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STUDIES ON ANTIMICROBIAL ACTIVITY BY EXTRACELLULAR AND INTRACELLULAR YEAST PROTEINS

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ABSTRACT

In the present study yeasts have been isolated from different sources like juice samples and soil. The antimicrobial activity for each of the isolate was checked against *B.subtilis, Pseudomonas, E.coli, Klebseilla, Salmonella, Serratia, Staphyloccous* and *Streptococcus.* The Isolates showed antimicrobial activity against most of the pathogens and had a greater zone of inhibition. *Klebseilla, P.aeuriginosa and Salmonella* was inhibited by all the isolates proving that *Saccharomyces cerevisiae* can be used as of the antimicrobial agent against those microorganisms. The proteins were purified from the isolates which showed better inhibition activity and they were checked whether the proteins were produced extracellular or intracellular. It was found that both intracellular and extracellular proteins had a greater impact on the inhibition of the microorganisms and the phosphate buffer A and ammonium sulphate precipitation method for the purification of the protein had an improved inhibition when compared to the other methods used to purify the proteins. The SDS-PAGE was carried out for the protein from the isolates and the intracellular proteins and extracellular proteins showed a wide range of proteins with different molecular weights.

KEYWORDS: *S.cerevasiea*, Antimicrobial activity, Proteins, SDS-PAGE.

INTRODUCTION

Saccharomyces cerevisiae is a eukaryotic microorganism classified in the kingdom Fungi, with 1,500 species currently reported.[1] Yeasts are unicellular and its size can be varying depending on the species.[2] Many different types of yeasts are currently used for making foods: baker's yeast in bread production; brewer's yeast in beer fermentation; yeast in wine fermentation and also for xylitol production. Yeasts are present in the gut flora of mammals and some insects.[3] Yeast is most widely used as model organisms for genetics and cell biology.[4] Yeast has potential to bioremediate toxic pollutants like arsenic from industrial effluent. The antimicrobial proteins play a very important in many living organisms like Insects, Fish, Plants and Mammals.[5,6,7] *Saccharomyces cerevisiae* shows inhibitor properties so yeast have been used for food preservation and resists undesired microorganisms during the production of beer and wine.[8]

MATERIALS AND METHODS

Isolation and culture media

The Isolation of the yeast was done from different soil sources and fermented juice products by serial dilution method. Samples were introduced into YPD and YM broth media and incubated at 28^0C for 24 hr. The samples were diluted with distilled water and then 0.1ml of dilutes were plated onto YPD agar media. After incubation at 28^0C, the appearing yeast colonies were purified. The pure cultures were maintained and subcultured on media including yeast extract peptone dextrose (YPD) broth medium (glucose, 10; yeast extract, 3; peptone, 3 g/L), yeast extract peptone dextrose (YPD) agar medium (YPD broth + 15 g agar). pH of the media was adjusted at 4.5. *Saccharomyces cerevisiae* were identification according to.[9-12]

Biochemical Identification of Yeast

The API 20 C AUX yeast identification system (BioMerieux Vitek, Inc., Hazelwood, Mo) was used for biochemical identification of the yeast isolates.

Antimicrobial Activity

The potential of the isolates to effect the growth of pathogenic microorganisms was determined by Agar diffusion method. The pathogenic microorganisms tested for antimicrobial activity were *B.subtilis, Pseudomonas, E.coli, Klebseilla, Salmonella, Serratia, Staphyloccous* and *Streptococcus*.

Protein Purification

The isolates showing antimicrobial activity which was determined by better zone of inhibition against most of the pathogens were selected and cultured on YPD broth and incubated for 24 hours at room temperature. The pure culture was prepared on slants and refrigerated for further investigation. The samples were then inoculated in broth for isolation of the proteins. The broth after incubation was centrifuged at 10,000rpm for 20min to separate the cells and the extracellular components. The cells were in the pellet whereas the extracellular components were in the supernatant after centrifugation. The cells and even the extracellular components were used for the further investigation to determine the proteins having the potential of antimicrobial activity were extracellular or intracellular.

The proteins were purified from the cells and the extracellular broth with different methods wherein they the cells in the pellet were lysed with Hepes buffer to breakdown the membrane and release out the components. The obtained extracellular components were directly used for the investigation. The extraction and purification of both the intracellular and extracellular proteins were carried out by phosphate buffer A, Phosphate buffer B and 50 % ammonium sulphate precipitation. The samples were dialysed in the buffer and then they were checked for antimicrobial activity to determine if the proteins were extracellular or intracellular.

