ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

FORMULATION OF COST EFFECTIVE ALTERNATIVE MICROBIAL CULTURE MEDIA USING FRUITS AND VEGETABLES WASTE: A NOVEL APPROACH

Nisha R. Mohane, P. M. Tumane, Durgesh D. Wasnik*

Post Graduate Teaching Department of Microbiology, L.I.T. Premises, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India

**Corresponding author: dwasnik75@gmail.com*

ABSTRACT

The present investigation reveals the formulation of cost effective alternative microbial culture media using fruits and vegetables waste. Vegetables and fruits peel used for formulation of alternative media like Drum stick, Orange, Potato, Corn, Banana, Pine apple, Pomegranate, Papaya, Coconut coir, Carrot and Beetroot were collected from local vegetable market. Peels and coir were sun dried for 2-3 days and formulated into media by standard protocol. Bacterial culture used for analysis were *E.coli, S.aureus, Pseudomonas spp, Klebsiella spp.* and *Bacillus spp.* Bacteria were streaked on the freshly prepared nutrient agar medium from the original stock culture. The best formulated media were Beetroot media, Papaya media, Pomegranate media and Potato media as they showed the luxuriant growth of all five bacteria. *Klebsiella spp* and *Pseudomonas spp* grows luxuriantly in all media. *S. aureus* only grows in papaya media, Beetroot media and Potato media. *Bacillus spp* grows well in Orange media, Pomegranate media, Coconut coir and Beetroot media. The colonial characteristics of both the commercially available nutrient agar and the formulated media were compared for each isolate. The results of this study suggest that Papaya peel agar and Potato peel agar could be used as an enrichment medium for the growth of bacteria. The growth results obtained using above formulated media proves its efficiency as an alternative cheap media for routine microbiological experimentation.

Keywords: Alternative media, Fruits and vegetable waste, Bacterial culture

1. INTRODUCTION

Microbiological studies depend on the ability to cultivate and maintain microorganisms under laboratory conditions by providing suitable culture media that offers favourable conditions. A nutrient media prepared for the growth of microorganisms in a laboratory is called culture media. Microorganisms can obtain energy directly from sunlight while carbon can be made available in organic forms such as carbohydrates or inorganic forms such as carbon dioxide and water. Nutrient agar medium is commonly used as general purpose medium for the cultivation of broad range of bacteria. It is a basic medium composed of peptic digest of animal tissue, beef extract and yeast extract, sodium chloride and agar. Commercially available media such as Nutrient Agar, Cetrimide Agar, MacConkey Agar are used for the growth of microorganisms but these are very expensive [1].

Ever increasing population and industrialization has resulted in sudden increase in pollution. Because of the detrimental effects of pollution on humans, animals and plants, the ever increasing pollution is causing concern all over the world. One of the major environmental concerns in urban areas today is the issue of Solid Waste Management. In India, the collection, transportation and disposal of solid waste is normally done in an unscientific and chaotic manner. Uncontrolled dumping of wastes on outskirts of towns and cities has created overflowing landfills, which are not only impossible to reclaim because of the haphazard manner of dumping, but also have serious environmental implications in terms of ground water pollution and contribution to global warming [2].

Environmental issues and concerns aimed at reducing the ambient pollution have boosted the search for clean technologies to be used in the production of commodities of importance to chemical, energy and food industries. This practice makes use of alternative materials, requires less energy and diminishes pollutants in industrial effluents, as well as being more economically advantageous due to its reduced costs. Considering this scenario, the use of residues from agro

industrial, forestry and urban sources in bioprocesses has aroused the interest of the scientific community lately. Nowadays the rise of the middle class and fast economic growth in India, different varieties of fruits produced in India and other countries are increasingly consumed. Due to the high consumption and industrial processing of the edible parts of fruit, fruit wastes such as banana, grapes, dragon fruit, orange, strawberry, lemon, watermelon, citrus, Pomegranate, pineapple residues, sugarcane bagasse and other fruit residues are generated in large quantities in cities sides area. Fruit waste has become one of the main sources of municipal solid wastes, which have been an increasingly tough environmental issue [3].

The fruit peel wastes contain simple and complex sugars that are metabolizable by microorganisms [4] and have much attention for their conversion to bio-ethanol, biogas and animal feed [5]. Designing treatment schemes for specific agricultural residue limits efficiency of waste collection and prolong treatment period. Therefore, adoption of a method that accommodates several fruit wastes is highly robust, cheap and realistic in ameliorating impediments associated with fruit waste disposal [6]. The cultivation of microbial cells (bacteria, yeast, and fungi) that converts fruit wastes into value added products such as biomass that can serve as animal feed supplement is a unique approach. Agar is a solidifying agent and very few studies have concentrated on replacing agar for solidification. By comparing other studies carried out in this area by a number of researchers, now it would be possible to use a number of sources as alternative culture media [7]. Even then, increasing cost of culture media has necessitated search for more readily available culture media at affordable price. Different media for the growth and isolation of organisms have been reported from different substrates [8]. Some vegetables and fruits have been used to cultivate both fungi and bacteria, such as Gooseberry [9], Carrot, Tomato, Cabbage, Pumpkin etc [10] with easily available low cost material as substitutes for Nutrient Agar. Some others have used cow pea, green gram and black gram as starch and protein substitutes to reduce the cost of microbial media [11]. In today's world waste disposal is also a major problem. So lot many researches are carried so as to use domestic waste for production of cheap media. Higher cost of cultivation media is a matter of concern [2]. Therefore, various alternative media are formulated and alternatives for agar are tested, so as to reduce the cost involved.

