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Biosynthesis of Culture Media for Microbial Growth

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Abstract

This study explores the use of plant-based culture media as sustainable alternatives to traditional nutrient agar for microbial cultivation. Two types of plant-based media were developed: one using China grass as a solidifying agent and the other incorporating agar. These were compared with conventional nutrient agar. Microbial cultures were inoculated onto each medium and incubated under optimal conditions, with growth patterns and colony characteristics observed and documented over time. The results showed distinct differences in microbial growth among the media types. Both plant-based media supported microbial growth, but variations in growth rates and colony morphology were noted compared to nutrient agar. These findings suggest that plant-based media could serve as environmentally friendly alternatives in microbiological studies, although further optimization and exploration of their compositions are necessary to enhance their utility.

Keywords: Plant-based culture media, sustainable alternatives, nutrient agar, microbial cultivation, China grass, agar, microbial growth, colony morphology, environmental sustainability, microbiological studies, growth patterns, media optimization

INTRODUCTION

This study evaluates the potential of plant-based culture media as sustainable alternatives to traditional nutrient agar for microbial growth. Nutrient agar, long established as a cornerstone for cultivating various microorganisms, relies on animal-based components, like beef extract, yeast extract, and peptone, which provide essential nutrients [1, 2]. However, ethical concerns about animal welfare, sustainability, and the high cost and limited availability of these components have driven interest in alternative plant-based media [3, 4]. The research focuses on comparing plant-based culture media solidified with China grass and agar against traditional nutrient agar [5, 6]. The study incorporates nutrient-rich plant ingredients, such as dal, green peas, spinach, sesame seeds, and flaxseeds to formulate media that support microbial growth. Each of these ingredients offers distinct nutritional benefits [7–9].

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- Dal and green peas are rich in proteins, carbohydrates, vitamins, and minerals, providing essential amino acids, energy sources, and cofactors for microbial metabolism.
- Spinach supplies vitamins, like A, C, and K and minerals, such as iron and calcium, which play critical roles in cellular processes and enzyme activity.
- Sesame seeds contribute oils, proteins, and minerals that enhance energy production, protein synthesis, and cellular integrity.
- Flaxseeds provide omega-3 fatty acids, lignans, and fiber, which may support membrane fluidity and promote beneficial microbial growth.

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To prepare the media, plant ingredients were powdered, mixed with distilled water, and boiled to extract soluble nutrients. This process mimics the nutrient composition of conventional agar while addressing the heat sensitivity and insolubility of certain nutrients [10–12]. Subsequent centrifugation removed insoluble particles, yielding a nutrient-rich plant broth. The broth was solidified into two media types: one using traditional agar and the other with food-grade agar agar (China grass) for cost-effective, animal-friendly solidification [11, 13]. Media preparation was conducted aseptically, poured into petri plates, and inoculated with bacteria from serially diluted tap water samples using the spread plate technique [14, 15]. Plates were incubated under optimal conditions to assess microbial growth patterns, colony morphology, and diversity [16, 17].

Results from this study aim to highlight the feasibility and efficacy of plant-based media in microbiological research. By providing a sustainable, ethical, and cost-effective alternative to traditional media, this approach promotes inclusivity and reduces reliance on animal-derived components. In conclusion, plant-based media represent a promising avenue for advancing microbial cultivation practices while addressing ecological and ethical concerns [18–22].

MATERIALS AND METHODS

Ingredients for Media: Dal, Green pea, Spinach, Sesame seed, Flaxseed, China grass, Agar powder, Nutrient agar powder.

Procedure

- *Preparation of Plant Materials:* Collect fresh plant materials, such as dal, green pea, spinach, sesame seed, and flaxseed. Wash thoroughly to remove dirt or debris. Air dry the materials in a well-ventilate area or use a food dehydrator (Figure 1).
- *Grinding and Weighing:* Once dry, grind the materials into fine powders using a blender or food processor. Ensure uniform texture.

Weigh the required amounts:

- *Dal:* 8.5 g.
- *Green pea:* 10 g.
- *Spinach:* 24 g.
- *Sesame seed:* 13 g.
- *Flaxseed:* 15.5 g.