Purification of Intracellular Enzyme

Ammonium Sulphate Precipitation

The different steps of protein purification were carried out at 4ºC. The precipitation of the proteins was performed according to the chart of Gomori 1955.

Dialysis

The resultant ammonium sulphate precipitate (in solution) was introduced to a special plastic bag called the dialysis tube. Dialysis was carried out to clear all the traces of the ammonium sulphate. Then 1ml of the sample was loaded in the dialysis tube and was kept in an upside-down position in a 500 ml beaker containing. And it was kept on a magnetic stirrer for 24h at room temperature and after every 6h the Tris HCl buffer in the beaker was changed.

SDS PAGE of Protein Extract

The Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed according to Laemmli (1970).

RESULTS AND DISCUSSION

Isolation of the Microorganisms: A total of 11 isolates were isolated from 12 different sources. These isolates were cultured and maintained on YPD agar medium for further investigation. The isolates were mainly obtained from juice samples and soil.

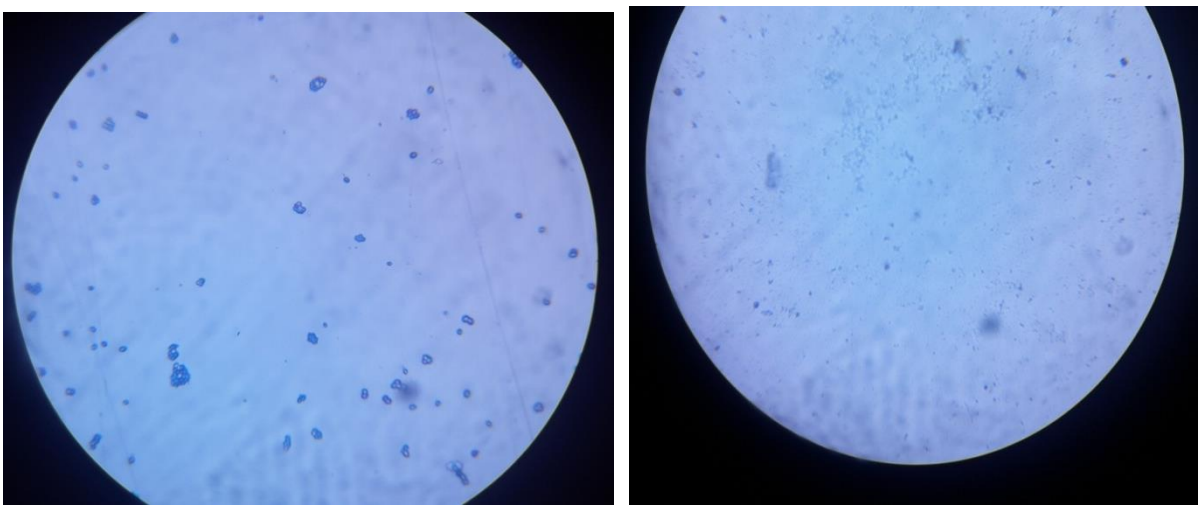

Fig. 1: Isolation of the Microorganisms.

Antimicrobial Activity

The antimicrobial activity for each of the isolate was checked against *B.subtilis, Pseudomonas, E.coli, Klebseilla, Salmonella, Serratia, Staphyloccous* and *Streptococcus.* The Isolates showed antimicrobial activity against most of the pathogens and had a greater zone of inhibition. *Klebseilla, Salmonella, P.aeuriginosa* was inhibited by all the isolates proving that *S.cerevasiea* can be used as of the antimicrobial agent against those microorganisms.

