World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

DETERMINATION OF MULTIENZYME AND ANTIBACTERIAL EFFICACY OF FERMENTATION BROTH FROM PINEAPPLE WASTES

***Barate D. L. and Gite P. G.**

Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola, India.

Corresponding Author: Barate D. L.
Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola, India.

Article Received on 22/06/2022 **Article Revised on 12/07/2022** **Article Accepted on 02/08/2022**

ABSTRACT

Waste utilization in fruits and vegetable processing industries is one of the important and challengeable jobs around the world. It is anticipated that the discarded fruits as well as its waste material could be utilized for future purposes viz. Fermentation. In the present study the pineapple waste consisting of pulp, crown leaves and peels were used. This study is carried out for analysis of multienzyme and antibacterial efficacy of fermented broth prepared by using *Saccharomyces cerevisae, Aspergillus niger* and *Bcaillus subtilis.* After fermentation enzyme activity was detected qualitatively by agar plate method for amylase, protease, lipase and cellulase activity and antimicrobial efficacy against *S. aureus, Enterococcus spp., Bacillus spp., E. coli and Klebsiella pneumoniae* were analyzed. The results showed presence of varied multienzyme activity and also susceptibility pattern of isolates toward the multienzyme preparation.

KEYWORDS: Fruits, Pineapple, Enzyme, multienzyme, antibacterial, Fermentation.

INTRODUCTION

Pineapple (*Ananas comosus* L. Merr.) is a tropical fruit belonging to the family of Bromeliaceae (Ayala-Zavala *et al.*, 2010) having a short stem and slender hard leaves that grow to medium to large-sized fruit. It is the third most important tropical fruit produced globally after bananas and mangos (Piana *et al.*, 2005; Jovanovic *et al.*, 2018). The processing of fruits results in the generation of big quantum of waste in the form of peel, pomace, stones and seeds. Such wastes are generally rich in bio-constituents such as fiber, pectin, cellulose, starch, phenolics, pigments and other useful materials. Biological conversion of such wastes into enzymes through solid-state fermentation have been worked out extensively (Raveendran *et al.*, 2018).There are reports of fermentative production of multienzyme using fruit peels of mango, pomegranate, apple, banana, orange, etc (Li *et al.*, 2015; Sagar *et al.*, 2018).

Recycling pineapple wastes by using pineapple waste as potential lead to discover new drugs to control some food poisoning and other infectious bacteria is a promising aim. Antimicrobial properties of various extracts from pineapple peel have recently been of great interest because of their possible use as natural additives emerged from a growing tendency to replace synthetic antimicrobials with natural ones (Lubiana *et. al.*, 2019).

The antibacterial activity revealed by the pineapple extracts might be due to presence of polyphenols, flavonoids, saponins and other secondary metabolites present in the extract. Flavonoids and polyphenols are more potent in inhibiting gram-positive bacteria. Both are phenolic compounds which have polar properties mostly work in the peptidoglycan layer in gram-positive bacteria rather than the non-polar lipid layer. Most plant phenolic compounds are non-toxic for human consumption therefore they could be used to prevent growth of many food-borne and food spoilage microorganisms. These phenols have the ability to denature the protein and its lipophilic nature helps them to attract the lipid molecules contained in the cell membranes and damage the bacterial cell membrane (Maurer *et al.*, 2001).

Fruit markets, vegetable markets, restaurants, and food processing industries are the prime contributors to pre-consumer type waste such as vegetables, fruits, and its peels in large quantities (Arunand Sivashanmugam, 2015). Sixty percent of pre-consumer type waste produced by food processing industries is made up of organic matters (Arun and Sivashanmugam, 2015). At present, the management of such organic waste is a significant global issue. Value added bio-products can be produced from the organic waste which also reduce the emission of greenhouse gas when disposed to landfill (Arun and Sivashanmugam, 2015).

In 2006, a researcher from Thailand named Rosukun developed a solution form product using organic solid waste and named it garbage enzyme (Arun and Sivashanmugam, 2015). This enzyme is a composite organic substance made up of organic acids, protein chains (enzyme), and mineral salts produced by the fermentation of waste vegetables, fruits or its peels, sugar, and water. The garbage enzyme can be applied to compose, decompose, transform, and catalyze (Selvakumar and Sivashanmugam, 2017). Besides, this enzyme solution works in the same way as commercial hydrolytic enzyme as it is able to achieve a high level of degradation within a short period. Thus, it can be utilized as a low-cost alternative to improve waste water treatment processes through the removal of impurities, harmful sludge, and bacteria, which in turn promotes recycling of waste back into the earth (Tang and Tong 2011). Thus the present study was put forth to study the multienzyme and antibacterial potential of fermentation broth from the pineapple fruit waste.

