PREPARATION OF A GALLIC ACID ENRICHED POMEGRANATE PEEL EXTRACT AND ITS APPLICATION IN CONTROLLING PATHOGENIC BACTERIA AND FUNGI

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Key words: Polyphenol, Gallic acid, Antimicrobial activity

Introduction:

Polyphenolics compounds are micronutrients, referred to as a diverse group of naturally occurring compounds containing multiple phenolic functionalities. (Amélia Martins Delgado et al., 2019)

They are classified based on the number of carbon atoms in conjunction with the structure of the basic phenolic skeleton into different groups such as:

- Flavonoids
- Phenols
- Phenyl
- Propanoids
- Benzoic acid

Flavonoids are the most abundant subclass of plant-derived polyphenolics and are of interest because of their apparent beneficial effects on human health, including antioxidant, anti-inflammatory, and antimicrobial activities. (Badriyah Shadid Alotaibi et al., 2022)

Background:

The background of this project revolves around the increasing demand for natural and safe alternatives to traditional antibiotics and antifungal agents.

With the rise of antibiotic-resistant bacteria and fungi, there is a need to explore new sources of antimicrobial compounds that can effectively control these pathogens.

Polyphenols are naturally occurring compounds found in fruits and vegetables that have been reported to possess antimicrobial properties.

These compounds have been shown to inhibit the growth of various pathogenic bacteria and fungi. Thus, the development of a polyphenolic-enriched extract from fruit peel could offer a promising solution for controlling these infectious microorganisms.

Objectives:

- Preparation of Polyphenolic enriched pomegranate peel extract using enzymatic method
- *In vitro* evaluation of the extract for antimicrobial activity against pathogenic bacteria and fungi using agar well diffusion and broth microdilution methods
- Assessment of cell viability by MTT assay and morphological studies using SEM analysis.

Methodology:

Preparation of Gallic acid enriched pomegranate peel extract

- Pomegranate peel was dried using hot air oven and powdered.
- 0.6g of the powdered peel in 100ml of 4.5pH of Citrate buffer was extracted with Viscozyme enzyme (Novozymes) along with Bioenzyme at 25°C and 24h in an incubator shaker.
- The Total polyphenolics contents (TFC) and Total Flavonoid content (TFC) of the extract was estimated using Folin- Ciocalteu spectrophotometric and Aluminium Chloride spectrophotometric methods. (Kharchoufi et al., 2018)

In vitro testing of antimicrobial activity of the pomegranate peel extract

The antimicrobial activity of the extract was evaluated using the following methods

- **Agar well diffusion**: Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. The agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm was punched and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is added into the well. Then, agar plates were incubated at different temperature with respect to the microorganism used. (Chan et al., 2018)
- **Broth microdilution and MIC determination**: Broth microdilution was performed at different dilutions of Gallic acid to determine the Minimum Inhibitory Concentration (MIC) effective in killing 50% of the microorganism. (Kharchoufi et al., 2018)

- **Cell viability assay by MTT**: It is a rapid colorimetric assay based on the cleavage of the tetrazolium ring of MTT (3-(4,5-dimethylthazolk-2-yl)-2,5-diphenyl tetrazolium bromide) by dehydrogenases in active mitochondria of living cells as an estimate of viable cell number. (Kumar et al., 2018)

- **Scanning electronic microscopic analysis**: The Scanning electron microscope works on the principle of applying kinetic energy to produce signals on the interaction of the electrons. SEM analysis is used to characterize the surface features and evaluate the morphological changes. (El-Kady et al., 2021)

Results:

Agar well diffusion

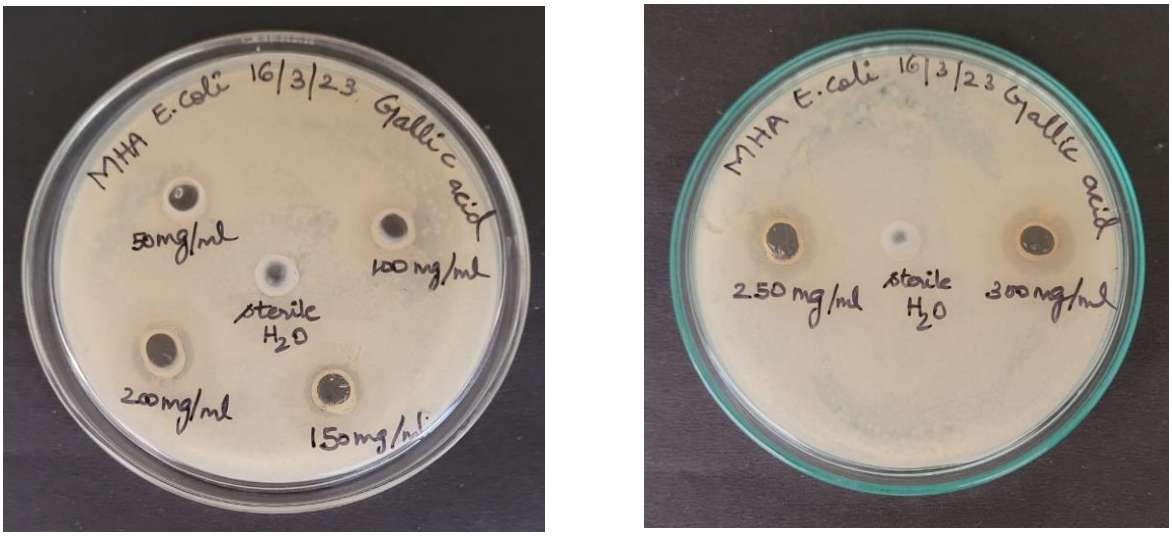


Figure: Antimicrobial activity of Gallic acid on *E.coli.* with different concentrations

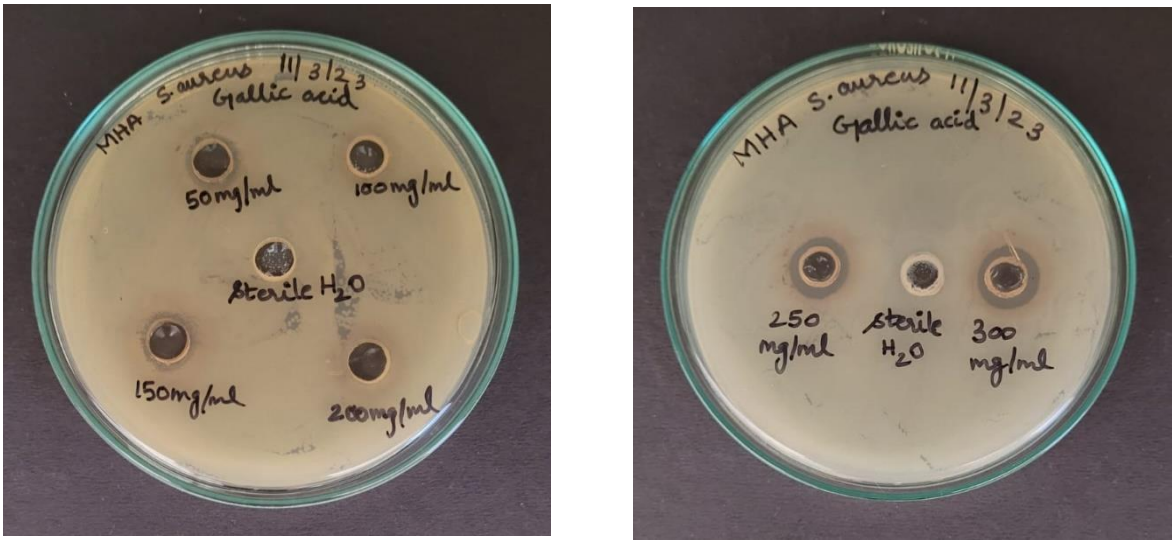


Figure: Antimicrobial activity of Gallic acid on *S.aureus* with different concentrations