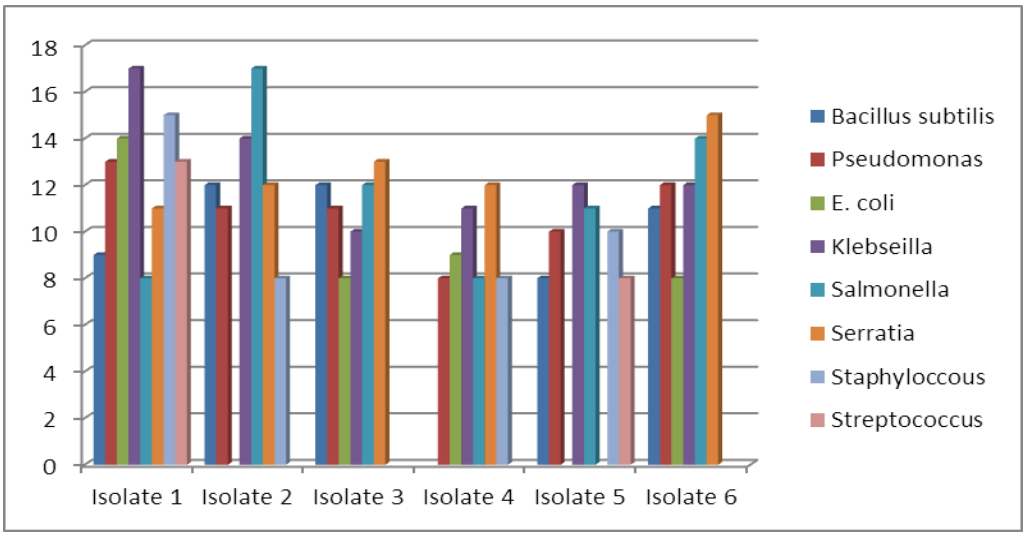

Fig.2: Antimicrobial Activity.

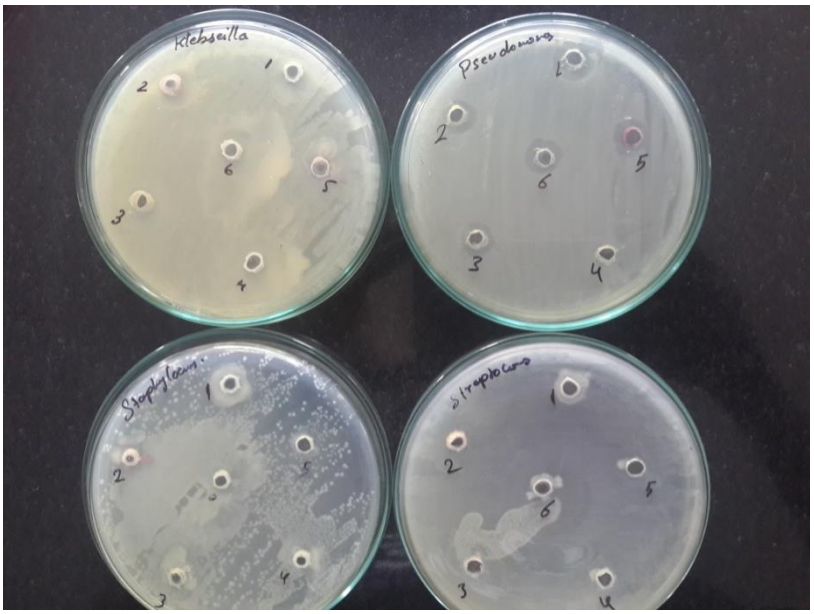

Fig. 3: Zone Of Inhibition.

Protein Purification and Antimicrobial Activity

All Isolates were selected for further investigation as they showed zone of inhibition against most of the pathogenic microorganisms. The intracellular and extracellular proteins were purified by 3 different ways and were checked for the antimicrobial activity to determine the proteins which play a role in reduction of the growth of the pathogens.