MATERIAL AND METHODS

Collection of sample

Pineapple waste was collected from local vegetable market and juice centres of Akola city. The waste consisting of pulp, crown leaves and peel was used. The samples were firstly washed with tap water and then with distilled water to remove any dust particles repeatedly. The sample was then homogenized and further used in experimental studies.

Production of multienzyme

The fermentation media was prepared by mixing (Pulp-100ml / Crown leaf-100 ml / Peel – 100g) substrate in Erlenmeyer flasks with D-glucose-1g; (NH2)2SO4 – 2.5g; KH_2PO_4 – 0.5g; $MgSO_4.7H_2O$ – 0.25g; NaCl – 0.05g; CaCl2 – 0.05g ; Distilled water - 500ml; pH – 5.5). The media was sterilized in autoclave at 121°C for 15 Lbs pressure prior to the fermentation process. Thereafter, it was cooled to room temperature. Then the culture of *Saccharomyces cerevisae*, *Aspergillus niger* and *Bacillus subtilis* was inoculated separately to the flask and kept for incubation for 30 days at room temperature for *Aspergillus niger*, *Saccharomyces cerevisae* and 37°C for *Bacillus subtilis*. After fermentation period, filtration and centrifugation process was done and filtrate was used for further process as crude multienzyme preparation. Protein content of Crude Multienzyme was determined by the Folin-Lowry method.

Screening of multienzyme activity of fermentation medium

(a) Qualitative Enzyme Assay for Amylase Activity

For amylase enzyme activity, agar-agar with 1% starch was prepared aseptically. With help of sterile cork borer, 5mm size wells were made in which 100µl of fermentation medium prepared from pineapple waste was inoculated then the plates were incubated for 24 hours at 37°C. Hydrolysis of starch was visualized as clear zones around the wells of plates against deep blue brown colour for starch by flooding with iodine solution (Emimol *et al.*, 2012). Diameter of the clear zone was measured and the activity level of the microorganisms was determined by the diameter of the clear zone formed.

(b) Qualitative Enzyme Assay for Cellulase Activity

The cellulase agar was prepared with 1% carboxyl methyl cellulose aseptically. With the help of sterile cork borer of 5mm size, wells were made in plates in which 100µl fermentation medium prepared from pineapple waste was inoculated in well and plates were incubated at 37°C for 24 hours - 48 hours, the plates were flooded with 0.3% congo red solution for 10 minutes.. Cellulase production is visualized by translucent zone around the well. Diameter of the translucent zone was measured and the activity level of the microorganisms was determined by the diameter of the translucent zone formed (Thirumurugan 2016).

(c) Qualitative Enzyme Assay for Protease Activity

The casein hydrolysis test was done by inoculation of the multienzyme to be tested on the agar plates containing 1% skimmed milk powder. With help of sterile cork borer of 5mm size wells, were made prepared in 100µl of fermentation medium prepared from pineapple waste was inoculated then the plates were then incubated at 37°C for 24 hours-48 hours. Formation of a clear zone was observed around the well and the diameter of the clear zone was measured. Diameter of the clear zone was measured and the activity level of the microorganisms was determined by the diameter of the clear zone formed (Sazci *et al.*, 1986).

(d) Qualitative Enzyme Assay for Lipase Activity

Egg yolk agar medium was prepared. With help of sterile 5mm cork borer, wells were made. The wells were labelled by the name of the sample to be inoculated. 100µl of each sample was added to well. The plates were at 37°C for 24 hours. After the incubation, the clear zone of hydrolysis was observed around well (Emimol 2012).