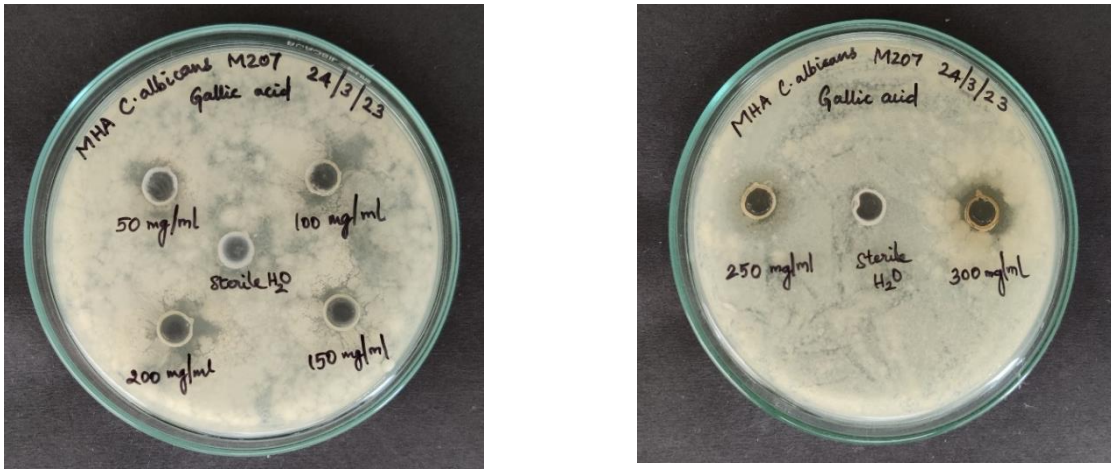


Figure: Antimicrobial activity of Gallic acid on *C.albicans* M207 with different concentrations