2. MATERIAL AND METHODS

2.1. Collection of Samples

Vegetables and fruits peel like Drum stick, Orange, Potato, Corn, Banana, Pineapple, Pomegranate, Papaya, Coconut coir, Carrot and Beetroot were collected from local vegetable market. The collected samples were transported to the laboratory and processed immediately [1].

2.2. Treatment of samples

Peels and coir were sun dried for 2-3 days. Dried material was grinded to powder using electronic blender. The powdered samples were kept in air tight containers until its use [1].

2.3. Formulation of media

Ten (10) gms of dry powder was kept in warm water for 30 min. Then filtered with the help of filter paper and filtrate was used to prepare different solid formulated media. Then agar, which is solidifying agent, was added in 100 ml distilled water. In all experiments pH of the media was adjusted to 6.5-7.0. The dissolved media was sterilized in autoclave at 121°C for 20 minutes under 15 psi pressures and were poured into sterile Petri dishes separately.

2.4. Preparation of fresh culture

Bacterial cultures used for cultivation were *E. coli, S. aureus, Pseudomonas spp, Klebsiella spp, Bacillus spp*. Then these bacteria were streaked on the freshly prepared Nutrient agar medium from the original stock culture. The cultures were allowed to incubate at 37°C for 24 hours.

2.5. Microbial inoculation into alternative media

The young cultures of test bacteria (*E.coli, S.aureus, Klebsiella, Pseudomonas, and Bacillus*) were inoculated in each alternative culture medium. Then all the plates were incubated at 37°C for 24 hours. After the incubation, all the plates were observed for microbial growth and pigmentation.

2.6. Analysis of Bacterial growth in formulated media

Bacterial growth was checked at 37°C for total duration of 24 hours. Fresh cultures of test organisms were

inoculated in Nutrient agar, agar powder and formulated medium. The presence/absence of bacterial growth in formulated media was visually observed [1].

2.7. Estimation of proteins and carbohydrates:

Protein was estimated by Folin Lowry's method while carbohydrates content was analyzed by DNSA method.

2.7.1. Folin Lowry's method

The phenolic group of tyrosine and tryptophan residues (amino acid) in a protein will produce a blue purple color complex, with maximum absorption in the region of 660 nm wavelength, with Folin- Ciocalteau reagent which consists of sodium tungstate molybdate and phosphate. Thus the intensity of color depends on the amount of these aromatic amino acids present and will thus vary for different proteins.

Different dilutions of BSA solutions were prepared by mixing stock BSA solution (1 mg/ml) and water in the test tube. The final volume in each of the test tubes was 5 ml. The BSA range was 0.05 to1 mg/ml. From these different dilutions, 0.2 ml protein solution to different test tubes was pipetted out and 2 ml of alkaline copper sulphate reagent was added and mixed well. This solution was incubated at room temperature for 10 mins. Then 0.2 ml of reagent Folin Ciocalteau solution was added to each tube and incubated for 30 min. Zero the colorimeter with blank and taken the optical density at 660 nm [13].

2.7.2. Dinitrosalicylic acid method (DNSA)

3, 5-Dinitrosalicylic acid (DNSA) is used extensively for the estimation of reducing sugars. It detects the presence of free carbonyl group (C=O) of reducing sugars. This involves the oxidation of the aldehyde functional group (in glucose) and the ketone functional group (in fructose). During this reaction DNSA is reduced to 3-amino5-nitrosalicylic acid (ANSA) which under alkaline conditions is converted to a reddish brown colored complex which has an absorbance maximum of 540 nm.

Glucose standard solution in 0.05 M acetate buffer (pH 4.8) was prepared and added 1 ml of each standard to separate tubes. To the tubes used as the blanks, added 1ml of 0.05 M acetate buffer. Unknown samples in an appropriate dilution were prepared. To each tube, 1 ml of 0.05 M acetate buffer was added and mixed. 3 ml DNS reagent was added to all the test tubes and mixed well. The tubes were placed in boiling water for 5 minutes, to room temperature and measured the absorbance at 540 nm [14].

3. RESULTS AND DISSCUSION

Bacterial cultures used for analysis were *E.coli, S.aureus, Pseudomonas spp, Klebsiella spp, Bacillus spp*. These bacteria were streaked on the freshly prepared Nutrient agar medium from the original stock culture.

The present study was aimed at replacing nutrient media by alternatives such as vegetable and fruit waste such as Carrot peels, Orange peels, Pomegranate peels, Beetroot peels, Potato peels, Corn peels, Banana peels, Drumstick peels, Pineapple peels, Coconut coir, Papaya peels was used in formulation of media. The above formulated medias supported growth of bacteria such as *E.coli, Pseudomonas spp, S.aureus, Klebsiella spp, Bacillus spp*. In preliminary study it was found that *E. coli, Pseudomonas and Klebsiella* grew well in all formulation in 24 hours. The best formulated media is Beetroot media, Papaya media, Pomegranate media and Potato media as it shows the luxuriant growth of all five bacteria. *Klebsiella spp* and *Pseudomonas spp* grows luxuriantly in all media. *S. aureus* only grows in papaya media, Beetroot media and Potato media. *Bacillus spp* grows well in Orange media, Pomegranate media, Coconut coir and Beetroot media. The colonial characteristics of both the commercially prepared agar and the formulated media were compared for each isolate. These results of the study suggest that Papaya peel agar and Potato peel agar could be used as an enrichment medium for the growth of bacteria.

