia grandis* fruits extracts was done by agar well diffusion method.(A. Sivaraj *et.al*.2011)[1].. Sterile MHA plates were prepared and inoculated by standard test pathogens. Solubilized fruits extracts in DMSO solvent were added in agar wells and allowed for diffusion followed by incubation. Observed the plates for antibacterial activity of *Coccinia grandis* fruits extracts against pathogens. Predominantly ethanol fruits extracts of *Coccinia grandis* showed higher antibacterial activity against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli* and *Pseudomonas aeruginosa.* Whereas other solvents extracts showed intermediate activity against test pathogens. Evaluated bacterial strains showed various patterns of inhibition on surface of agar and measured zone of inhibition in mm. Results were shown in table no. 2.

Prepared the experimental setup for the determination of MIC of *Coccinia grandis* fruits extracts by broth dilution test (V.A. Gargade et. al. 2015)[14]. Sterile nutrient broth tubes were diluted by different concentration of *Coccinia grandis* fruits extract were ranging from 100 µg/ml to 1000 µg/ml .The minimal inhibitory concentration of *Coccinia grandis* ethanol extract was most effective against *Staphylococcus aureus, Escherichia Coli, Salmonella typhimurium* and *Klebsiella pneumoniae* at concentration 500 µg/ml. The methanol extract was also most potent against *Staphylococcus aureus* and *Escherichia Coli* at 600 µg/ml. The least activity was recorded with other solvent extracts against human pathogens. Sterile DMSO solution in nutrient broth kept as negative a control. MIC values were shown in table 3.

In present study antibacterial activity of *Coccinia grandis* fruits extracts against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia Coli, Salmonella typhimurium* and *Klebsiella pneumoniae* and showed satisfactorily antimicrobial activity against selected standard drug resistance pathogens. MIC of extracts was also carried out by broth dilution method and calculated the MIC value and also confirmed the chemical constituents in fruits extracts by various chemical methods. Earlier work was done and published on *Coccinia grandis* leaf extracts against selected bacterial strains *E.coli, Bacillus cereus, Klebsiella Pneumoniae, Staphylococcus aureus* and *Streptococcus pyogenes* (A. Sivaraj *et.al*.2011)[1] . *Coccinia grandis* leaf extracts were prepared in various solvents and tested antimicrobial activity against pathogens. Leaf extracts showed antibacterial activity against all selected bacterial strains. Among the all Solvent extracts hexane leaf extracts showed high antibacterial activity whereas other solvents extracts were satisfactorily. MIC of leaf extracts was also tested and most potent against *S.aureus, E.coli, K.pneumoniae* at a concentration below 31.25µg/ml. As the *Coccinia grandis* fruits used by traditional therapeutic purpose to cure various diseases (Arshad Hussain et.al. 2010)[5]. All these results compared with the present research and evaluated and studied the antimicrobial activity of *Coccinia grandis* fruits extracts against potent pathogens.

**CONCLUSION**

*Coccinia grandis* fruits extracts were macerated in different solvents and extracted extractives of phytoconstituents in solvent. Revealed phytoconstituents of fruits extracts were responsible for the inhibition of growth of test pathogens. In experiments *Coccinia grandis* fruits extracts showed effective antimicrobial activities against selected test pathogens such as *Staphylococcus areus* (MCC 2408), *Escherichia coli* (MCC 2412), *Pseudomonas aeruginosa* (MCC 3458), *Klebsiella pneumoniae* (MCC 2570) *and Salmonella typhimurium* were derived from patients. Further extracts were determined the MIC values of fruits extract and confirmed its required concentration against possible human pathogen with use of this study. Multi drug resistance bacteria can be treated by the use of this preparation with herbal and lesser side effects. Fruits extract containing possible phytochemicals were evaluated and these are having different antimicrobial, analgesic, antipyretic, antidiabetic and antimalarial activities. These research findings have given good opportunity for the preparation of herbal drugs with use of *Coccinia grandis* plant extracts against possible diseases caused by various pathogens. In future study extend up to aqueous extract of *Coccinia grandis* is having more potential to cure possible infections caused by various viruses.