Intracellular Proteins

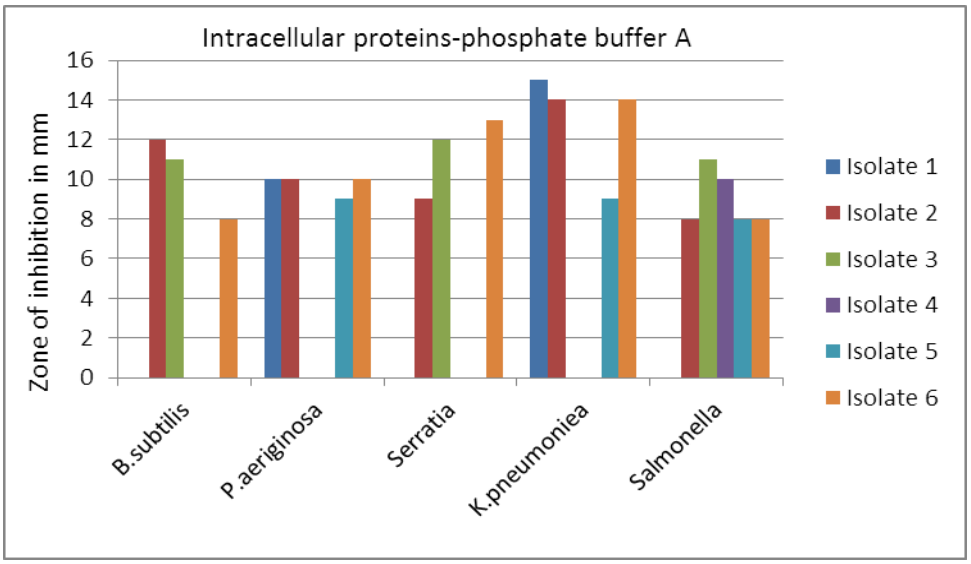

Fig. 4: Zone of inhibition of Intracellular proteins with phosphate buffer A.

The intracellular proteins from all the isolates showed activity against *Salmonella* when eluted by phosphate buffer A.

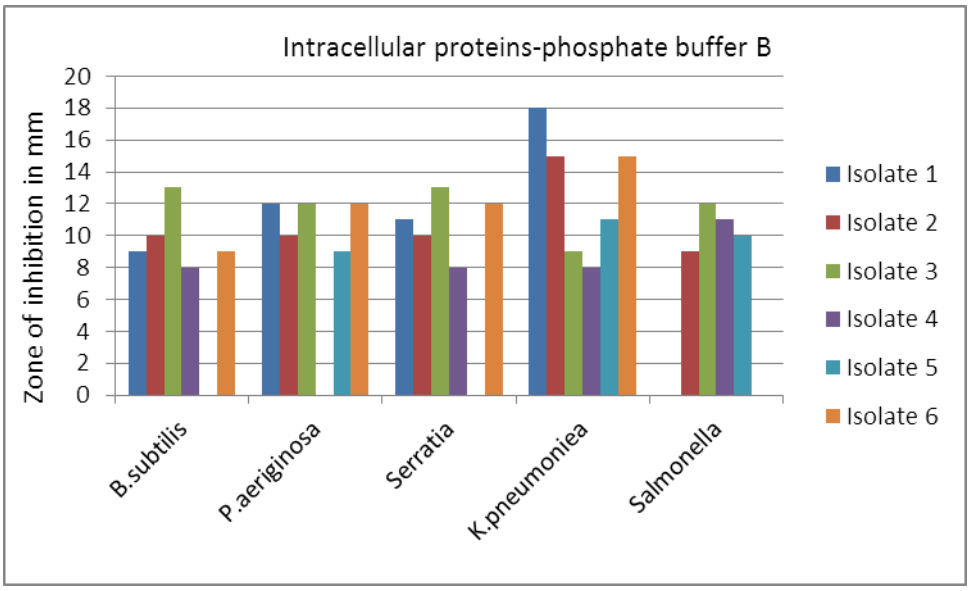

Fig. 5: Zone of inhibition of Intracellular proteins with phosphate buffer B.

K.pneumoniea was majorly affected by all the isolates when purified with phosphate buffer B. *Salmonella* showed the least antimicrobial activity.