In the work of Wasa Alibe *et al*., [11], Banana peel and maize cob were collected from different locations within Gombe main market from various banana and maize sellers. The collected materials were air dried, grinded into fine particles using mortar and pestle and then sieved with 0.8 mm sieve size. Bacteria isolates (*Escherichia coli, Staphylococcus aureus, Shigella* sp., *Salmonella* spp., *Klebsiella* sp., and *Pseudomonas aureginosa*) were collected from Federal Teaching Hospital Gombe and confirmed using relevant biochemical tests and the fungi isolates were isolated from spoilt bread and orange using potato dextrose agar (PDA) and the formulated media. Proximate analysis carried out, show that the banana peel contained 18.56% moisture, 3.05% ash, 7.20% fat, 16.54% protein, 15.42% crude fibre and 45.23% carbohydrate, while the corresponding values for maize cob is 10.14% moisture, 2.86% ash, 2.20% fat, 14.18% protein,

13.26% crude fibre and 59.36% carbohydrate. The bacterial isolates were sub-cultured onto commercially prepared nutrient agar medium and the formulated banana peel and maize cob media. The colonial characteristics of both the commercially prepared agar and the formulated media were compared for each isolate. Fungi were also isolated from spoilt bread and orange using commercially prepared potato dextrose and the formulated banana peel and maize cob media. Three fungi were isolated and identified; the cultural characteristics of each fungus on each medium were compared. The result of the study suggest that banana peel agar could be used as an enrichment medium for the growth of fungi, while maize cob agar could be more useful in bacterial growth medium [11].

In the work of Dr. Chanda V. Berde *et al*., [15] GCO media supported the growth of bacteria, yeast as well as fungi. There was no significant variation in the colony morphology of bacterial and yeast cultures. The pigmentation was slightly affected in case of *Pseudomonas* sp. but not in *Sarcina* sp. The extracellular pigment might be reacting with the media components leading to the change in colour of the pigment produced by *Pseudomonas* sp. intracellular pigment of *Sarcina* sp. remains unaffected. No variation was observed in the growth of yeast *Saccharomyces cerevisiae* as well as *Candida* sp. on Sabauraud's agar and GCO agar media. The protein and sugar composition was estimated by standard methods and showed presence of 24.2 mg/dl and 28 mg/dl of sugar and protein, respectively. Thus, being rich in these components, the media is able to support growth of microorganisms. The growth results obtained using GCO media prove its efficiency as a alternative cheap media for routine microbiological experimentation [15].

In the work of Nidhi Tripathi *et.al.* [16], it was observed that maximum growth of all the isolates was observed in the media containing Coconut Coir. With their findings it was concluded that if plant friendly microorganism is supplemented with aqueous exact of coir fibers it would be beneficial in the soilless hydroponics of vegetables. Bacteria were isolated from the roots of vegetables. The bacterial isolates were sub-cultured onto commercially prepared nutrient agar medium, minimal media and formulated coconut coir media. They concluded that results of the present study revealed that proper use of coir powder and coir aqueous extract with microorganisms will be beneficial for plant growth. This study opens the new idea for further studies on the effect of coir powder and coir extract on soilless culture of vegetables and pot experiments on vegetables soil inoculated with plant friendly microorganisms [16].

Table 1: Morphological Characteristics

Sr.No	Test Organism	Gram Staining	Motility
1.	*Escherichia coli*	Gram-negative	Motile
2.	*Staphylococcus aureus*	Gram-positive	Non-motile
3.	*Klebsiella spp*	Gram-negative	Non-motile
4.	*Pseudomonas spp*	Gram-negative	Motile
5.	*Bacillus spp*	Gram-positive	Motile

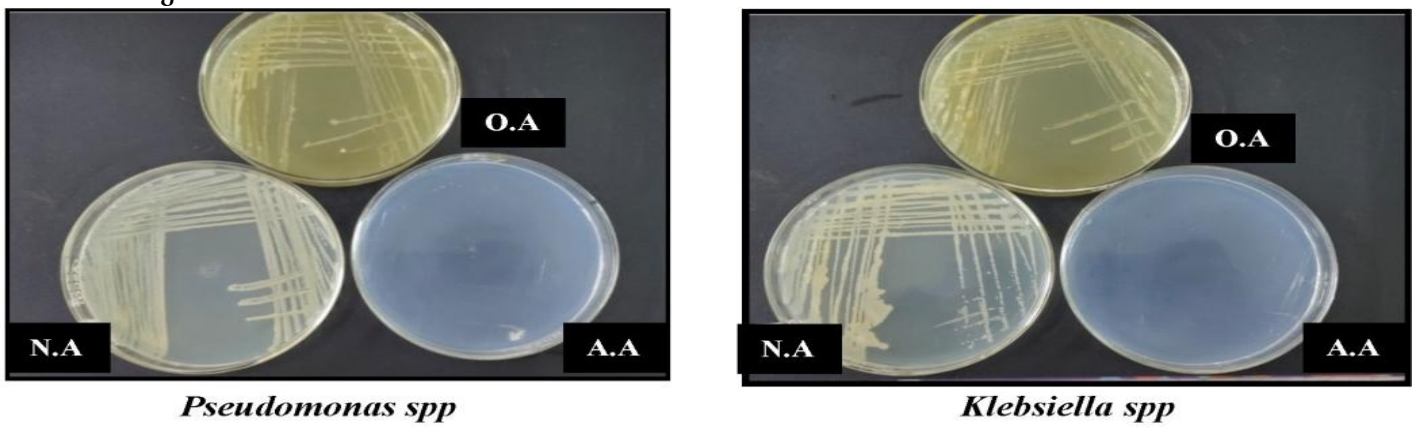
Table 2: Analysis of Bacterial growth in formulated media