Screening of antibacterial potential of fermentation broth containing crude multienzyme

The crude extract of multienzyme was screened for its antimicrobial activity i.e. determination of zone of inhibition against tested organisms by agar well diffusion method as given by Balouiri *et al.*, (2016). According to CLSI 2012, 3- 4 fresh bacterial culture colonies were inoculated in nutrient broth and incubated for 4 hours then compared its turbidity standard 0.5 McFarland. The culture was inoculated on Muller Hinton Agar plates evenly. Then with the help 5mm sterile cork borer, wells were made in the inoculated media plates. The 100µl of the suspension of different mixture of enzyme was inoculated into the well with the help of micropipette. The plates were then left for half an hour and incubated at 37°C overnight. After incubation, the plates were viewed for the zone of inhibition around the well.

RESULTS AND DISCUSSION

The production media were prepared using different substrates from pineapple waste such as pulp, crown leaves and peels and inoculated with above said organisms and incubated for period of 30 days. After incubation the fermented broth medium was analyzed for presence of enzyme activity for amylase, cellulase, protease and lipase along with determination of protein into the sample. The protein content into the crude multienzyme sample was analyzed by Folin Lowry Method. Standard graph of bovine serum albumin was prepared (Fig 1) for determination of protein content into the samples. It was found that the protein content into the crude enzyme prepared using *S.cerevisae* with pulp was 162 ug/ml, peels 143 ug/ml and leaves 160 ug/ml. The crude enzyme prepared from *A.niger* with substrate peels was found to have protein content 180 µg/ml followed by pulp 135 ug/ml and leaves 168 ug/ml. while in case of *B.subtilis* crude enzyme preparation from peels showed 182 ug/ml, pulp showed 92 ug/ml and leaves showed 111 ug/ml protein content (Fig 2).

Qualitative determination of multienzyme activity of crude sample from pineapple waste for amylase, protease cellulase and lipase activity is shown in (Fig 3) It was found that using *S.cerevisae* for pulp as substrate amylase and protease showed the same activity which was 20mm and also cellulase and lipase showed the same activity by producing zone 10mm. In case of leaves substrate amylase and cellulase showed same activity which was 20mm and also protease and lipase showed same activity i.e. 15mm. In case of peels as a substrate amylase, protease cellulase and lipase showed same activity which was 15mm zone of clearance.

In case of *A.niger* in which pulp used as a substrate amylase and cellulose showed the same activity which was 10mm followed by protease 15mm and lipase 20mm zone of clearance. In case of leaves substrate protease showed the highest activity (20mm) and amylase and lipase showed same activity (15mm) followed by cellulase (10mm). In case of peels as a substrate amylase showed the highest activity (20mm) and protease, cellulase, lipase showed slight and same activity10mm. In case of *B.subtilis* used for production using pulp as substrate amylase, protease and lipase showed same activity (15mm) followed by cellulase (10mm). In case of leaves as substrate amylase and protease showed same activity (10mm) and cellulase and lipase showed same activity (15mm). In case of peels as substrate amylase and lipase showed the same activity which was (20mm) followed by protease and cellulase which showed 16mm and 10 mm zone of clearance.

The antibacterial activity of crude multienzyme was also checked against *Enterococcus spp., S. aureus, Bacillus spp., E. coli and Klebsiella pneumoniae* in (Table1). It was found that using *S. cerevisiae* and pulp as substrate showed the highest activity against *Bacillus spp*. which showed 15 mm zone of inhibition followed by *Enterococcus spp*. which showed 11mm zone of inhibition while against *S. aureus, E.coli and Klebsiella pneumoniae* no activity was recorded. In case of leaves as substrate highest zone of inhibition was recorded against E. coli 16 mm followed by *Enterococcus* spp. 15mm, *S. aureus* 10mm and *Bacillus spp*. 12mm while no zone was produced against *Klebsiella pneumoniae*. In case of peels as substrate *Enterococcus spp*. showed highest zone of inhibition which was 21mm followed by *S. aureus* 10mm, *Bacillus spp.* 16mm and *E.coli* (15mm) while no zone was produced against *Klebsiella pneumoniae*.

