Utilization Of Dragon Fruit Peel As Microbial Growth Media And Determining Its Antimicrobial Activity

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Abstract—Their is increase in dragon fruit peel waste and hence in organic waste. This study introduces the use of dragon fruit peel as microbial growth medium and as means of reducing the buildup of organic waste. Based on proximate study of total sugar content, the ideal DFP media is - 29.5 gram DFP powder suspended in 1 liter distilled water. After 18 hours of incubation at 37°C on DFP agar media, colonies of 5 bacteria and 2 fungus are observed to determine their viability. The growth of Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger, and Fusarium oxysporum shows comparable result. This finding is corroborated by measuring growth curve at optical density of 600 nm in DFP broth over a 48-hour period at 37°C temperature. The present DFP agar is still lacking in providing suitable growth nutrients for Staphylococcus aureus and Klebsiella pneumonia as it presents lesser growth.

The main effect of diabetes is diabetic foot as it runs a higher risk of mortality or amputation. The purpose of this study is to ascertain whether red dragon fruit peel has any antibacterial properties against the bacteria that cause diabetic ulcers. Red dragon fruit extract was prepared. Streptomycin, chloramphenicol and amoxicillin are used as controls. Zone of inhibition is observed in Pseudomonas aerugenosa, Escherichia coli and staphylococcus aureus, which proves red dragon fruit peel to be a good antimicrobial agent.

Keywords: antibacterial agent, diabetic ulcer, dragon fruit peel, microbial growth media, organic waste utilization

I. INTRODUCTION

Huge productions of fruit and vegetables are mobilized worldwide thanks to modern, effective agriculture procedures. Most of these waste products are frequently disposed of inappropriately, which causes serious environmental problems. There are about 200 tons of waste, the majority of which is organic waste. Fruit peels make up a significant amount of this organic waste (up to 40%) Sites where fruit waste is dumped give harmful bacteria, yeast, and viruses the fuel they need to flourish [1].

Fruit peels have been widely researched in terms of waste utilisation and are being converted into value-added goods such dietary fibre supplements, plant fertiliser, and microbiological agar media [2]. According to several research, fruit peel waste from banana and pineapple peels has been effectively used as an alternative microbial agar growth and shows potential [3].

The study described earlier also revealed that fruit peels include carbohydrates (either in simple or complex form), protein, fibre, as well as vitamins and minerals [4]; these are thought to be the essential elements needed in microbial agar to support the growth of microorganisms. It has been observed that some fungi cultivated on pineapple trash produce cellulase when used as a substrate for growing cultures for the creation of value-added products.

The price of all microbiological medium is rising rapidly. Some innovative, cost-effective microbiological media should be developed to address this issue. Using dragon fruit peel waste as the foundation for microbial media can help achieve this. [5].

Infection of the lower limb, the development of ulcers, and the destruction of deep tissue are all common effects of peripheral vascular disease and neuropathy, including diabetic foot. The incidence and prevalence of diabetic foot are, respectively, 1.0–4.0% and 5.3–10.5%. On the toes occur about 20–30% of diabetic foot ulcers. Due to a number of reasons, including thin subcutaneous fat and skin, slow blood flow, the resistance of major blood arteries, and hidden leg sutures, foot ulcers in diabetics are both common and difficult to treat. Leg bones are also involved in foot ulcers, which raises the possibility of death or amputation [6].

82 positive cultures were found in an Indian study of 100 patients with persistent diabetic ulcers. The dominating organism is Staphylococcus Aureus, followed by Pseudomonas Aeruginosa. In a series of diabetic pus ulcers, Staphylococcus spp. (92.9%), Klebsiella spp. (75.4%), Proteus spp. (73.7%), Shigella spp. (68.4%), Escherichia coli spp. (42.1%), and Pseudomonas spp. (10.5%) were the most prevalent bacteria [7].

The red dragon fruit is one of the plants that is known to have antibacterial properties. Red dragon fruit is a Cactaceae fruit that is popularly consumed. Osteoporosis, hypertension, diabetes, and lowering cholesterol can all be regularly prevented and treated using red dragon fruit [8].

Therefore, the purpose of this study was to use DFP as an alternative microbial agar medium and as an antimicrobial agent using a variety of experimental techniques and analysis.