Sr. No	Peel wastes	*E.coli*	*S.aureus*	*Klebsiella spp*	*Pseudomonas spp*	*Bacillus spp*
1.	Orange peel	++	-	+++	+++	+++
2.	Pomegranate peel	+++	+	+++	+++	+++
3.	Pineapple peel	+++	-	+++	+++	-
4.	Papaya peel	+++	+++	+++	+++	+
5.	Drumsticks peel	++	-	+++	+++	-
6.	Corn peel	+	-	+++	+++	-
7.	Banana peel	+++	-	+++	+++	-
8.	Coconut coir	+	-	+++	+++	+++
9.	Carrot peel	+++	+	+++	+++	-
10.	Beetroot peel	+++	++	+++	+++	+++
11.	Potato peel	+++	++	+++	+++	+

Note: *+++ : Luxuriant growth, ++ : Moderate growth, + : Poor growth, -: No Growth,*

3.1. Formulated Media

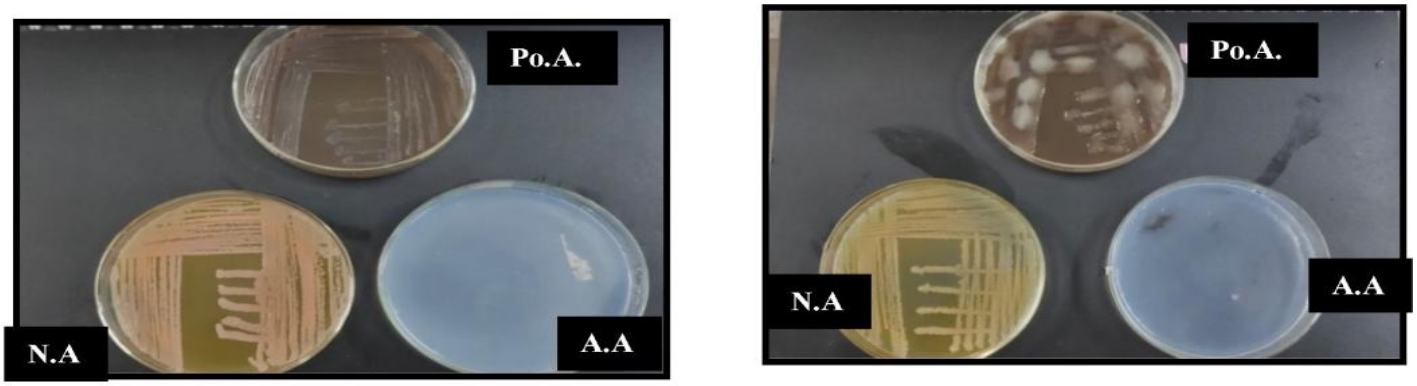
3.1.1. Orange Peel Media



Pseudomonas spp *Klebsiella spp*

Fig. 1: Bacteria streaked on orange peel media, NA and Agar Agar respectively

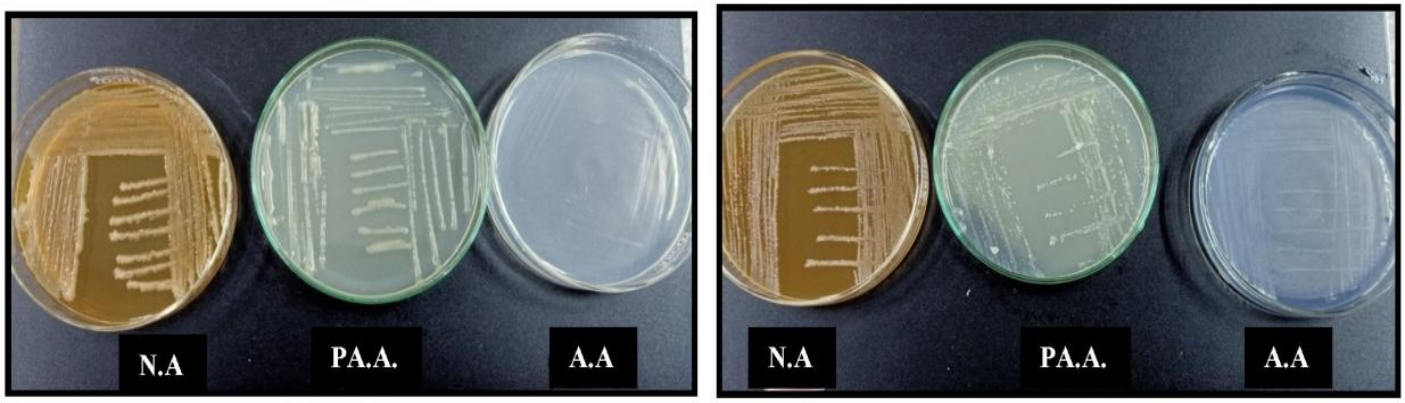
3.1.2. Pomegranate peels media



E.coli *Klebsiella spp*

Fig. 2: Bacteria streaked on Pomegranate peel media, NA and Agar Agar respectively