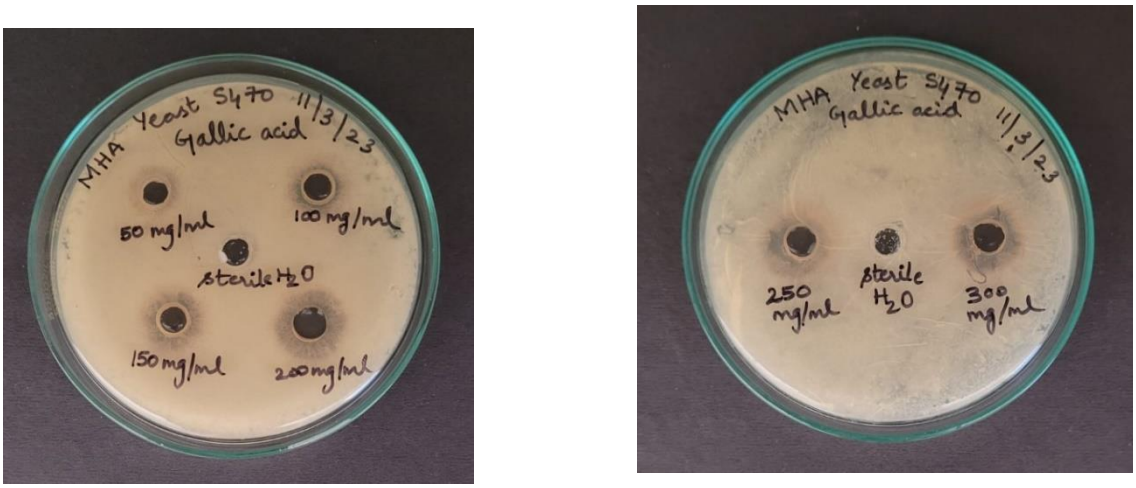


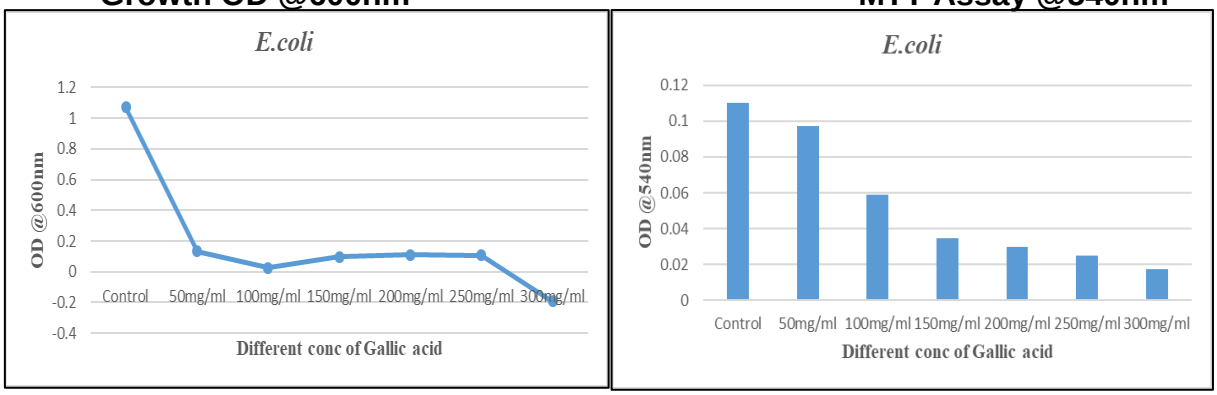
Figure: Antimicrobial activity of Gallic acid on *C.albicans* S470 with different concentrations

Table: Zone of inhibition for Gallic acid by agar well diffusion

Zone of inhibition (in cm)						
Microorganisms	**50mg/ml**	**100mg/ml**	**150mg/ml**	**200mg/ml**	**250mg/ml**	**300mg/ml**
E.coli	**0.6**	**0.7**	**0.7**	**0.7**	**1**	**1**
S.aureus	**0.5**	**0.6**	**6**	**0.8**	**0.8**	**0.8**
C.albicans M207	**0.6**	**0.6**	**0.6**	**0.6**	**0.7**	**1**
C.albicans S470	**0.7**	**0.7**	**0.8**	**0.9**	**1**	**1**

Broth Microdilution Results of Gallic acid:

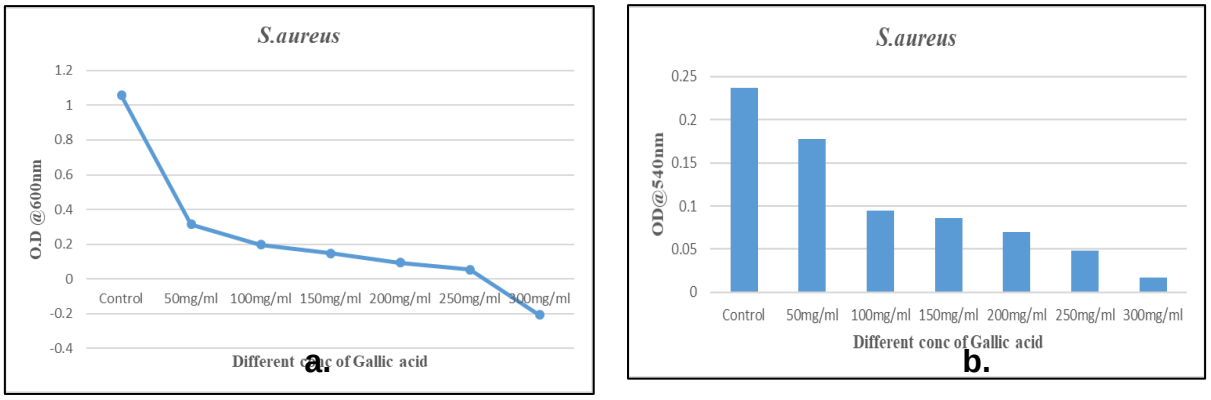
Growth OD @600nm **MTT Assay @540nm**



a. **b.**

Figure: Broth Microdilution of *E.coli* treated with different concentrations of Gallic acid