In case of *A. niger* and as pulp substrate crude fermentation broth showed the highest activity against *E.coli* (16mm) followed by *Enterococcus* spp. (12mm), *S. aureus* (15mm), *Bacillus* spp. (12mm) and no activity was recorded against *Klebsiella pneumoniae*. In case of leaves as substrate *Enterococcus* spp. showed highest zone of inhibition (20mm) and *S.aureus* showed less zone (11mm) followed by *Bacillus* spp. (17mm), *E.coli* (16mm) and *Klebsiella pneumoniae* (12mm). In case of peels as substrate *Enterococcus spp. and Klebsiella pneumoniae* showed the same activity which was 10mm while *S.aureus* and *E.coli* showed same activity i.e. 12 mm followed by *Bacillus spp*. with 16 mm zone of inhibition. In case of B. subtilis using pulp as substrate no activity was recorded against *Enterococcus spp, S.aureus and Bacillus spp*. Only *E.coli* and *Klebsiella pneumoniae* showed the activity with 11mm, 12mm zone of inhibition. In case of leaves as substrate *E.coli* and *Klebsiella pneumoniae* showed the same activity which was 11mm followed by *S.aureus* 10mm zone of inhibition while against *Enterococcus spp*. and *Bacillus spp*. no activity was recorded. In case of peels as substrate showed the prominent activity against *E.coli* and *Klebsiella pneumoniae* which showed 16 mm zone of inhibition followed by *S.aureus* and *Bacillus* spp. which showed 12mm and 13mm zone of inhibition while against *Enterococcus* no activity was recorded.

Fig. 1 : Standard Graph of Protein estimation of Bovin Serum Albumin (BSA)

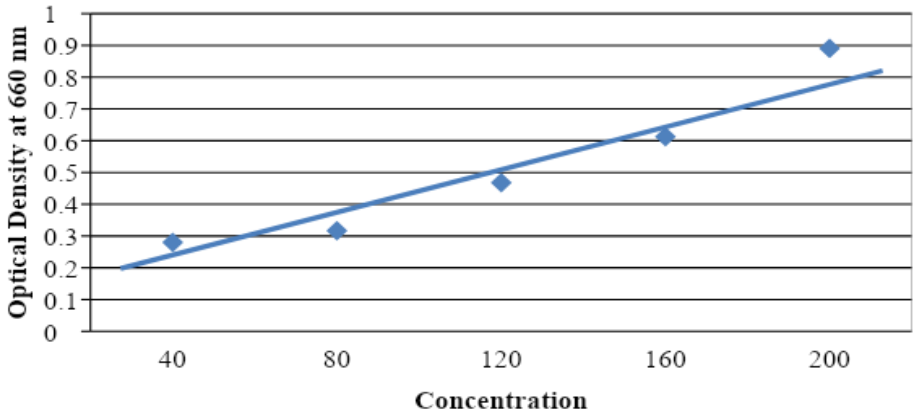


Fig. 2 : Protein estimation of crude multienzyme from pineapple waste

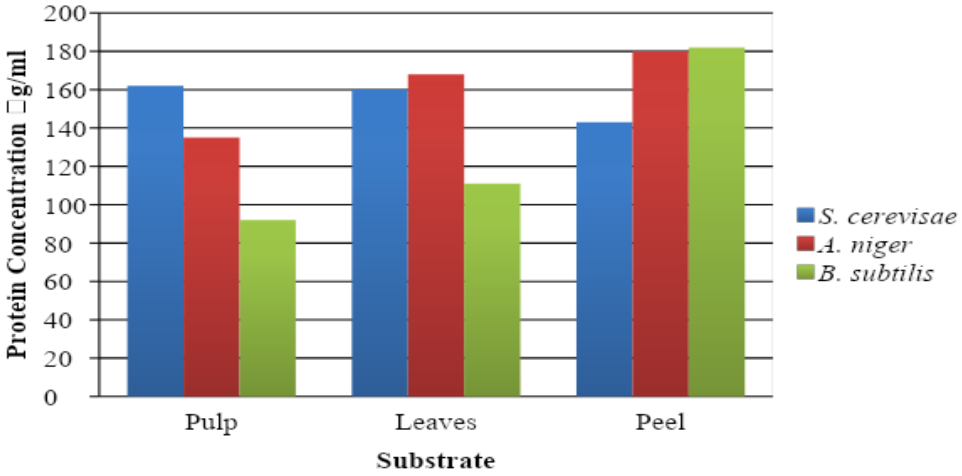


Fig. 3 : Qualitative assay for multienzyme activity from pineapple waste using *S. cerevisae*