II. MATERIALS AND METHOD [9, 10, 11, 12]

White dragon fruit was used for making microbial growth media. Dragon fruit peel agar (DFPA) and dragon fruit peel broth (DFPB) were made. Red dragon fruit extract was made for antimicrobial activity.

II.I. MICROBIAL GROWTH MEDIA SAMPLE PREPARATION

Dragon fruits were obtained from regular fruit shop and their flesh was separated from the skin. Skin or the peels (waste) were chopped into fine pieces and kept in hot air oven for 5 hrs at 95□C. The dried peels are converted to fine powder using kitchen mixer.

II.II. SUGAR CONTENT ANALYSIS

A solution was made by mixing dragon fruit in distilled water and sugar content was known by DNSA method. Spectrophotometer was used for analysis [9].

II.III. PROTEIN CONTENT ANALYSIS

Protein content of dragon fruit was calculated using biuret method. Analysis were done using spectrophotometer [10].

II.IV. DFP MICROBIAL MEDIA PREPARATION

After applying a few formulas and doing certain adjustment, it was known that 29.5 grams of dragon fruit peel powder was required in 1 liter of agar medium.

II.V. DFP LIQUID MEDIA

DFP powder was mixed in distilled water. To ensure homogenous mixing, it was heated at low flame and continuously stirred for 5-7 min. After cooling, filtration was done using filter paper. Then, the solution was centrifuged at 3000 rpm for 40 min. The supernatant was then transferred to Erlenmeyer flask and pellet was discarded.

II.VI. DFPB (DRAGON FRUIT PEEL BROTH) MEDIA

The solution prepared above was sterilized at 121□C for 15 min and then it was ready for analysis.

II.VII. DFPA (DRAGON FRUIT PEEL AGAR) MEDIA

7 grams of agar powder was required in 1 liter of DFP solution which was used as DFPA. Media was sterilized at 121□C for 15 min before doing analysis.

Bacterial and Fungal Cultures - Samples of Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Aspergillus niger and Fusarium oxysporum weres obtained from sevashram hospital, Parul University. Bacteria were serially diluted (10−4) in Phosphate Buffer Saline.

II.VIII. INCUBATION OF BACTERIA AND FUNGI ON DFPA

2 drops of each bacteria was taken and spread plated on DFPA. The plates were incubated at 37□C and growth was observed. Spores of fungi were placed in the center of DFPA. The plates were incubated at 37□C for 6 to 7 days and growth was observed [11].

II.IX. MICROBIAL GROWTH RATE ON DFPB

1 loop of each bacteria and fungi was transferred to test tubes having 10ml of DFPB from the serial dilution. Test tubes are kept in shaker at 25□C for 48 hrs. Optical density was measured by spectrophotometer after every one hour interval.

1 loop spores of both the fungus were transferred to test tubes having 10 ml of DFPB. Test tubes were kept in shaker at 25□C for 4 days. Optical density was measured by spectrophotometer in every one hour interval.

II.X. ANTIMICROBIAL ACTIVITY DISC PREPARATION

Whatman Filter Paper was used for disc diffusion method. It was cut into small round disc using paper punching machine and then kept in hot air oven at 80□C for 20 min to ensure sterility. Red dragon fruit was washed properly. Then, the pulp was separated from the skin and the peel was converted to fine paste using kitchen mixer or blender. This was followed by filteration to get red dragon fruit extract. This extract was then boiled at low flame for 5-7 min. Disc diffusion method was used for testing antimicrobial activity. 2 drops of each bacterial.

culture prepared before is poured for spreading and left for 15 min so that the bacterial culture seeps into the Mueller–Hinton Agar.

Controls - Streptomycin, chloramphenicol and amoxicillin are placed. Disc was dipped into the red DFPE and placed in the MHA plate using sterile forcep and incubated at 37□C for 24 hrs. Using a dark background on MHA medium, the diameter of the inhibition zone was measured with a ruler. The clear area surrounding the paper disc, measured from one end to the other, serves as the resistance zone diameter measurement [12].

III. RESULT AND DISCUSSION

III.I. MICROBIAL GROWTH MEDIA

As mentioned in table-1 a whole fruit of 560 grams would contain 201 grams of the peel. Afterdrying the peel and making its powder, up to 93% of the weight is reduced due to loss of moisture from drying process. Hence, the final weight of the dragon fruit peel powder was 14.07gram.

