RESEARCH ARTICLE OPEN ACCESS

Antimicrobial Activity of Fermented Citrus Fruit Peel Extract

Geetha Saramanda[1] and Jyothi Kaparapu[2]
[1]*Department of Microbiology,Andhra University,Visakhapatnam*
[2]*Department of Botany,Andhra University,Visakhapatnam*
Corresponding author:jyothikaparapu@gmail.com

ABSTRACT:
Wastes generated from fruits and vegetables are organic in nature and contribute a major share in soil and water pollution. Also, green house gas emission caused by fruit and vegetable wastes is a matter of serious environmental concern. The aim of the present work is to evaluate the antimicrobial potency of fermented Citrus fruit peels extract.The antimicrobial activity was done by agar well diffusion assay against five bacteria and three fungi. The citrus peel extracts showed highest zone of inhibition against pathogens, compared with the control Chloramphenicol and Griseofulvin used. Citrus peel extract showed good antimicrobial activity indicating its potency as a promising source of natural antimicrobials. As microorganism are becoming resistant to present day antibiotics, our study focuses on antimicrobial activity and future prophylactic potential of the lemon peel.

Key words: Antimicrobials, Bacteria, Citrus, Fungi and Pollution

---

Date of Submission: 16-11-2017 Date of acceptance: 30-11-2017

I. INTRODUCTION

Decomposable waste such as fruits, vegetables and its peels in huge quantities are produced by food processing industries, vegetable markets and restaurants. Globally, management of these organic waste is a major issue. Greenhouse gases like methane and nitrous oxides were produced by the disposal of these decomposable wastes either in the landfill or by composting. The decomposable waste which is thrown into the environment can be used to produce value added bio-product which in turn reduces the production of greenhouse gas from it. One of such products was developed by researcher Dr. Rosukon from Thailand using organic solid waste in the year 2006 and named as garbage enzyme. This enzyme is a complex organic substance of protein chains (enzyme), organic acids and mineral salts produced by fermentation of waste fruits, vegetables or its peels, sugar and water. The garbage enzyme functions similarly to enzymes in achieving a high degree of degradation within a shorter time.

In garbage enzyme the sugar is used frequently as a substrate in fermentation processes, in the production of lactic acid, polyhydroxybutyrate, ethanol, pullan, xanthan gum and molasses has been widely used as a substrate in fermentation processes (Bae et al. 2004). The proponents of the garbage enzyme describes it as a complex organic substances of protein chains, mineral salts and juvenile hormones and also claim that it functions to decompose, transform as well as catalyze reactions. It is also claimed that the garbage enzyme functions differently in different concentrations (Arun et al.,2015)

The main objective is extraction, identification of antimicrobial compounds and demonstration of antimicrobial activity of fermented lemon (***Citrus lemon L***.) peel against bacteria. Biologically active compounds present in the medicinal plants have always been of great interest to scientists. The peel of citrus fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants. These compounds, not only play an important physiological and ecological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries. The citrus peel oils show strong antimicrobial activity.

The prime objective of this study is to analyze the biocatalytic activity of garbage enzyme produced by fermentation of lemon fruit peels, jaggery and water. Successively antimicrobial potential of garbage enzymes on five major pathogenic microorganisms *E. coli , Staphylococcus aureus , Sterptococcus pyogens, Salmonella typhi* and *Pseudomonas aeruginosa* were the bacterial strains *; Aspergillus niger, Fusarium* and *Cladosporium* sps. were studied.

II. MATERIALS AND METHODS

Preparation of garbage enzyme:

A large batch of garbage enzyme had been produced for this study, from the methodology and recipes published by Dr.Rosukan *et al*. for production of 5 liters of garbage enzyme extract, 1 kg of jaggery, 3kg of sweet lemon peel and 5 liters of water were mixed well in air-tight containers (Joeanoon, 2008). The container was placed in a cool, dry and well ventilated area for complete degradation of organic matter. The fermentation was conducted for three months. After three months the solution was filtered and characteristics of pure garbage enzyme solution were analyzed (Fig.1). Lowry protein assay (Lowry et al., 1951) was used for quantitative determination of protein concentration in garbage enzyme.

Fig.1. Production of garbage enzyme

Medium preparation

28.0 g nutrient agar (NA-Himedia) medium for bacteria and 39.0 g potato dextrose agar (PDA-Himedia) medium for fungiwere taken in two different conical flask containing 1000 ml distilled water. The conical flask with medium, petri plates are sterilized using autoclave at 15 lb pressures for 15 min. Well mixed sterilized medium in conical flask was poured into sterile petri plates in sterile conditions and then petri plates were stored in the incubator.

Antimicrobial assay

The antimicrobial potential of garbage enzyme was investigated to confirm the pathogen killing property of garbage enzyme. Antimicrobial assay was done by agar well diffusion method using garbage enzymes (5, 10and 15%). Petri plates were prepared by pouring 30 ml of NA or PDA medium for bacteria or fungi respectively. The test organism was inoculated on a solidified agar plate. The surfaces of medium were inoculated with bacteria/fungi. *E. coli* (Gram negative), *Staphylococcus aureus* (Gram positive), *Sterptococcus pyogens* (Gram positive), *Salmonella typhi (*Gram negative) and *Pseudomonas aeruginosa* (gram negative) were the bacterial strains ; *Aspergillus niger*, *Fusarium* and *Cladosporium* were the fungal strains used as test microorganisms in this study and these were obtained from the P.G microbiology Dept.Visakha Government DegreeCollege for Women,Visakhapatnam.