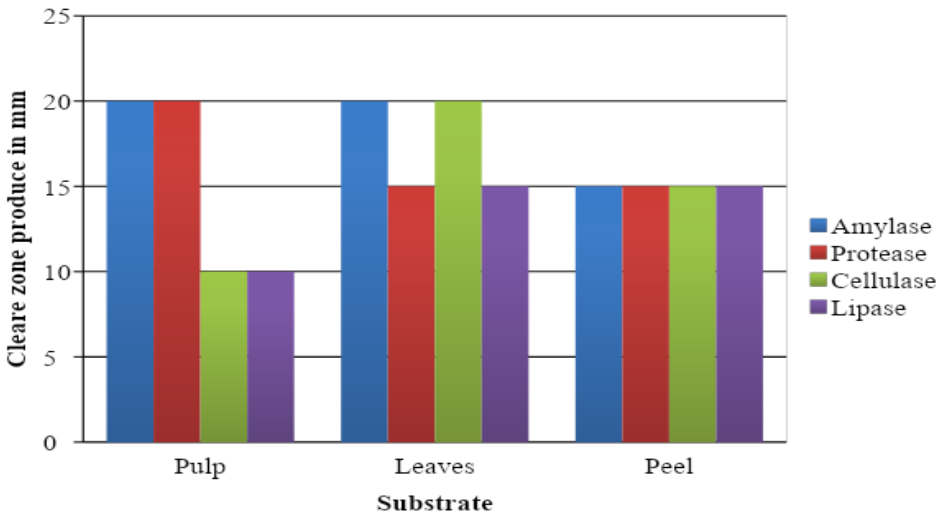
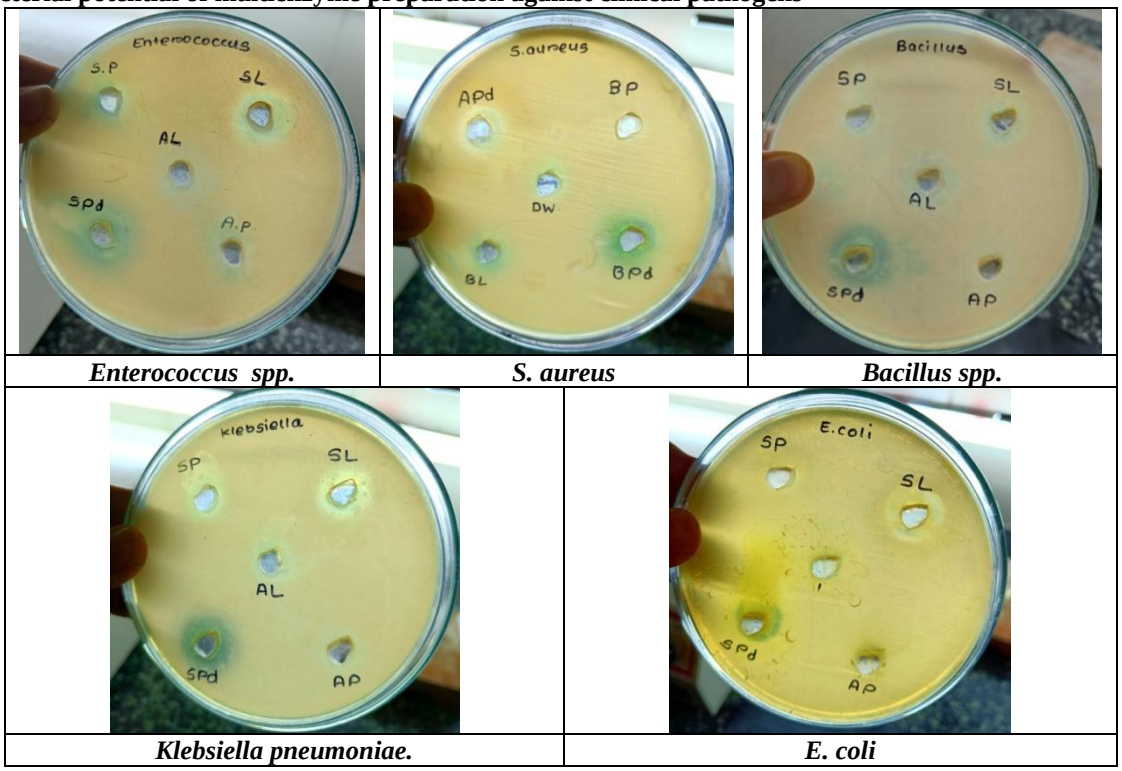


Table 1: Antibacterial activity of crude multienzyme from Pineapple waste.