Sugar analysis (fig 1) done by DNSA method showed that dragon fruit peel had enough amountof carbohydrate to support the nutrient/energy requirement of microorganisms needed for growth. As stated in table-2, sugar content percentage or brix value of dragon was 1.45 which was more than nutrient agar and many fruits like pineapple and banana [13]. Therefore, a significant benefit of using dragon fruit peel was that less peel/waste is required to create an equivalent amount of microbial growth media.

Protein analysis (fig 2) done by biuret method showed that dragon fruit peel lacked in sufficientprotein content which was required for DNA and RNA synthesis. As stated in table-2, protein content percentage of dragon fruit was 1.4 which was much less than nutrient agar [13].

Table 1. Proportion of Dragon Fruit Peel

Parameters	Value
Whole fruit	560 gm
Peel	201 gm
Dried peel	14.07 gm

Table 2 – Sugar and Protein Analysis

Parameters	Value
Sugar	1.45
Protein	1.4

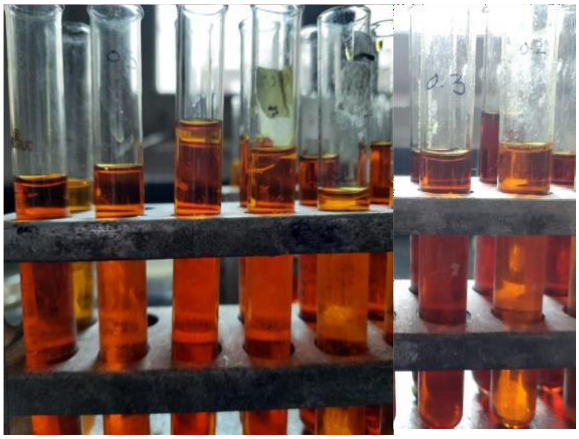


Figure 1. Sugar Analysis

Figure 2. Protein Analysis

III.II. GROWTH OF MICROORGANISM IN DFPA

Viable colonies of bacteria was visible. Growth of fungus was also observed after 7 days due tohigh sugar content.

Escherichia coli (fig.3), *Bacillus subtilis* (fig.4) and *Pseudomonas aeruginosa* (fig.5) had bacterialgrowth and *Aspergillus niger* (fig.8) and *Fusarium oxysporum* (fig.9) had fungal growth. In spiteof the fact that DFPA had already supplied the microorganisms abundant carbon sources to develop, it lacked protein, which might make the colonies appear stunted (according to the Biuret'smethod analysis in Table 2). Essential amino acids serve as nitrogen sources for microorganisms that can contribute to the production of DNA and RNA, according to a study by Todar (2012) [14].The more DNA and RNA produced, the more bacteria will proliferate, increasing the size of the colonies.

Staphylococcus aureus (fig.6) *and Klebsiella pneumonia* (fig.7) did not have enough bacterial growth. The distinct type type of sugar source present in DFPA, that fails to reflect the type of sugar required by the bacteria, may, hypothetically, be the origin of this finding. In addition, certainamino acids that are crucial for promoting growth may not have been present in the peel of the dragon fruit, which

could have decreased the nutritional components for growth. Due to these issues, DFPA is unable to effectively feed sufficient nutrients for bacterial growth. In spite of that,there is bacterial growth, though in small amount which did not cover the entire petriplate.