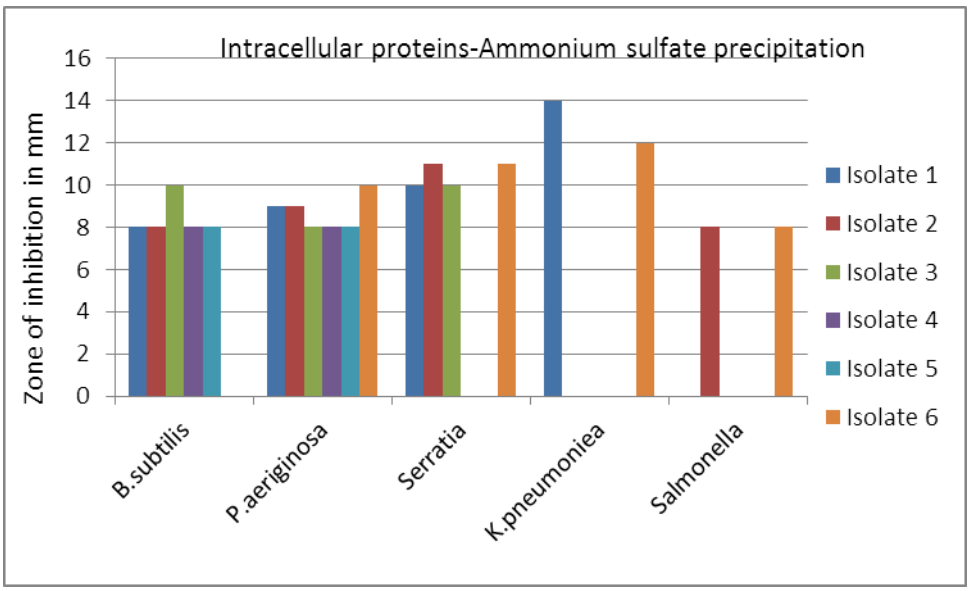

Fig. 6: Zone of inhibition of Intracellular proteins with Ammonium sulfate precipitation.

The ammonium sulphate method showed better antimicrobial activity against *B.subtilis* and *P.aeriginosa*. The intracellular proteins did not show much of the activity against *K.pneumoniea* and *Salmonella*.

Extracellular Proteins

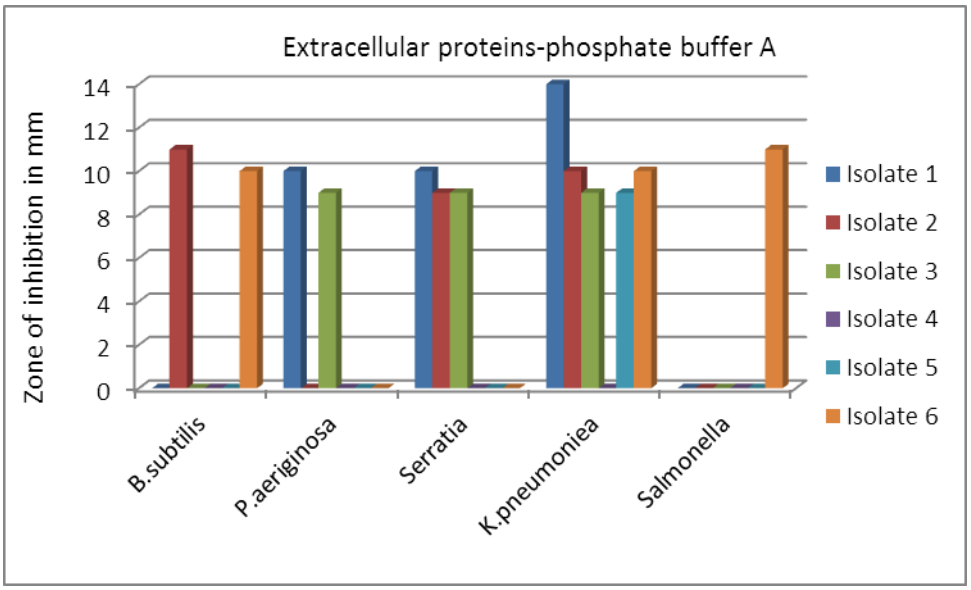

Fig. 7: Zone of inhibition of Extracellular proteins with phosphate buffer A.

The extracellular proteins eluted using phosphate buffer A had higher antimicrobial activity against *K.pneumoniea* and isolate 1 showed maximum inhibition with a zone of 14mm.