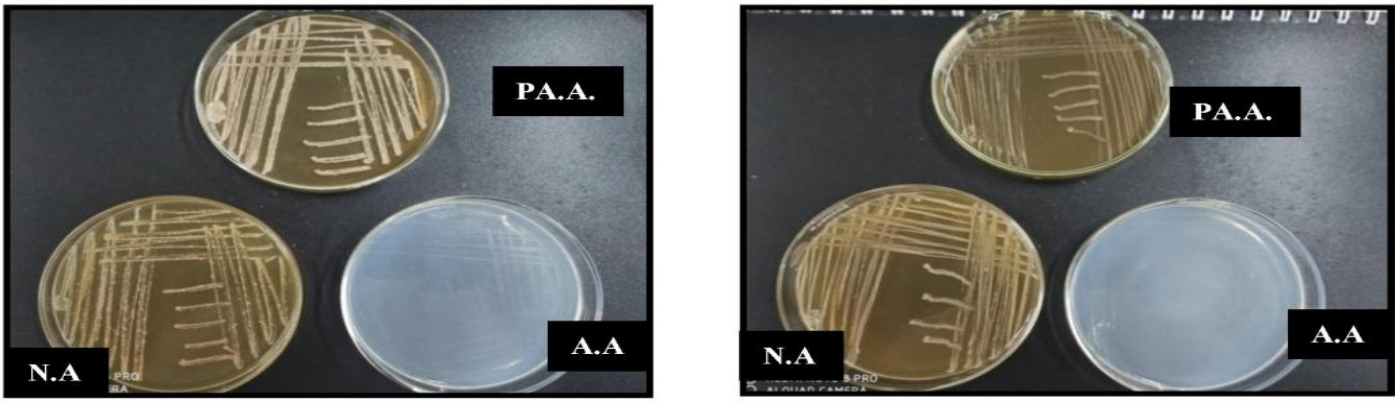
3.1.3. Pineapple peels media



Klebsiella spp *Pseudomonas spp*

Fig. 3: Bacteria streaked on Pineapple peel media, NA and Agar Agar respectively

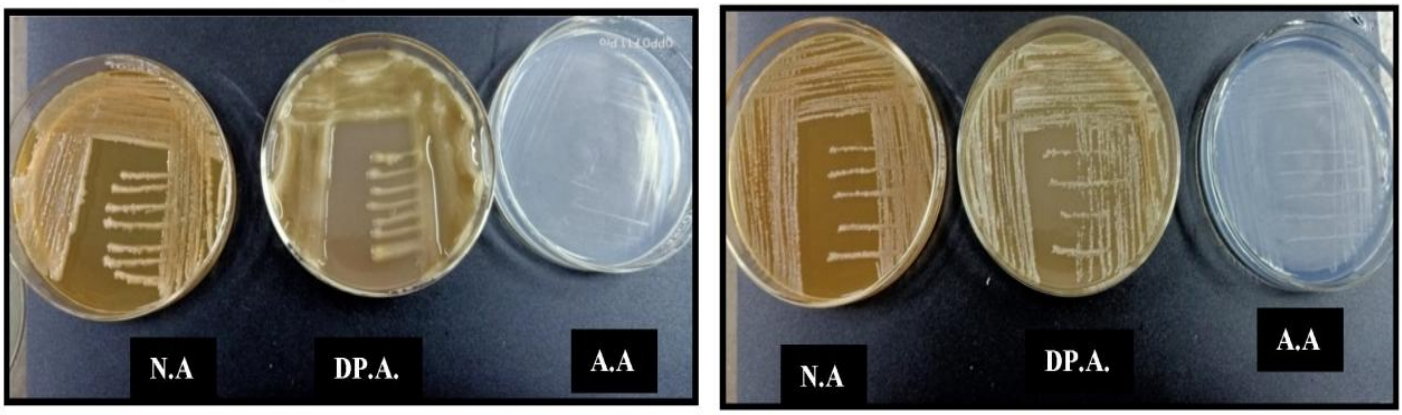
3.1.4. Papaya peels media



Pseudomonas spp *S. aureus*

Fig. 4: Bacteria streaked on Papaya peel media, NA and Agar Agar respectively

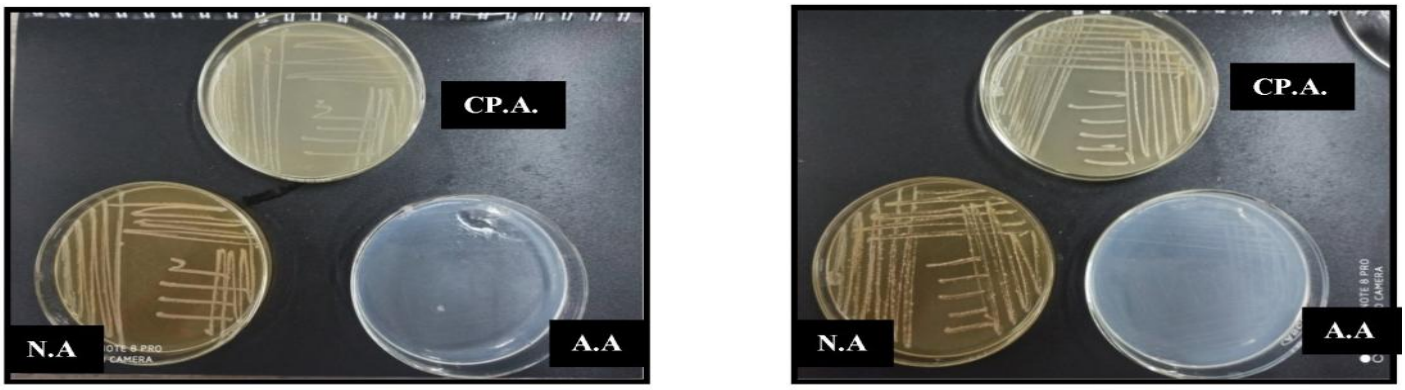
3.1.5. Drumstick peels media



Klebsiella spp *Pseudomonas spp*

Fig. 5: Bacteria streaked on Drumstick peel media, NA and Agar Agar respectively

3.1.6. Carrot peels media



E.coli *Pseudomonas spp*

Fig. 6: Bacteria streaked on Carrot peel media, NA and Agar Agar respectively

3.1.7. Beetroot peels media

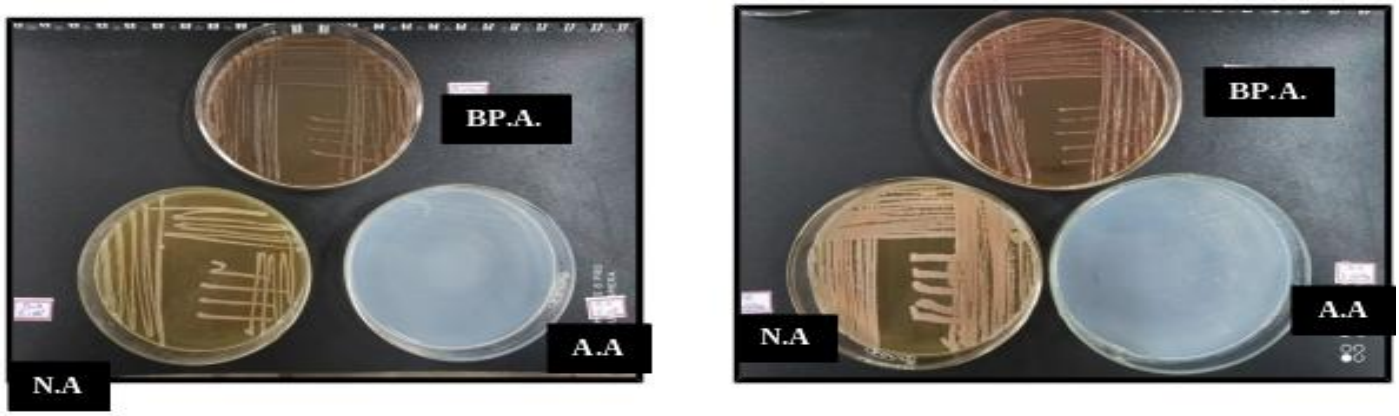