1. *Heating and Dissolution:* Add distilled water to the powdered mixture in a container. Heat on a hot plate while stirring continuously for 5–10 minutes until dissolved. Cool the mixture to room temperature.
2. *Centrifugation for Extraction:* Transfer the mixture into centrifuge tubes and centrifuge at 8000 rpm for 7–10 minutes. Collect the supernatant (liquid extract) and transfer it to a clean container.
3. *Preparation of China Grass Solution:* Weigh 2 g of China grass for every 50 ml of water.
4. Soak the grass in water and heat until fully dissolved.
5. *Incorporating China Grass into the Extract:* Mix the China grass solution with the extract.
6. Heat the mixture while stirring until consistent.
7. *Preparation of Agar Solution:* Weigh the appropriate amount of bacteriological agar. Heat the mixture until dissolved, forming a clear solution.
8. *Incorporating Agar into the Extract:* Combine the agar solution with the extract. Heat and stir to achieve uniformity.

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Preparation of Bacteriological Nutrient Agar

Weigh and mix the following:

- *Beef extract:* 0.3 g.
- *Peptone:* 0.5 g.
- *Agar:* 1–2 g.
- *Sodium chloride:* 0.5 g.

Add to 100 ml distilled water, heat, and stir until dissolved.

- *Media Sterilization:* Transfer the media into containers and plug them with cotton. Autoclave at 121°C and 15 psi for 15–20 minutes. Allow the media to cool inside the autoclave.
- *Sample Collection:* Collect water samples in sterile bottles.
- *Serial Dilution:* Label sterile tubes for dilutions (e.g., 10^{-1}, 10^{-2}). Perform sequential dilutions using sterile water or saline.
- *Media Pouring:* Pour cooled agar media into Petri dishes (20 ml per dish). Allow plates to solidify and label them.
- *Plating the Samples:* Using a sterile pipette, transfer 0.1 ml from the 10^{-6} dilution onto an agar plate. Spread the inoculum evenly without puncturing the agar.
- *Incubation:* Leave plates with lids slightly ajar to evaporate moisture. Incubate plates at 37°C.
- *Colony Counting:* After 24 hours, observe and record bacterial colony growth.

RESULTS AND DISCUSSION

The following table presents the incubation period, colony morphology, and the number of colonies formed on different media types:

Nutrient Agar Medium

The nutrient agar medium consistently promoted robust bacterial growth, with a steady increase in the number of colonies over time. This confirms its reliability and effectiveness as a culture medium for bacterial proliferation (Figures 2 and 3).

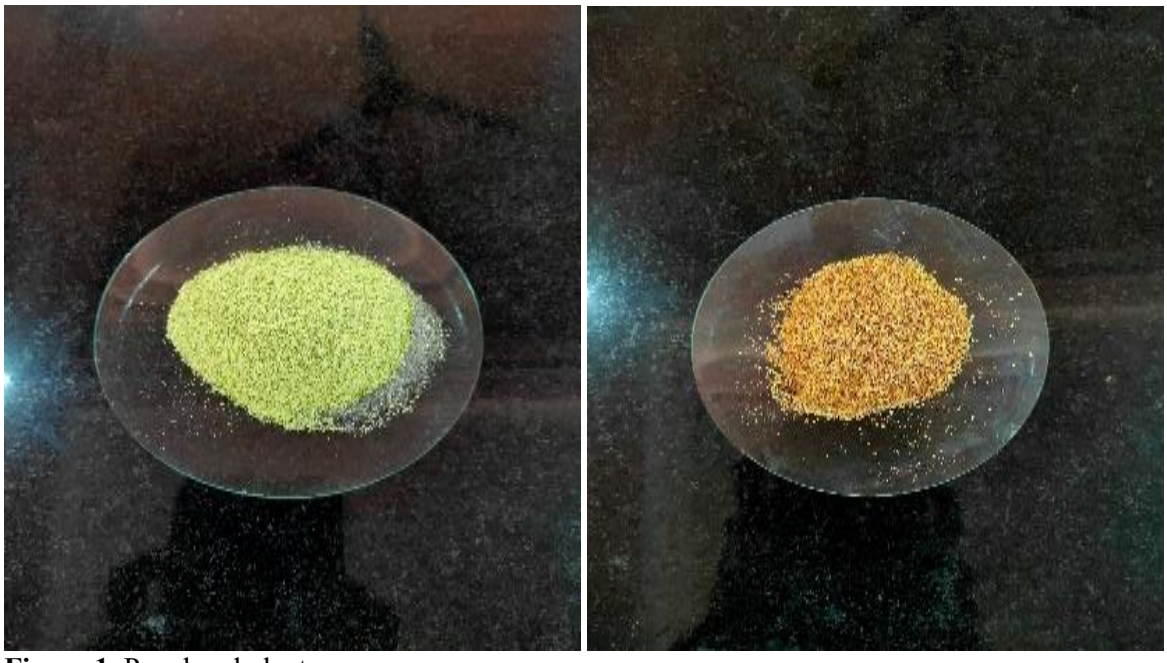

Figure 1. Powdered plant sources.