Inoculums containing *E. coli, S. aureus* , *Sterptococcus pyogens, Salmonella typhi* and *Pseudomonas aeruginosa* were spread on nutrient agar plates and *Aspergillus niger, Fusarium* and *Cladosporium* sp. were spread on potato dextrose agar. Using a sterile cup-borer, wells of diameter of 17.78mm were made and labeled on the back of the plate. To this, 15µl of each samples was dispensed in respective labeled wells. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature for 48 h for fungi strains. Each sample was tested in triplicate. The antibacterial activity of each extract was expressed in terms of mean of diameter, for the zone of inhibition (mm) formed by addition of each extract.

III. RESULTS AND DISCUSSION

Garbage enzyme was prepared using lemon peel, jaggery and water in airtight containers. After three months, the solution was filtered and separated from solid residues. The solution obtained was centrifuged for 30 min with 3000 rpm. The supernatant was used as the garbage enzyme source. During fermentation, carbohydrates were converted into volatile acids and in addition, organic acids present in waste material also leached out into fermented solution since the pH of garbage enzyme was acidic in nature. Nazim and Meera (2013) produced garbage enzyme using the simple fermentation of fresh vegetable waste, brown sugar and water for two months .

The antimicrobial activity of garbage enzyme on bacteria and fungi are shown in Tables 1 and 2 respectively. When compared with positive control standards, the bacterial and fungal zone of inhibition for 150 µl of garbage enzyme solution is higher. It was observed that when using 150 µl of 15% garbage enzyme solution (pH 3.6), zone of inhibition for *E. coli* , *S. aureus* , *Sterptococcus pyogens*, *Salmonella typhi* and *Pseudomonas aeruginosa* are 11mm,10mm,10mm, 13 mm and 9mm Respectively. The zone of inhibition for fungi *Aspergillus niger*, *Fusarium sps* and *Cladosporium sps* are 21 mm, 22mm and 30mm respectively. This observation clearly reveals that garbage enzyme has the highest power to reduce or inhibit the pathogen because the acidic nature of garbage enzyme helps to extract extracellular enzymes from the organic waste materials into the solution during fermentation (Bhavani Prakash, 2011). These extra-cellular enzymes are likely responsible for the lytic action towards the pathogens which are commonly found in waste. (Straub et al., 1993). This observation confirms that garbage enzyme possesses pathogen killing/inhibiting property. Puupponen-Pimia et al., 2008, observed the enhanced antimicrobial activity of garbage enzyme by increasing the pH. The results obtained in this study identified that garbage enzyme has both biocatalytic and pathogen inhibiting property. Therefore it has the potential to enhance the stability of sludge by removing the solids and suppressing the activity of microbes in the sludge.

Table.1. Anti-microbial activity of fermented lemon peel extract on bacterial sps

Name of the organism	Zone of inhibition
E.coli	11mm
Staphylococcus	10mm
Streptococcus	10mm
Pseudomonas	13mm
Salmonella	9mm

Table.2. Anti-microbial activity of fermented lemon peel extract on bacterial sps

Name of the organism	Zone of inhibition
Aspergillus niger	21mm
Fusarium sps	22mm
Cladosporium sps	30mm

In fungal species, the maximum zone of inhibition was observed in *Cladosporium sps* and minimum zone of inhibition was observed in *Aspergillus.niger*. Compared to the bacterial and fungal species, the anti-microbial activity highly observed in fungal species. These are samples isolated from air and dust particles.

IV. CONCLUSION

The antimicrobial activity of garbage enzyme was found to be active on most of the clinically isolated microorganism and fungi, as compare with standard drugs. The present study justified the claimed uses of garbage enzyme in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the eco-friendly fermented citrus peel extract as the antimicrobial agents.

REFERENCES

[1]. Arun, C and Sivashanmugam P.. Investigation of biocatalytic potential of garbageenzyme and its influence on stabilization ofindustrial waste activated sludge. Process Safety and Environmental Protection 9 4 (2 0 1 5) 471–478

[2]. Bae, S., and Makoto, S. Bacterial cellulose production by fed-batch fermentation in molasses medium. Biotechnol Prog. Vol. 20, pp. 1366- 1371, 2004

[3]. Bhavani Prakash, 2011. How to make and use garbage enzyme. http://www.ecowalkthetalk.com (accessed 17.12.13).

[4]. Joean oon, 2008. http://veg4planet.blogspot.in/2008/07/garbage-enzyme-anti-greenhouse-effect.html (accessed 17.10.12).

[5]. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275

[6]. Nazim, F., Meera, V., 2013. Treatment of synthetic grey water using 5% and 10% garbage enzyme solution. Bonfring Int. J. Ind. Eng. Manage. Sci. 3 (4), 111–117.

[7]. Puupponen-Pimia, R., Nohynek, L., Ammann, S.,Oksman-Caldentey, K.M., Buchert, J., 2008. Enzyme-assisted processing increases

antimicrobial and antioxidant activity ofbilberry. J. Agric. Food Chem. 56,681–688.
[8]. Straub, T.M., Pepper, I.L., Gerba, C.P., 1993. Hazards frompathogenic micro-organisms in land-disposed sewage sludge.Rev. Environ. Contam. Toxicol. 132, 55–91.

International Journal of Engineering Research and Applications (IJERA) is **UGC approved** Journal with Sl. No. 4525, Journal no. 47088. Indexed in Cross Ref, Index Copernicus (ICV 80.82), NASA, Ads, Researcher Id Thomson Reuters, DOAJ.

Geetha Saramanda Antimicrobial Activity of Fermented Citrus Fruit Peel Extract." International Journal of Engineering Research and Applications (IJERA) , vol. 7, no. 11, 2017, pp. 25-28.