Fig. 7: Bacteria streaked on Beetroot peel media, NA and Agar Agar respectively

Table 3: Analysis of Bacterial growth in different combination of formulated compound media

Sr. No	Peel wastes	*E.coli*	*S.aureus*	*Klebsiella spp*	*Pseudomonas spp*	*Bacillus spp*
1.	Orange peel, Drumstick peel, Papaya peel, Potato peel, Banana peel	++	-	+++	+++	+++
2.	Corn peel, Beetroot peel, Carrot peel, Pomegranate peel, Pineapple peel	+++	+	+++	+++	+++
3.	Orange peel, drumstick peel, papaya peel, Potato peel, Banana peel, Corn peel, Beetroot peel, Carrot peel, Pomegranate peel, Pineapple peel	+++	-	+++	+++	-

3.1.8. Mix 1: Orange peel, Drumstick peel, Papaya peel, Potato peel, Banana peel

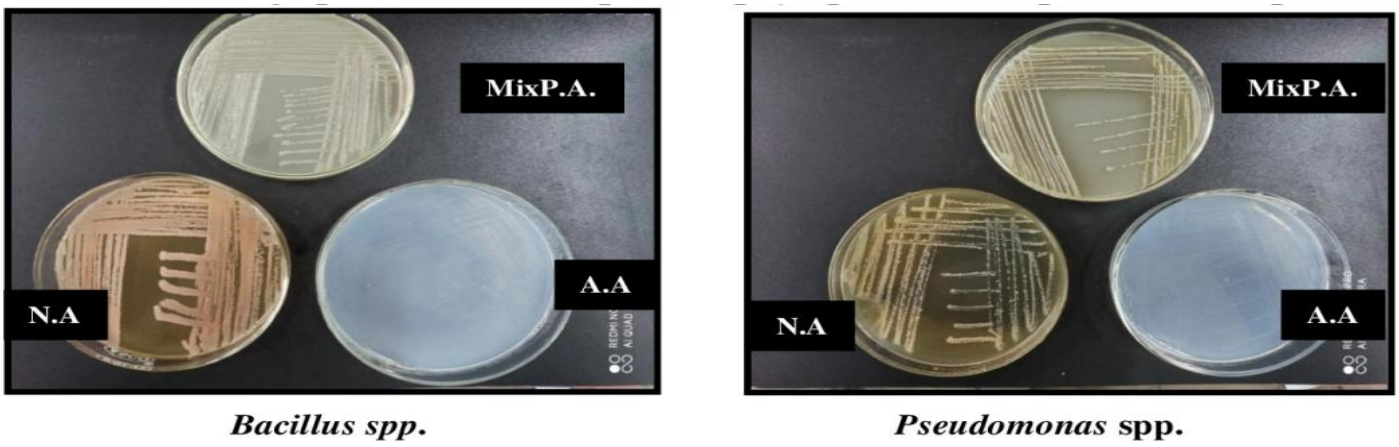
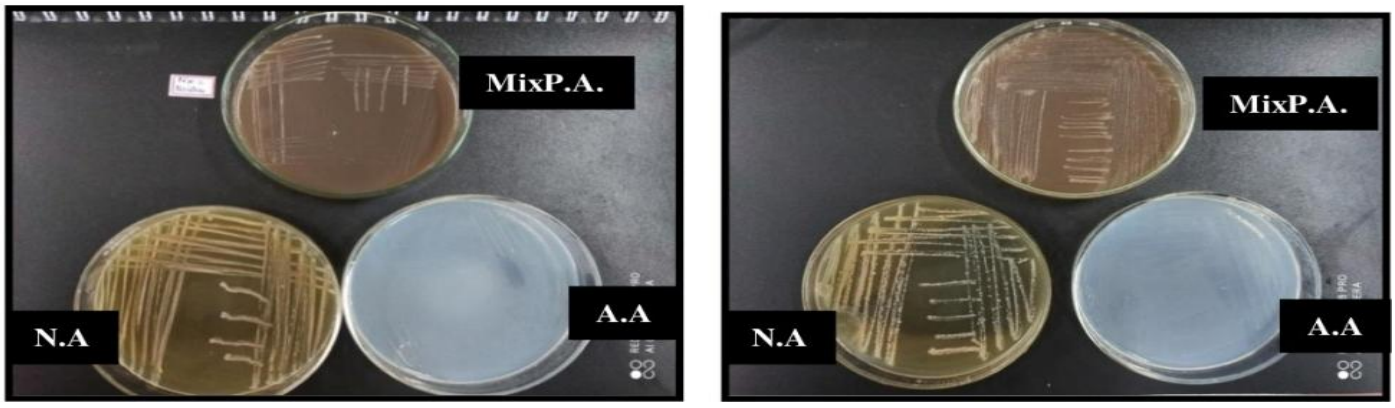