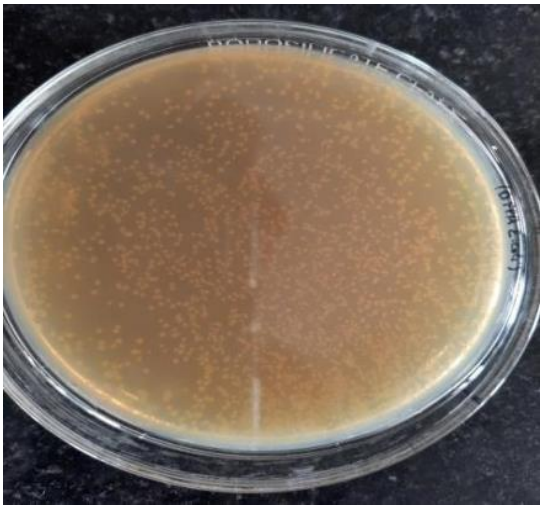


Figure 3. ***E.coli***

Figure 4. ***Bacillu***

Figure 5. ***Pseudomonas aeruginosa***

Figure 6. ***Staphylococcus aureus***

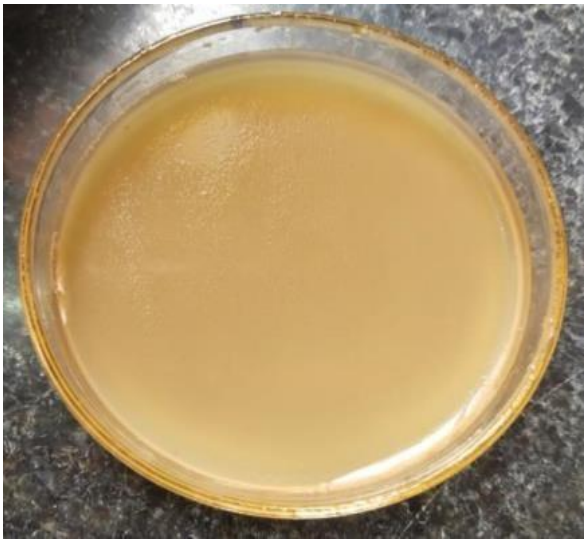


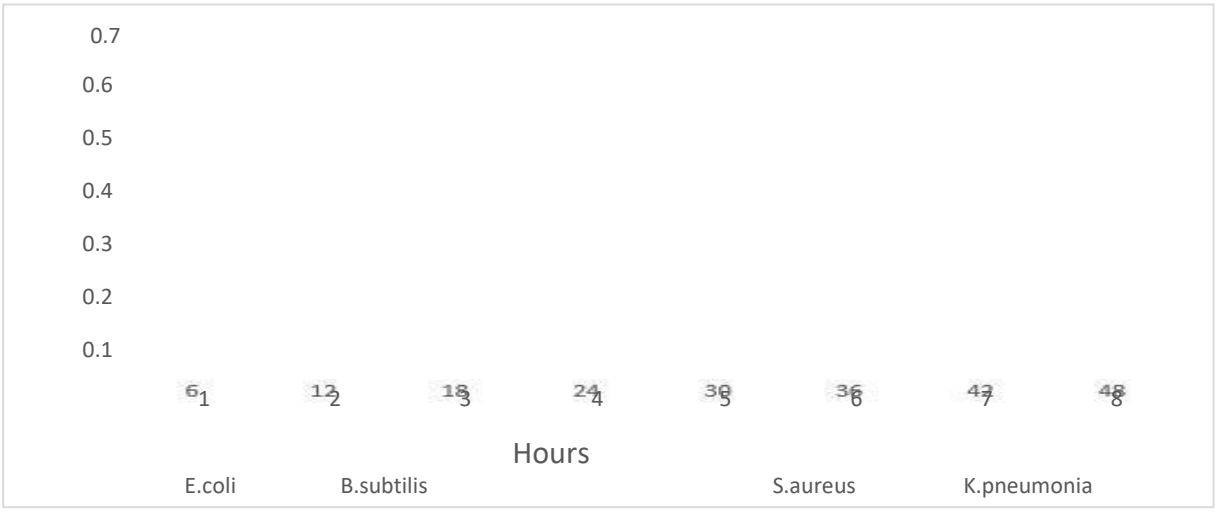
Figure 7. ***Klebsiella pneumonia***

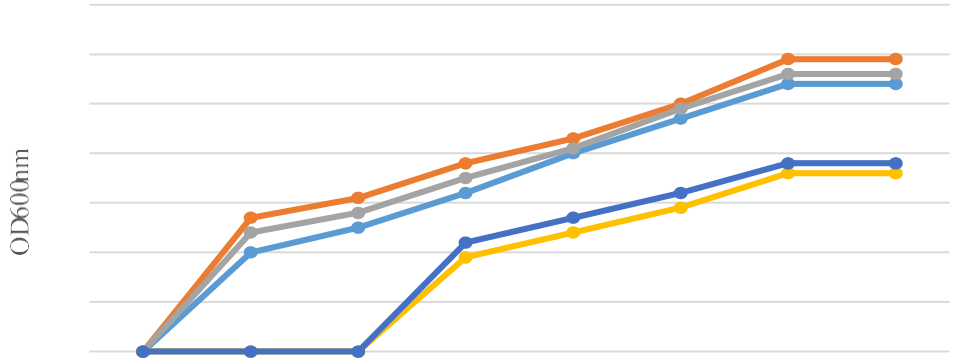
Figure 8. ***Aspergillus niger***

Figure 9. ***Fusarium oxysporum***

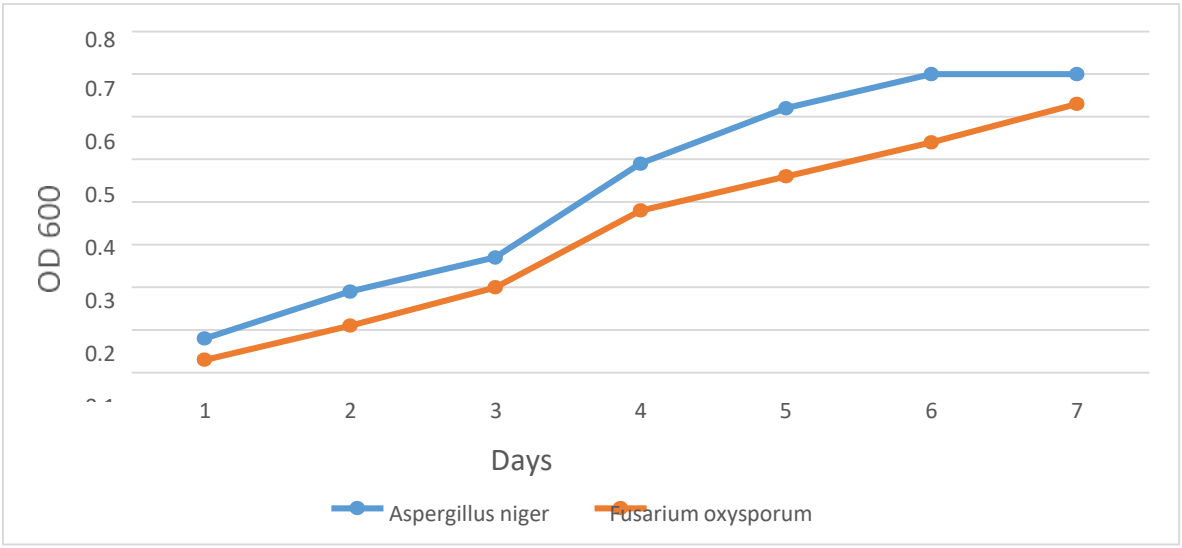
III.III. GROWTH OF MICROORGANISM IN DFPB

It was observed that *Escherichia coli, Bacillus subtilis and Pseudomonas Aeruginosa* showed a similar pattern. They had a lag phase of about 6 hrs, followed by exponential phase of 36hrs and ended in stationary phase after 42hrs of incubation period. So these three bacteria showed markablegrowth.





Graph 1. Growth Curve of Bacteria



Graph 2. Growth Curve of Fungus

However, the growth curve of *Staphylococcus aureus and Klebsiella pneumonia* had a longer lag phase and also presented comparatively lesser growth. Lag phase lasted for about 18hrs followedby 24hrs of exponential phase and then ended in stationary phase after 42hrs of incubation period. Therefore, growth of these bacteria was not markable.

Aspergillus niger had lag phase of 1 day, followed by exponential growth for 5 days and then itattained stationary phase after 6 days of incubation period. *Fusarium oxysporum* also had lag phase of 1 day followed by exponential phase of 6 days and ended in stationary phase after 7 days incubation period.

III.IV. ANTIMICROBIAL ACTIVITY

DFPE showed zone of inhibition in *Escherichia coli* (fig. 10), *Pseudomonas aeruginosa* (fig. 11) and *Staphylococcus aureus* (fig. 12) which means it was effective enough against these bacteria. DFPE however did not show zone of inhibition against *Klebsiella pneumonia* (fig. 13)and *Bacillus subtilis* (fig.14) which means it proved to be ineffective against these two bacteria.