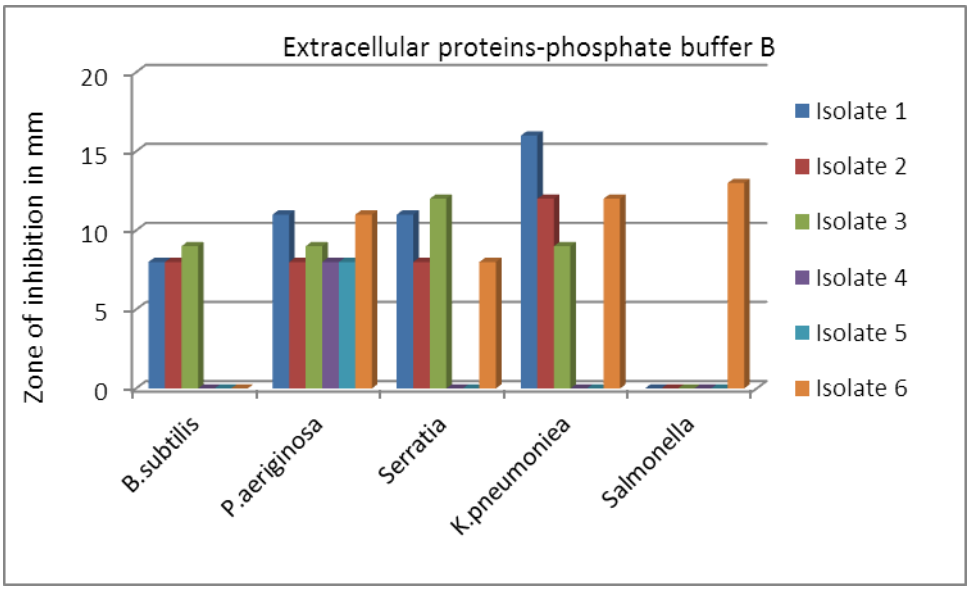

Fig. 8: Zone of inhibition of Extracellular proteins with phosphate buffer B.

Isolate 1 showed a higher zone of inhibition against *K.pneumoniea* when eluted using phosphate buffer B. the extracellular proteins eluted with buffer B did not show much activity against *Salmonella*.

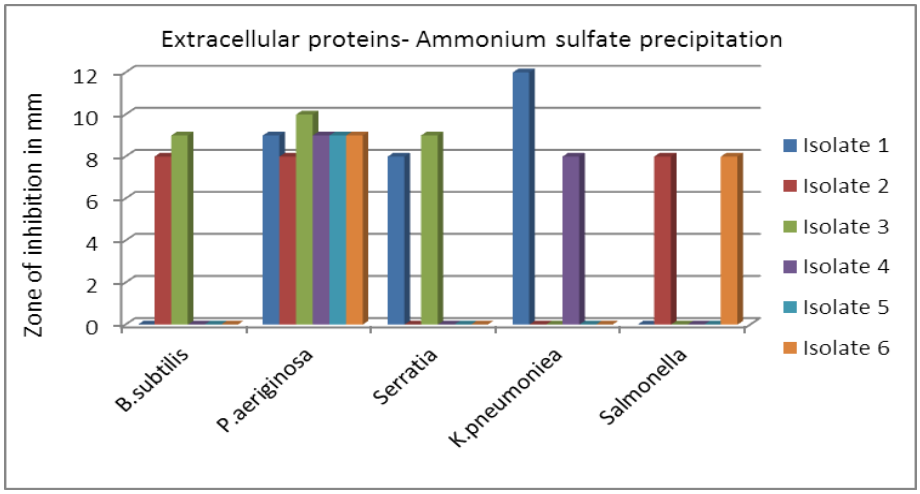

Fig. 9: Zone of inhibition of Extracellular proteins with Ammonium sulfate precipitation.

The extracellular proteins extracted using ammonium sulphate precipitation had a greater antimicrobial activity against *P.aeriginosa*. Isolate 1 showed higher zone of inhibition against *Klebsiella pneumoniea* when the extracellular proteins were precipitated with Ammonium sulphate.