Fig. 8: Bacteria streaked on Mix 1 media, NA and Agar Agar respectively

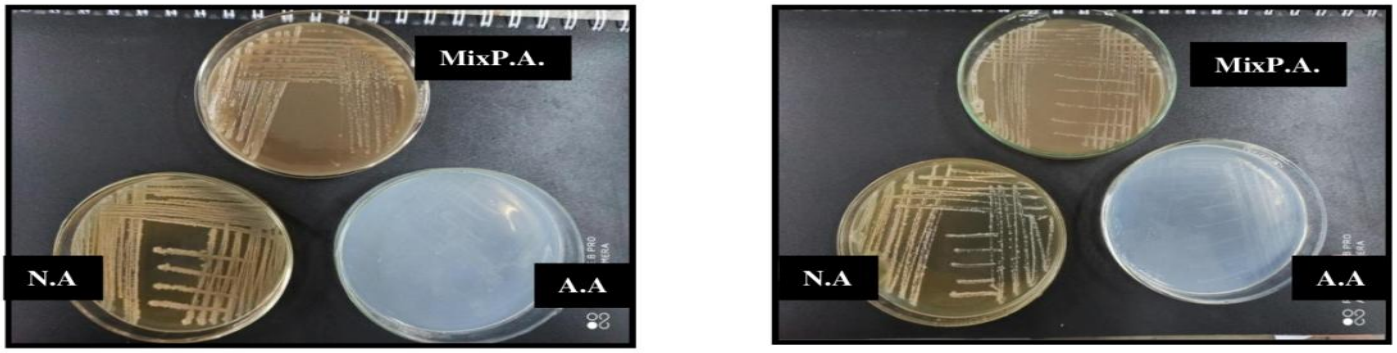
3.1.9. Mix 2: Corn peel, Beetroot peel, Carrot peel, Pomegranate peel, Pineapple peel



Bacillus spp. *Pseudomonas* spp.

Fig. 9: Bacteria streaked on Mix 2 media, NA and Agar Agar respectively

3.1.10. Compound Media: Orange peel, drumstick peel, papaya peel, Potato peel, Banana peel, Corn peel, Beetroot peel, Carrot peel, Pomegranate peel, Pineapple peel



Klebsiella spp. *Pseudomonas* spp.

Fig. 10: Bacteria streaked on Compound media, NA and Agar Agar respectively

Table 4: Concentration of sugar and protein in alternative media

Sr. No	Alternative media	DNSA (Reducing sugar) mg/ml	Folin Lowry (Protein) mg/ml
1.	Orange peel media	89	46
2.	Pomegranate peel media	119	46
3.	Pineapple peel media	86	38
4.	Papaya peel media	81	60
5.	Drumstick peel media	23	70
6.	Corn peel media	88	28
7.	Banana peel media	83	55
8.	Coconut coir media	50	30
9.	Carrot peel media	91	51
10.	Beetroot peel media	44	115
11.	Potato peel media	50	18