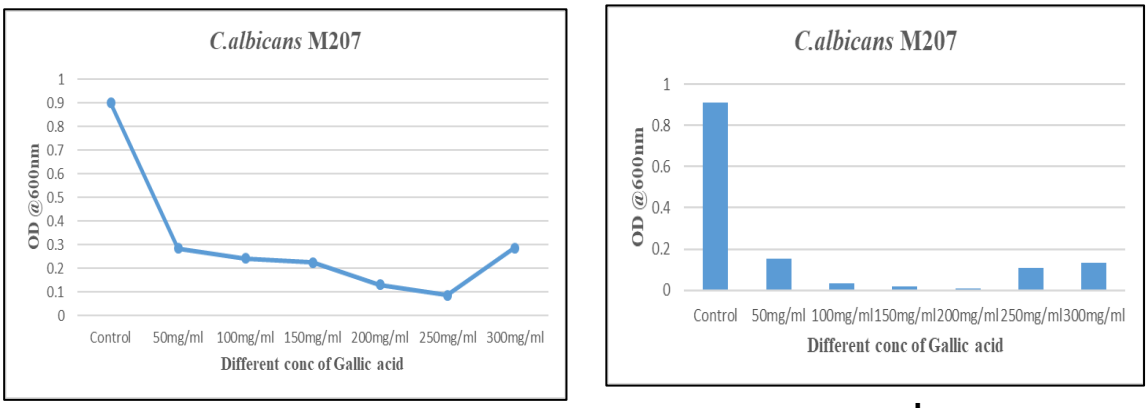
a) Growth OD @600nm b) MTT assay @540nm



a. **b.**

Figure: Broth Microdilution of *S.aureus* treated with different concentrations of Gallic acid

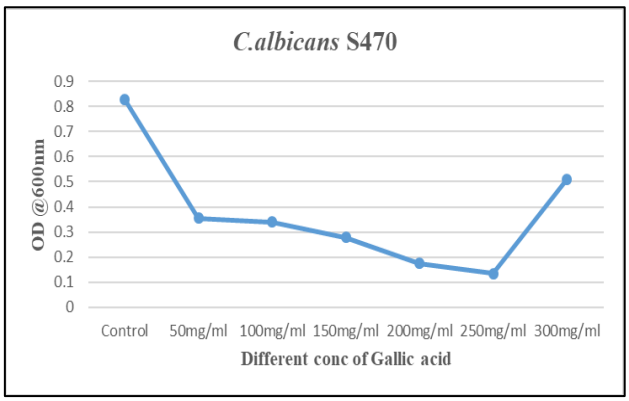
a) Growth OD @600nm b) MTT assay @540nm

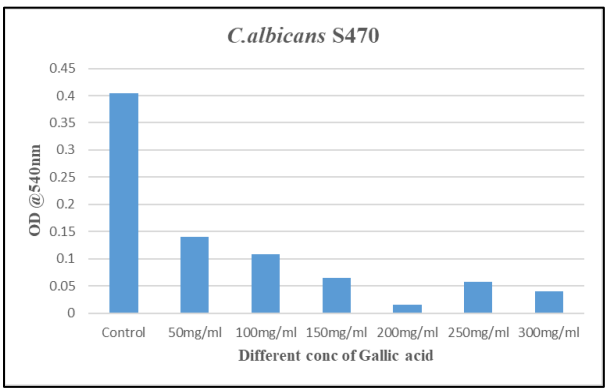


a. **b.**

Figure: Broth Microdilution of *C.albicans* M207 treated with different concentrations of Gallic acid