**Table 1: Qualitative analysis of phytochemicals from *Coccinia grandis* fruits extract**

| Name of Phytochemical | Name of solvent extract | | | |
|---|---|---|---|---|
| | Ethanol | Methanol | Acetone | Chloroform |
| Alkaloids | + | + | - | + |
| Tannins | - | - | - | - |
| Glycosides | + | + | + | + |
| Terpenoids | + | + | - | - |
| Saponins | + | + | - | + |
| Flavonoids | + | + | + | + |
| Carbohydrate | + | + | + | + |
| Sulphuric Acid | - | - | - | - |

**+ = Presence, - = Absence**

**Table 2. Antibacterial activity of fruits extract of *Coccinia grandis***

| Organisms | Solvent extracts with Zone of Inhibition in (mm) | | | |
|---|---|---|---|---|
| | Ethanol | Methanol | Acetone | Chloroform |
| *Staphylococcus aureus* | 12.5± 0.10 | 7.30± 0.10 | NA | 6.30± 0.10 |
| *Escherichia coli* | 10.4± 0.15 | 6.50±0.10 | NA | 5.50± 0.10 |
| *Pseudomonas aeruginosa* | 9.85± 0.05 | NA | 4.55±0.10 | 4.60± 0.05 |
| *Klebsiella pneumoniae* | 10.20± 0.10 | NA | 5.50± 0.03 | NA |
| *Salmonella typhimurium* | 13.2± 0.05 | 4.85± 0.05 | NA | NA |

**NA= No Activity**

**Table 3. Minimum Inhibitory concentration of *Coccinia grandis* fruits extract against human pathogens**

| Organisms | Solvent extract with MIC value in µg/ml | | | |
|---|---|---|---|---|
| | Ethanol | Methanol | Acetone | Chloroform |
| *Staphylococcus aureus* | <500 | >600 | >1000 | >700 |
| *Escherichia coli* | <500 | >600 | >800 | >1000 |
| *Pseudomonas aeruginosa* | >1000 | >1000 | >1000 | >1000 |
| *Klebsiella pneumoniae* | <500 | >1000 | >1000 | >1000 |
| *Salmonella typhimurium* | <500 | >1000 | >1000 | >1000 |