3.2. Protein estimation

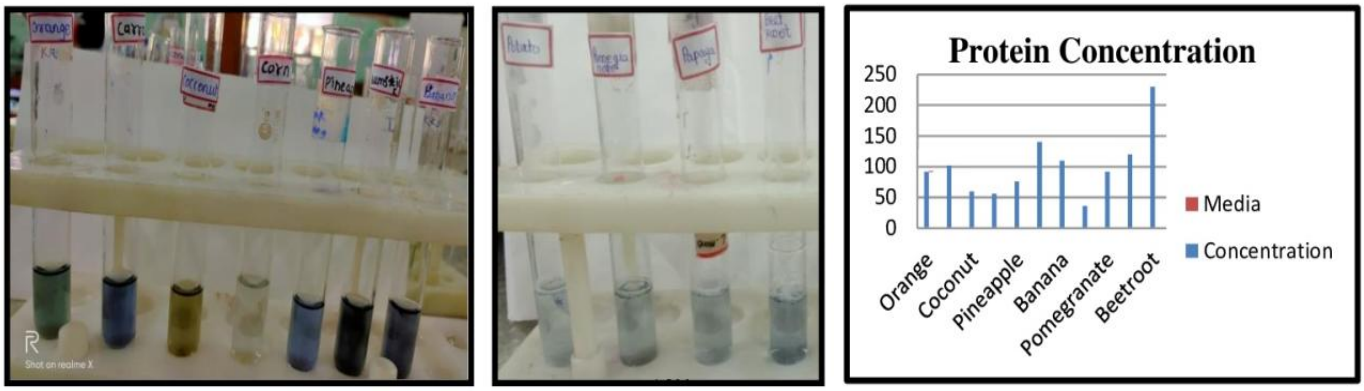


Fig. 11: Protein estimation by Folin Lowry method

3.3. Carbohydrate Estimation

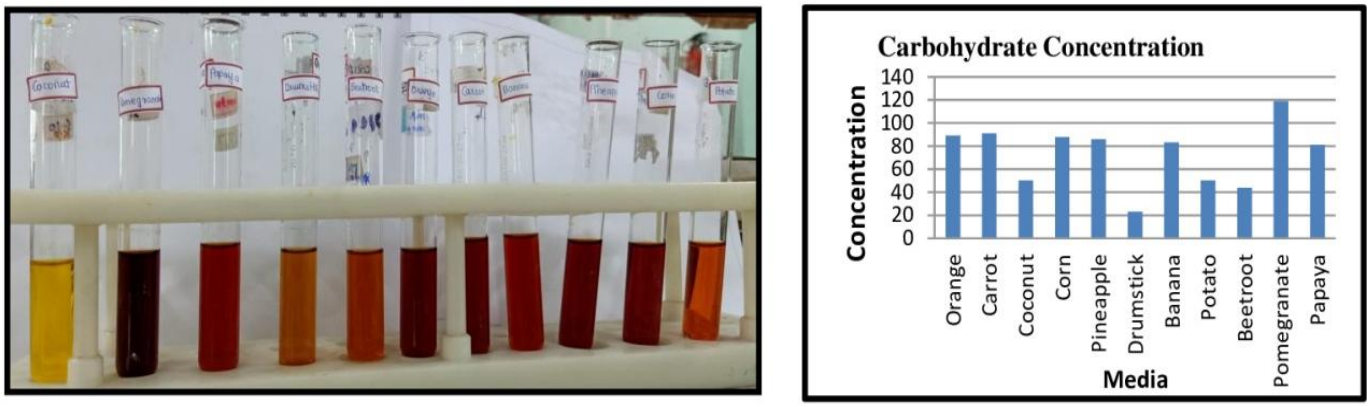


Fig. 12: Carbohydrate estimation by DNS (Dinitro salicylic acid) method

4. ACKNOWLEDGEMENT

Authors would like to express their gratitude to all colleagues and all the technical staff for their assistance in the laboratories of Department of Microbiology.

Conflicts of Interest

There are no conflicts of interest.

5. REFERENCES

1. Jadhav P, Sonne M, Kadam A, Patil S, Dahigaonkar K, Oberoi JK. *International Journal of Current Research and Review*, 2018; **2(10)**:6-11.
2. Gao LM, Sun H, Liu XZ, Che YS. *Mycological Research*, 2007; **111**:87-92.
3. Saranraj P, Anbu S. *International journal of innovation and applied studies*, 2017; **2(1)**:59-71.
4. Saheed OK, Jamal P, Karim MIA, Alam Z, Muyibi SA. *J Biol. Sci*, 2013; **13(5)**:379-385.
5. Tijani IDR, Jamal P, Alam M, Mirghani M. *Int Food Res J.* 2012; **19(2)**:427-432.
6. Aggelopoulos T, Katsieris K, Bekatorou A, Pandey A, Banat IM, Koutinas AA. *Food Chem*, 2014; **145**:710-716.
7. Ravathie A, Sevvel P, Nirmala R, Kularajany N. *Journal of Natural Product and Plant Resources*, 2012; **2**:697-700.
8. Basu S, Bose C, Ojha N, Das N, Das J, Pal M, Khurana S. *Bioinformation*, 2015; **11(4)**:182-184.
9. Sathiya VS, Vasantha RS, Senthil KRP, Jagannathan. *Journal of Applied Pharmaceutical Science*, 2013; **3 (11)**:040-044.
10. Deivanayaki M, Iruthayaraj AP. *Inter J Biosci.*, 2012; **2**:47-51.
11. Wasas AD, Huebner RE, Klugman KP. *Journal of Clinical Microbiology*, 1999; **37(6)**:2045-2046.
12. Adesemoye AO, Adedire CO. *World J Microbial & Biotech.*, 2005; **21**:329-336.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. *Journal of Biological Chemistry*, 1951; **193(1)**:265-275.
14. Miller Gail Lorenz. *Anal. Chem.*, 1959; **31(3)**:426-428.
15. Berde CV, Berde VB. *World Journal of Pharmacy and Pharmaceutical Sciences,* 2015; **5(4)**:1488-1499.
16. Tripathi N, Choudhary A Rawat R. *Inter. J. of Applied and Pure Sci. and Agriculture*, 2018; **1(4)**:31-34.