As presented in table 3, in *Escherichia coli*, DFPE showed inhibition zone of 9mm. Streptomycin was positive control and chloramphenicol and amoxicillin were negative controls.In *Pseudomonas aeruginosa*, DFPE showed inhibition zone of 13mm. chloramphenicol was positive control and streptomycin and amoxicillin were negative controls. In *Staphylococcus aureus*, DFPE showed inhibition zone of 8mm. streptomycin and chloramphenicol werepositive controls and amoxicillin was negative control.

In both, *Klebsiella pneumonia* and *Bacillus subtilis* DFPE did not show zone of inhibition which meant that DFPE was unable to kill these two bacteria. Both had just streptomycin as their positivecontrol and chloramphenicol and amoxicillin as negative controls.

Table 3. Antimicrobial Activity

Organism	Streptomycin	Chloramphenicol	Amoxicillin	Dragon Fruit Extract (Zone of Inhibition)
Escherichia coli	+	-	-	9mm
Pseudomonas aerugenosa	-	+	-	13mm
Staphylococcus aureus	+	+	-	8mm
Klebsiella pneumonia	+	-	-	-
Bacillus subtilis	+	-	-	-

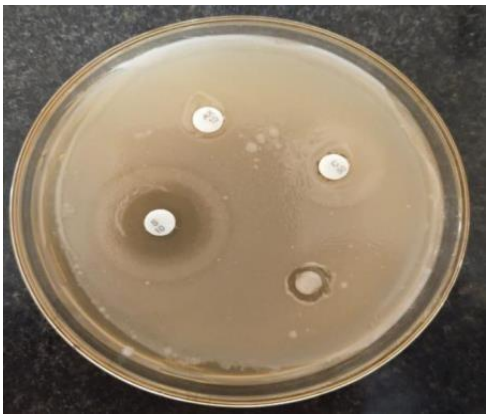


Figure 10. ***E.coli***

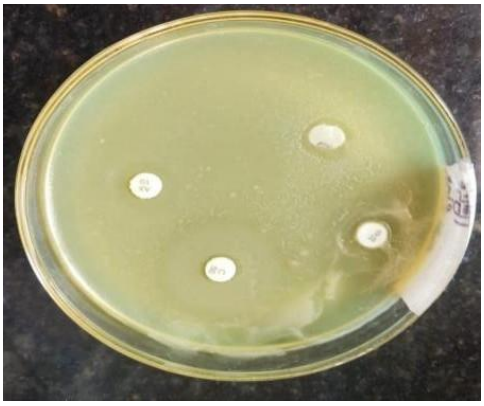


Figure 11. ***Pseudomonas aeruginosa***

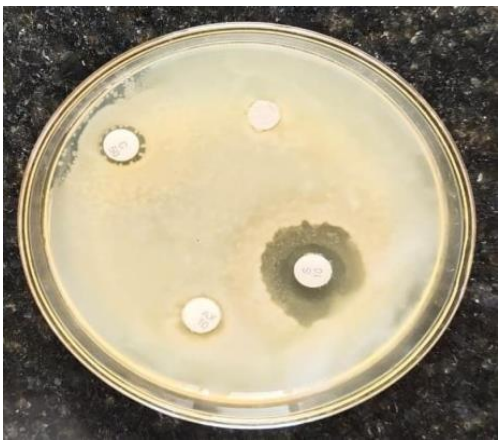


Figure.12 - ***S.aureus***

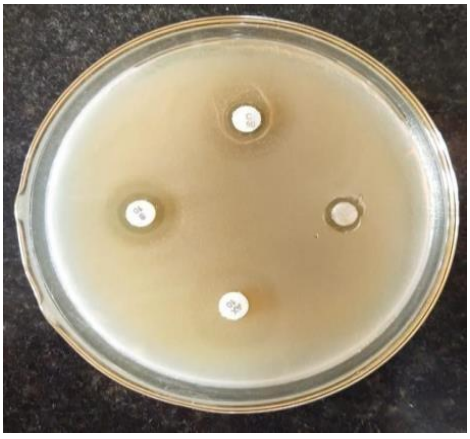


Figure.13 - ***klebsiella pneumoniae***

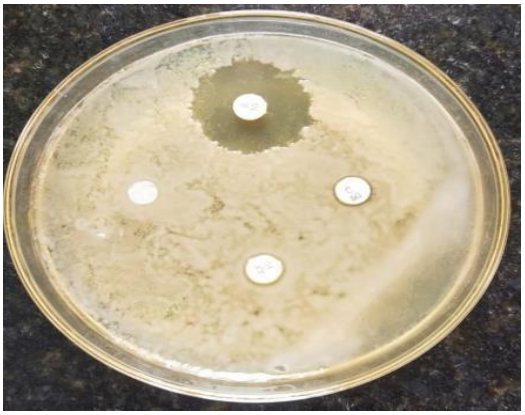


Figure 14. ***Bacillus subtilis***

Three processes underlie the antimicrobial activity of flavonoid compounds: inhibition of nucleic acid production, inhibition of cell membrane function, and inhibition of energymetabolism. The rings A and B play a significant role in the process of interconnection

or hydrogen bonding by playing an important role in the accumulation of nucleic acid bases that inhibit the formation of DNA and RNA, which is how flavonoids work againstbacteria by inhibiting the synthesis of nucleic acids. A key factor in the antibacterial activity of flavonoids is the positioning of hydroxyl groups in rings B at positions 2', 4' or 2', 6' and 5.7' in ring A. Due to interactions between flavonoids and bacterial DNA, flavonoids reduce the permeability of bacterial cell walls, microsomes, and lysosomes [15].

By combining with extracellular and dissolved proteins to generate complex compoundsthat can harm bacterial cell membranes, flavonoids hinder the function of cell membranes. This is accompanied by the release of intracellular chemicals. According to additional research, flavonoids interfere with the permeability of cell membranes and prevent enzymes like ATPase from binding, which limits the function of cell membranes.Moreover, flavonoids can stop bacteria from using oxygen, which can stop energy metabolism. Cytochrome C reductase is blocked by flavonoids, which stops metabolic production. Bacteria require energy for macromolecular production [15].