a) Growth OD @600nm b) MTT assay @540nm



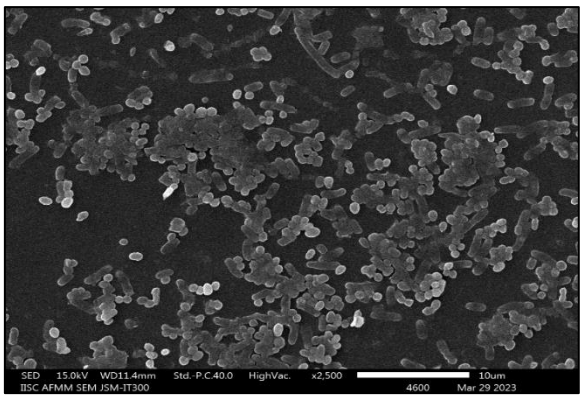


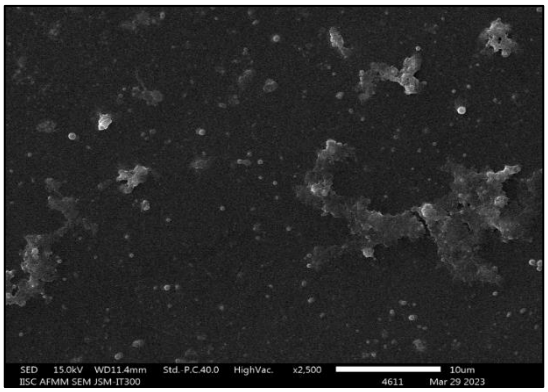
a. **b.**

Figure: Broth Microdilution of *C.albicans* S470 treated with different concentrations of Gallic acid

a) Growth OD @600nm b) MTT assay @540nm

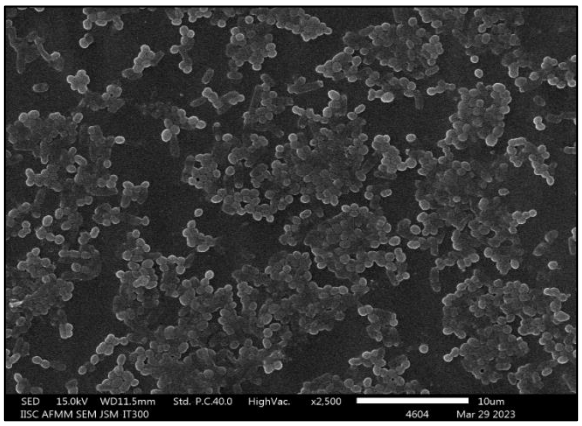
SEM Images:

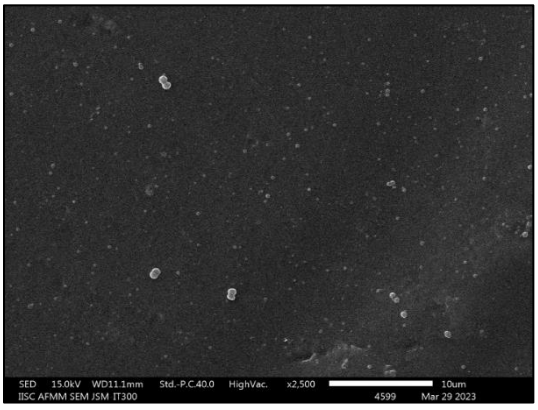




a. **b.**

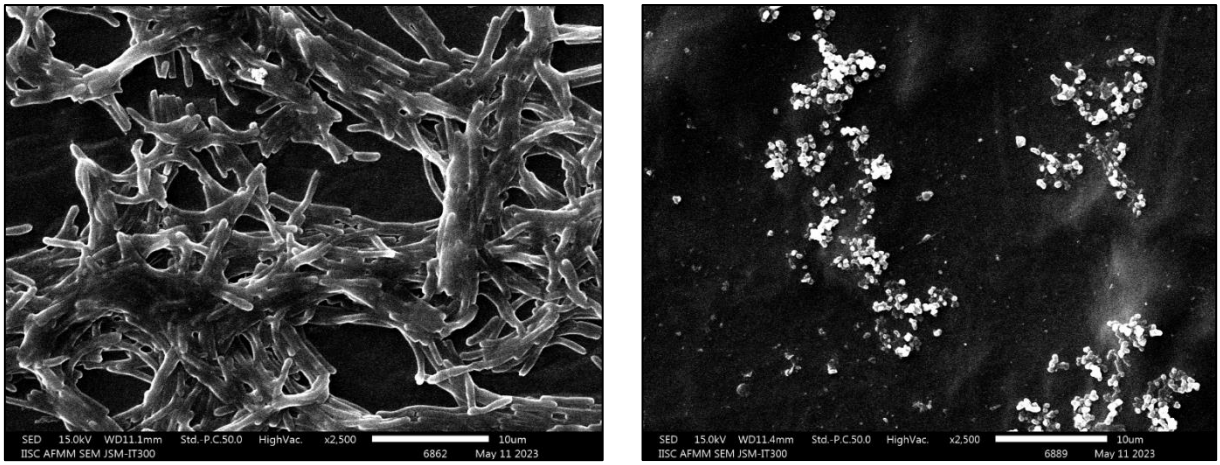
Figure: SEM images of *E.coli* treated with Gallic acid at 250mg/ml concentration, observed at 2500X magnification. a) *E.coli* Control b) *E.coli* Treated with Gallic acid