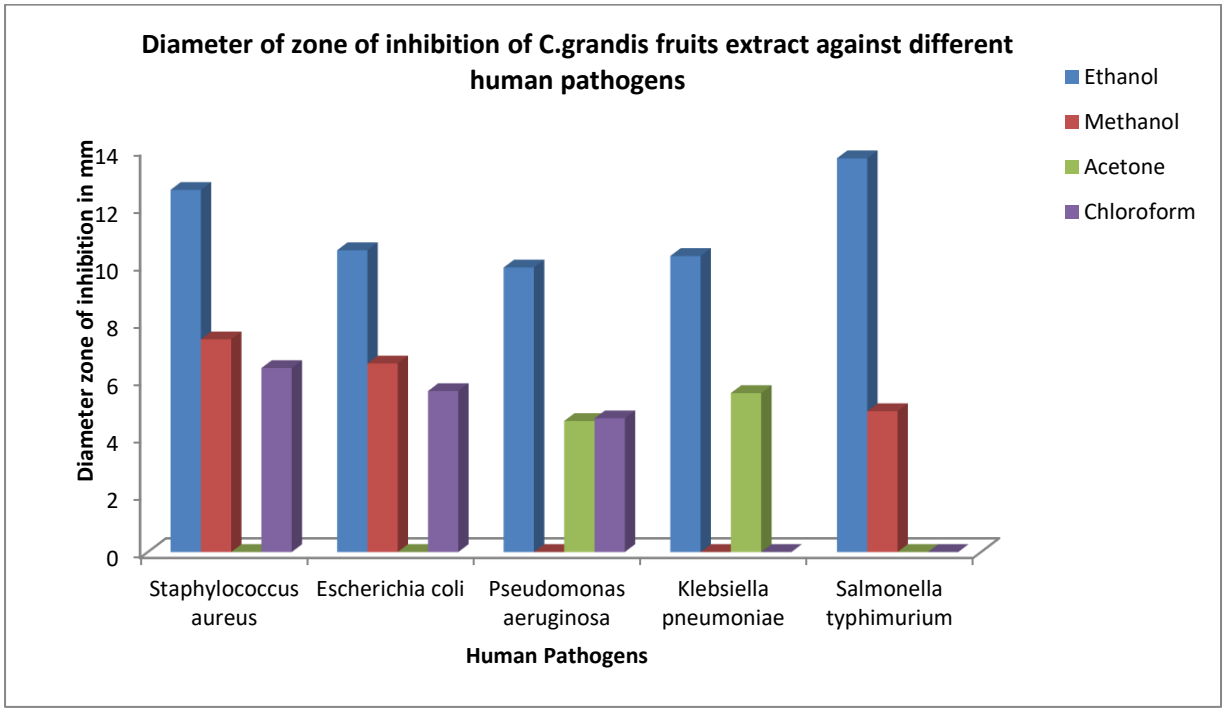



**Fig 1. Antibacterial activity of fruits extract of *Coccinia grandis* against**

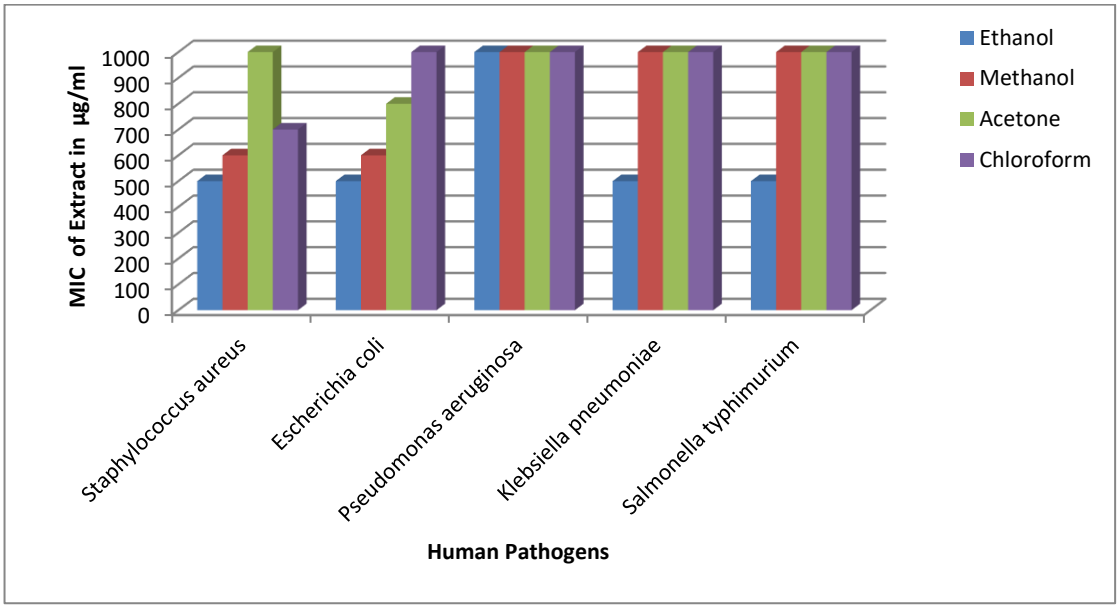
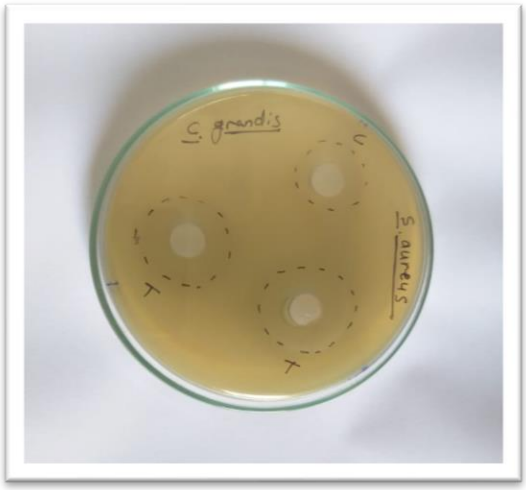
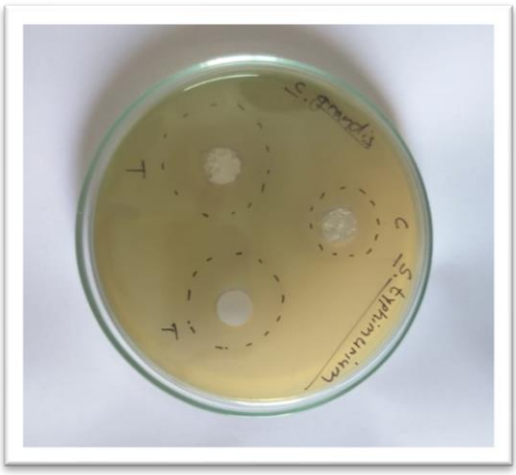



**Fig. 2. Minimum Inhibitory concentration of *Coccinia grandis* fruits extract against human pathogen**

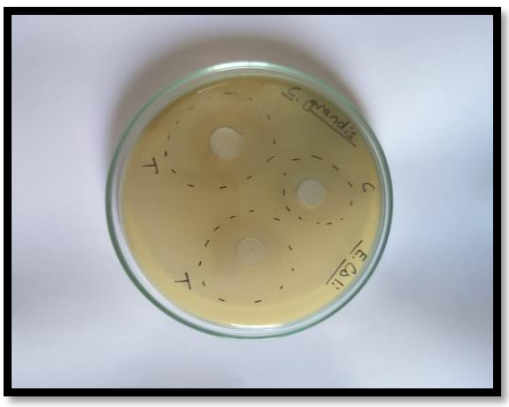
**Fig. 3. Images of antimicrobial activity of *Coccinia grandis* extract against human pathogens**



***Staphylococcus aureus***



***Salmonella typhimurium***



***Escherichia coli***

***Pseudomonas aruginosa***


***Klebsiella pneumoniae***

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