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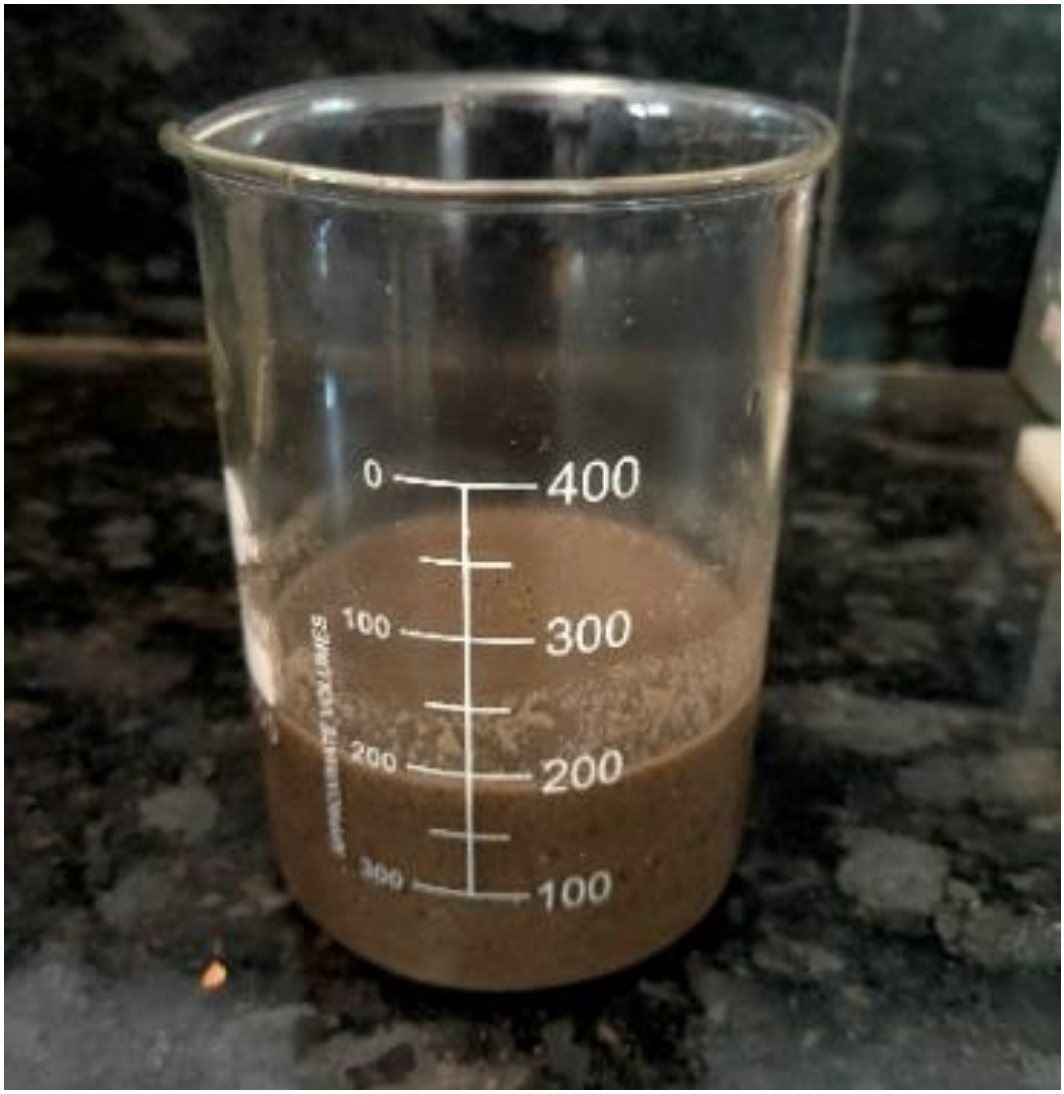

Figure 2. Plant based nutrient broth.

Figure 3. Sterilized media.

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Plant Agar and Plant China Grass Media

Both plant-based media supported bacterial growth at slower rates compared to nutrient agar. During the initial incubation period (48 hours), nutrient agar showed a moderate number of colonies as bacteria adapted to the environment (lag phase) (Table 1). Plant agar supported modest bacterial growth, with visible colonies forming. In contrast, plant China grass medium initially lacked colonies but showed progress with one colony observed after 48 hours (Figure 4).