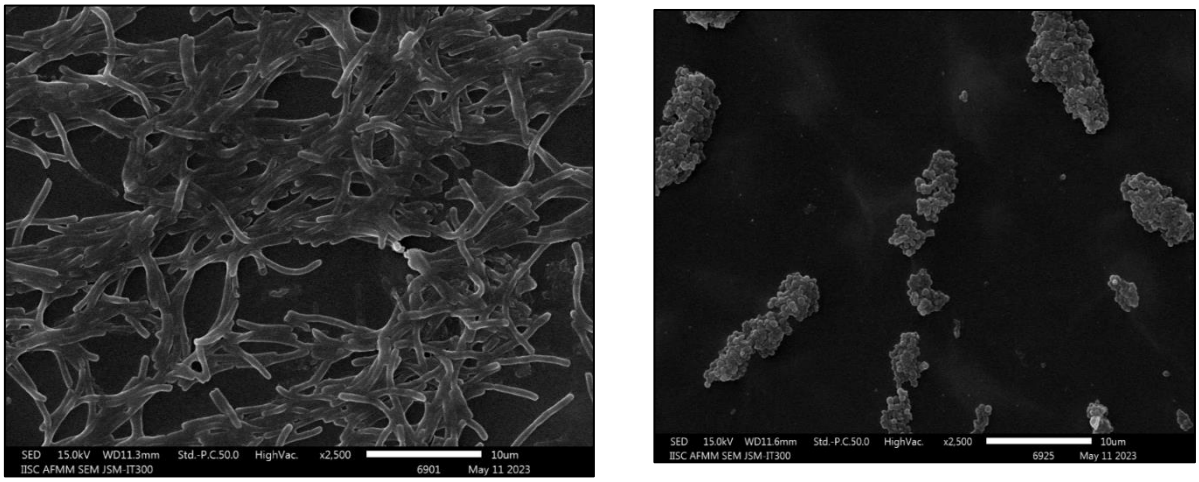
a. **b.**

Figure: SEM images of *S.aureus* treated with Gallic acid at 250mg/ml concentration, observed at 2500X magnification. a) *S.aureus* Control b) *S.aureus* Treated with Gallic acid



a. **b.**

Figure: SEM images of *C.albicans* M207 treated with Gallic acid at 200mg/ml concentration, observed at 2500X magnification. a) *C.albicans* M207 Control b) *C.albicans* M207 Treated with Gallic acid



a. **b.**

Figure: SEM images of *C.albicans* S470 treated with Gallic acid at 200mg/ml concentration, observed at 2500X magnification. a) *C.albicans* S470 Control b) *C.albicans* S470 Treated with Gallic acid

Conclusions:

- Pomegranate peels are economical and effective source of high value polyphenolics.
- Gallic acid enriched extract have excellent antimicrobial activity against pathogenic high biofilm forming drug resistant bacteria and yeast.
- The extract showed a dose dependent antimicrobial activity against the tested bacteria and yeast.
- The results for agar well diffusion were corroborated with broth micro dilution experiments indicating dose dependent response.

- SEM images reveal that Gallic acid effectively inhibits *C.albicans* biofilm and triggers a morphological change from biofilm to yeast mode.

Scope for future work:

- Through this study we have identified that the polyphenolics present in the pomegranate extract exhibit antimicrobial activity against the pathogenic bacteria *S. aureus* and *E. coli* and the fungus *C. albicans*.

- Polyphenolics enriched pomegranate extract possessing antimicrobial property have potential application in functional foods, pharmaceutical and cosmetic preparations.