Sr. No.	Isolate used	Substrate used	Zone of inhibition (in mm)				
			Enterococcus spp.	*S. aureus*	*Bacillus spp.*	*E. coli*	*Klebsiella pneumoniae*
1.	*S. cerevisae*	Pulp	11	-	15	-	-
		Leaves	15	10	12	16	-
		Peel	21	10	16	15	-
2	*A. niger*	Pulp	12	15	12	16	-
		Leaves	20	11	17	16	12
		Peel	10	12	16	12	10
3	*B. subtilis*	Pulp	-	-	-	11	12
		Leaves	-	10	-	11	11
		Peel	-	12	13	16	16

Antibacterial potential of multienzyme preparation against clinical pathogens



Enterococcus spp. | ***S. aureus*** | ***Bacillus spp.***

Klebsiella pneumoniae. | ***E. coli***

DISCUSSION

In the present investigation the utilization of pineapple waste such as pulp, peels and crown leaves was analyzed for production of multienzyme. In the present study the multienzyme production was analyzed which showed the amylase, protease, cellulase and lipase activity from all the medium prepared using different substrates and organisms. The results obtained were supported by other researchers who also reported the production of multienzymes as Neupane and khadka (2019) reported the amylase, gelatinase, protease, lipase and cellulase production from different fruits and vegetable to which they termed as garbage enzyme. This study also used pineapple as one of the substrate for the production. Other studies of Thirumurugan (2016); Madhumithah *et al.,* (2011); Reji and Pradeep (2019); Mavani *et al.,* (2020) have also reported production of multienzymes using various waste such as fruits and vegetables amongst pineapple, orange and papaya ecoenzymes have been shown potential for various purposes.

In the present study antibacterial activity was also determined against some common human pathogens. The results showed presence of varied susceptibility pattern of isolates toward the multienzyme preparation this is similar with other studies who also reported the antimicrobial potential of multienzyme preparations (Bhavani Prakash 2011; Arun and Sivashanmugam 2015; Neupane and Khadka 2019; Saramanda and Kaparapu 2017; Mavani *et al.,* 2020; Aartheeswari and Kirthiga 2021).

CONCLUSION

It can be concluded that pineapple waste can be a good source for production of multienzyme. The finding of the study confirms the enzymatic activities of fermented

broth for amylase, protease, cellulase and lipase which found as a crude mixture of these multienzymes. Further the study also concludes that the multienzyme preparation also exhibits the antibacterial potential. These eco-friendly multienzyme preparations would be effective in many ways to the environment and society as it may useful in various applications, can be environment friendly and produced from cheap sources.