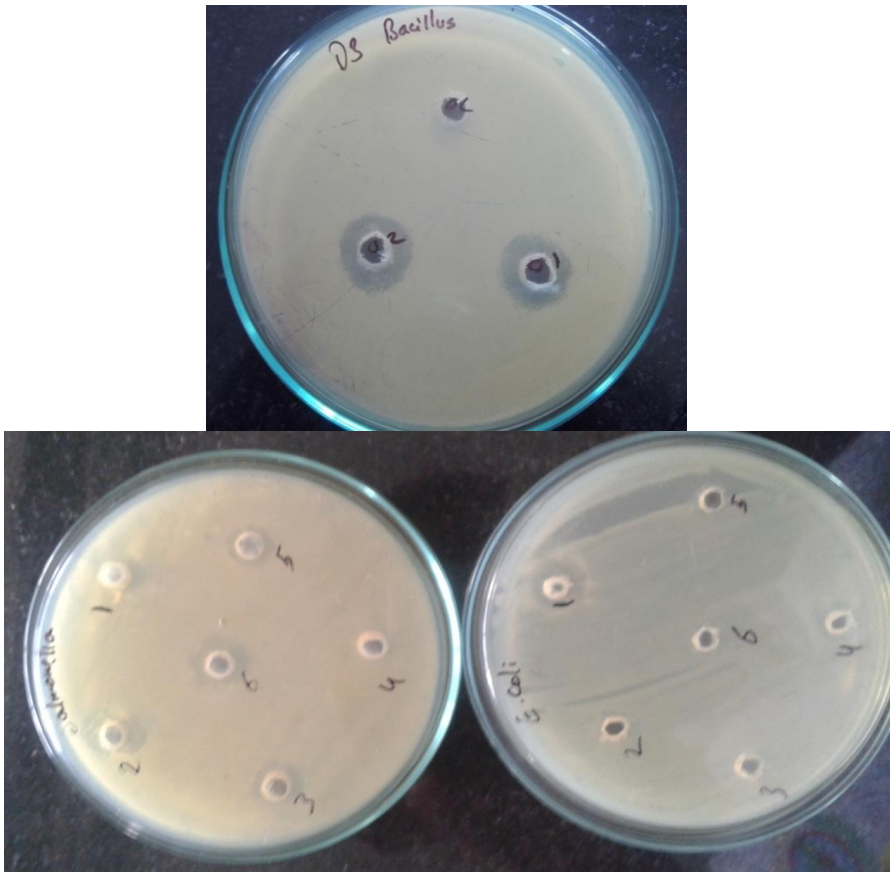

Fig. 10: Zone of inhibition against *Klebsiella pneumoniea* of the extracellular proteins precipitated with Ammonium sulphate.

SDS-PAGE

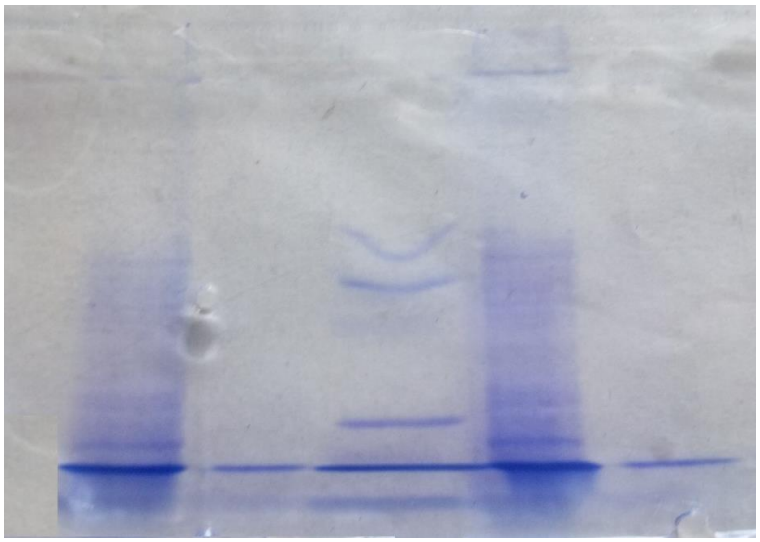

Fig. 11: SDS-PAGE for the protein from the isolates and the intracellular proteins and extracellular proteins.

The SDS-PAGE was carried out for the protein from the isolates and the intracellular proteins and extracellular proteins showed a wide range of proteins with different molecular weights.

CONCLUSION

Based upon our results we conclude that antimicrobial activity for each of the isolate was checked against *B.subtilis, Pseudomonas, E.coli, Klebseilla, Salmonella, Serratia, Staphyloccous* and *Streptococcus.* The isolates shown antimicrobial activity against most of the pathogens and had a greater zone of inhibition. *Klebseilla, Salmonella, P.aeuriginosa* was inhibited by all the isolates proving that *S.cerevasiea* can be used as of the antimicrobial agent against those microorganisms and isolate 1 showed higher zone of inhibition against *K.pneumoniea* when the extracellular proteins eluted using phosphate buffer A and B and also shown higher zone of inhibition against *Klebsiella pneumoniea* when the extracellular proteins were precipitated with Ammonium sulphate. The SDS-PAGE was carried out for the protein from the isolates and the intracellular proteins and extracellular proteins showed a wide range of proteins with different molecular weights. These proteins may be the reason for the antimicrobial activity against the pathogens.

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