Alkaloids are antimicrobial compounds that, in addition to flavonoids, have pharmacologic effects on people and animals. This is due to the fact that alkaloids can stop bacterial protein synthesis enzymes in their tracks. Impaired bacterial metabolism can result from this enzyme's activity being inhibited [15].

IV. CONCLUSION

As per this study, advantage of using dragon fruit peel as microbial growth media was observed. In microbial growth media through viability analysis, DFPA was found to provide necessary nutients for *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus niger* and *Fusarium oxysporum* to grow. This result was supported through OD value. The viability, morphology and growth rate of *Klebsiella pneumonea* and *Staphylococcus aureus* were not supportive enough. The existence of different nutrient compositions in growth media, such as those containing different amounts of protein, vitamins, and minerals, might theoretically accountfor this scenario. In spite of the fact that DFPA had already given the microorganisms ample carbon sources to develop, it lacked protein, which might have affected the growth of *Staphylococcus aureus* and *Klebsiella pneumonia*. Essential amino acids give microorganisms nitrogen sources so they can participate to the production of DNA and RNA. The more DNA andRNA produced, the more bacteria will proliferate, increasing the number of the colonies. There could have been increase in the growth of *Staphylococcus aureus* and *Klebsiella pneumonia* if weadded 4-5 drops of bacterial culture instead of 2 drops. Another way was by carrying serial dilutionof 10^{-2} instead of serial dilution of10^{-4}.

Antimicrobial Activity's Results showed that the inhibition zone was seen in *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* but was missing in *Klebsiella pneumonia* and *Bacillus subtilis*. There was variation in the inhibition zone of all the 3 positive results. Researchers assume that the content of flavonoids, vitamin C, tannins, alkaloids, steroids, and saponins, terpenoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, carotene, and phytoalbumin and alkaloids is what makes a major contribution in inhibiting the growth and development of *Pseudomonas aeruginosa, E.coli* and *staphylococcus aureus* bacteria and these elements were present in Red Dragon Fruit Peel. Future research on DFPA and DFPB nutrients may lead to the creation of a supplemented form of these nutrients that can be utilised as a suitablemedium for other fastidious microorganisms, such as starter cultures for the fermentation of foodsand for making of antimicrobial disc and medicines.

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