REFERENCES

1. Aartheeswari. S and Dr. B. Kirthiga Production of an Ecofriendly Enzyme Biocleaner from Fruit Wastage. *International Journal for Research in Engineering Application & Management*, 2021; 06I1272034: 285-290.
2. Abriouel, H., Franz, C. M., Omar, N. B., & Gálvez, A. Diversity and applications of Bacillus bacteriocins. *FEMS microbiology reviews*, 2011; 35(1): 201-232.
3. Arsyada, I. F., Rianti, D., & Munadziroh, E. Antibacterial activity of mixed pineapple peel (Ananas comosus) extract and calcium hydroxide paste against Enterococcus faecalis. *Dental Journal (Majalah Kedokteran Gigi)*, 2018; *51*(1): 20-24.
4. Arun C and Sivashanmugam P (2015). Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge. *Process Saf. Environ. Prot.* 94 : 471
5. Ayala-Zavala, J.F., Rosas-Domínguez, C., Vega-Vega, V. and González-Aguilar, G.A. (2010) Antioxidant Enrichment and Antimicrobial Protection of Fresh-Cut Fruits Using Their Own by Products: Looking for Integral Exploitation. *Journal of Food Science*, 75, R175-R181.
6. Balouiri, M., Sadiki, M., &Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2), 71-79.
7. Bernfeld, S. (1951). Sigmund Freud, MD, 1882—1885. *International Journal of Psycho-Analysis*, 32, 204-216.
8. Bezerra, R.P., Borba, F.K.S.L., Moreira, K.A., Lima-Filho, J.L., Porto,A.L.F., Chaves, A.C., 2006. Extraction of amylase fromfermentation broth in poly(ethylene glycol) salt aqueous two-phase system. *Braz. Arch. Biol. Technol*. 49 (4), 547–555.
9. Bhawani Prakash, (2011). How to make and use garbage enzyme. *Retrieved*, 2(09), 2012.
10. Chopra, L., Singh, G., Kumar Jena, K., & Sahoo, D. K. (2015). Sonorensin: a new bacteriocin with potential of an anti-biofilm agent and a food biopreservative. *Scientific reports*, 5(1), 1-13
11. De Vries, R. P., Riley, R., Wiebenga, A., Aguilar-Osorio, G., Amillis, S., Uchima, C. A., & Grigoriev, I. V. (2017). Comparative genomics reveals high biological diversity and specific adaptations in the industrially and medically important fungal genus Aspergillus. *Genome biology*, 2018; 18(1): 1-45.
12. Emimol, A., Ganga, G., Parvathy, R., Radhika, G., & Nair, G. M. Screening of microbes producing extracellular hydrolytic enzyme from corporation waste dumping site and house hold waste for the enhancement of bioremediation methods. *IOSR-JPBS*, 2012; 4: 54-60.
13. Horst Feldmann, *Yeast. Molecular and Cell bio*. Wiley-Blackwell. ISBN 978-3527326099, 2010.
14. Jovanovic, M., Milutinovic, M., Kostic, M., Miladinovic, B., Kitic, N, Brankovic, S. and Kitic, D. Antioxidant Capacity of Pineapple (Ananascomosus (L.) Merr.) Extracts and Juice. *Lekovite Sirovine*, 2018; 38: 27-30.
15. Kunamneni, A., Permaul, K., & Singh, S. Amylase production in solid state fermentation by the thermophilic fungus Thermomyceslanuginosus. *Journal Of Bioscience And Bioengineering*, 2005; 100(2): 168-171.
16. Ladd, J. N., & Butler, J. H. A. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil biology and Biochemistry*, 1972; 4(1): 19-30.
17. Laderman, K.A., Davis, B.R., Krutzsch, H.C., Lewis, M.S., Griko, Y.V., Privalov, P.L. and Anfinsen, C.B. The purification and characterization of an extremely thermostable α-Amylase from the hyperthermophilic Archaebacterium *Pyrococcus furiosus*. *Journal of Biological Chemistry*, 1993; 268(32): 24394-24401.
18. Li PJ, Xia JL, Shan Y, Nie ZY Comparative study of multi-enzyme production from typicalagro-industrial residues and ultrasound-assisted extraction of crude enzyme in fermentation with *Aspergillus japonicus* PJ01. Bioprocess. *BiosystEng*, 2015; 38: 2013-22. doi.org/10.1007/s00449-015-1442-3.
19. Lubaina, A. S., Renjith, P. R., & Kumar, P. Antibacterial potential of different extracts of pineapple peel against gram-positive and gram-negative bacterial strains. *Asian Journal of Pharmacy and Pharmacology*, 2019; 5(S1): 66-70.
20. Madhumithah, C. G., Krithiga, R., Sundaram, S., Sasikumar, C. S., Guhathakurta, S., & Cherian, K. M. Utilization of vegetable wastes for production of protease by solid state fermentation using *Aspergillus niger*. *World Journal of Agricultural Sciences*, 2011; 7(5): 550-555.
21. Mamma. D., Kourtoglou, E. and Christakopoulos, P. Fungal multiennzyme production on industrial by products of the citrus processing industry. *Bioresource Technology*, 2008; 99(7): 2373- 2383.
22. Manimozhi, D.M., Sankaranarayanan, S. and Sampathkumar, G. Evaluating the Antibacterial Activity of Flavonoids Extracted from Ficusbenghalensis. *International Journal of Pharmaceutical and Biological Research*, 2012; 3: 7-18.
23. Maurer, H.R. Bromelain: Biochemistry, Pharmacology and Medical Use. *Cellular and Molecular Life Sciences*, 2001; 58: 12341245.

24. Mavani, H. A. K., Tew, I. M., Wong, L., Yew, H. Z., Mahyuddin, A., Ahmad Ghazali, R., & Pow, E. H. N. Antimicrobial efficacy of fruit peels eco-enzyme against *Enterococcus faecalis*: an in vitrostudy. *International Journal Of Environmental Research And Public Health*, 2020; 17(14): 5107.
25. Miller, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*, 1959; 31(3): 426-428.
26. Nawawai Mohd MG, Othman N, Sadikin AN, Khalid SS, Yusof N Development of empty fruit brunch filter with addition of chitosan for pre-treatment of palm oil mill effluent. *J. Chem. Nat. Res. Eng.*, 2008; 2: 8.
27. Neupane, K., & Khadka, R. Production of garbage enzyme from different fruit and vegetablewastes and evaluation of its enzymatic and antimicrobial efficacy. *Tribhuvan University Journal of Microbiology*, 2019; *6*: 113-118.
28. Nicholson WL Roles of Bacillus endospores in the environment. *Cell Mol Life Sci*, 2002; 59: 410–416.
29. Park, H. S., Jun, S. C., Han, K. H., Hong, S. B., & Yu, J. H. Diversity, application, and synthetic biology of industrially important Aspergillus fungi. *Advances in applied microbiology*, 2017; 100: 161-202.
30. Piana, C.F., Featherstone, A. and Boland, M. Vertical Integration in Ecuador: The Case of Fresh-Cut Pineapples. *Review of Agricultural Economics*, 2005; 4: 593-603.
31. Raper, K. B., & Fennell, D. I. The Genus *Aspergillus baltimore*, Maryland: Williams and Wilkins. *Field view with a diseased cotton plant showing coagulated fibers*, 1965.
32. Raveendran, S., Parameswaran, B., Ummalyma, S. B., Abraham, A., Mathew, A. K., Madhavan, A., & Pandey, A. Applications of microbial enzymes in food industry. *Food Technology And Biotechnology*, 2018; 56(1): 16.
33. Reji, S. R., & Pradeep, N. S. Isolation and selection of fungal strains for multienzyme production from Western Ghats. *International Journal of Agriculture, Environment and Biotechnology*, 2019; 12(1): 2332.
34. Sagar NA, Pareek S, Sharma S, Elhadi M, Yahia,Maria LG Fruit and vegetable waste: bioactive compounds, their extraction, and possible utilization. *Compr Rev Food Sci Food Saf*, 2018; 17: 512-531.
35. Samson RA, Houbraken J, de Vries RP, Modern taxonomy of biotechnologically important Aspergillus and Penicillium species. *Adv. Appl. Microbiol*, 2014; 86: 199–249.
36. Saramanda G and Kaparapu J Antimicrobial activity of fermented citrus fruit peel extract. Int Journal of Engineering Research and Application, 2017; 7: 25-28.
37. Sazci A, Erenler K and Radford A Detection of cellulolytic fungi by using Congo red as an indicator: a comparative study with the dinitrosalicyclic acid reagent method. *J Appl.Bacteriol*., 1986; 61(6): 559–562.
38. Selvakumar P and Sivashanmugam P. Optimization of lipase production from organic solid waste by anaerobic digestion and its application in biodiesel production. Fuel Process. *Technol.,* 2017; 165: 1.
39. Sharma, D., & Bisht, D. M. tuberculosis hypothetical proteins and proteins of unknown function: hope for exploring novel resistance mechanisms as well as future target of drug resistance. *Frontiers In Microbiology*, 2017; 8: 465.
40. Sullivan L, Ross RP, Hill C. Potential of bacteriocin-producing lactic acid bacteria for improvements in food safety and quality. *Biochimie*, 2002; 84: 593–604.
41. Tang FE and Tong CW A study of the garbage enzyme's effects in domestic wastewater. *Int. J. Environ. Chem, Ecol, Geol and Geop Eng.*, 2011; 5: 887.
42. Thirumurugan P. Production and analysis of enzyme bio-cleaners from fruit and vegetable wastes by using yeast and bacteria. Student project Report (D.O.Rc.No.1082/2015A; Project No: 28) submitted to Tamil Nadu State Council for Higher Education (TANSCHE), India, 2016; 4-6.
43. Varga, J., Frisvad, J. C., Kocsubé, S., Brankovics, B., Tóth, B., Szigeti, G., & Samson, R. New and revisited species in Aspergillus section Nigri. *Studies in Mycology*, 2007; 58(1): 33-56.
44. Yong, F. M., & Wood, B. J. B. Biochemical changes in experimental soy sauce koji. *International Journal of Food Science & Technology*, 1977; 12(2): 163-175.