Table 1. Bacterial growth over time on different plant-based and nutrient-agar media at 37°C.

Incubation Period (37°C)	Agar-Based Plant Source	China Grass Based Plant Source	Nutrient-Agar Based
48 hours	1	1	3
72 hours	4	1	9
96 hours	9	4	16
120 hours	13	6	25
144 hours	17	9	32

In Table 1, the number of colonies observed in each media.

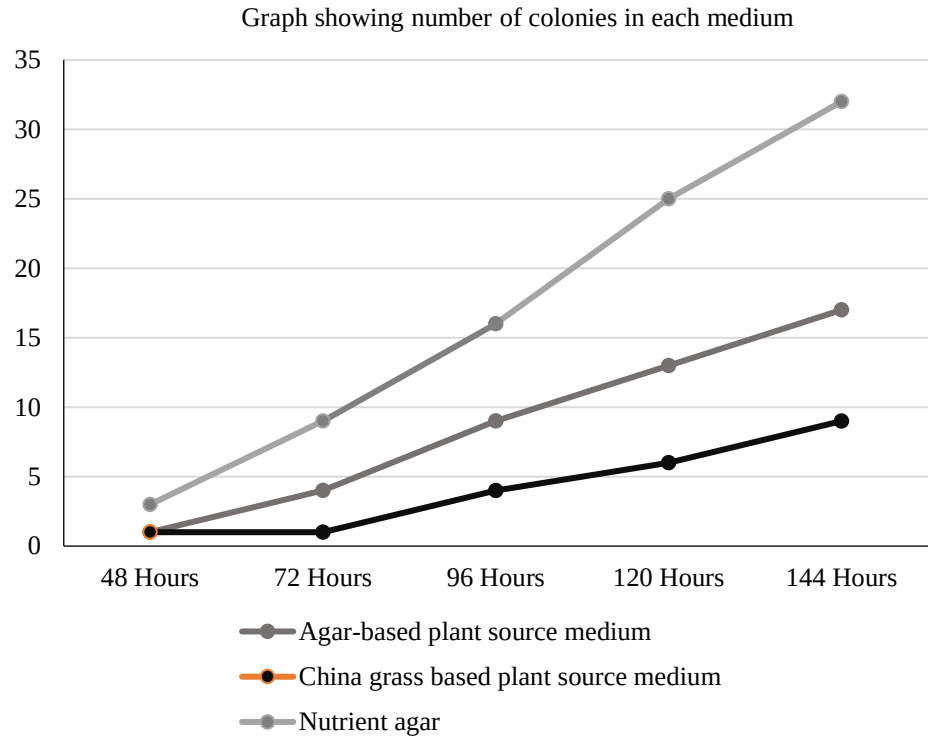

Figure 4. Graph showing the number of colonies in each medium.

Extended Incubation (72–144 Hours)

Over time, plant agar exhibited a gradual increase in colony numbers, while plant China grass media demonstrated slower but sustained growth. Nutrient agar outperformed both plant-based media, with a higher and faster proliferation rate.

CONCLUSIONS

The experiment demonstrated that plant-based culture media, incorporating ingredients, such as dal, green pea, spinach, sesame seed, and flaxseed, along with China grass or agar as solidifying agents, can effectively support microbial growth. When compared to conventional nutrient agar, the plant-

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based media showed comparable or superior performance in facilitating microbial proliferation. The findings suggest that plant-based media offer a viable alternative or complement to traditional nutrient agar in microbiological research and applications. The use of natural, renewable plant materials aligns sustainability goals by reducing reliance on synthetic or animal-derived components.

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REFERENCES

1. Adesemoye AO, Adedire CO. Use of cereals as basal medium for the formulation of alternative culture media for fungi. World journal of microbiology and biotechnology. 2005;21:329–336.
2. Ahmed E, Sarhan MS, Nemr RA, Elsawey H, Abdel-Fadeel MR, Sibroe-Daanaa HA, et al. Culturing without culture media: Echoing the plant inoculum effect into the plant-only-based culture media. In: Conference: The Plant Microbiome: Exploration of Plant-Microbe Interactions for Improving Agricultural ProductivityAt: Technische Universität Berlin Campus El Gouna, El Gouna, Hurghada, Red Sea, EgyptAffiliation: Cairo University 2018. doi: 10.13140/RG.2.2.15017.95846.
3. Arya SS, Salve AR, Chauhan S. Peanuts as functional food: A review. J Food Sci Technol. 2016;53(1):31–41. doi: 10.1007/s13197-015-2007-9.
4. Barreca D, Nabavi SM, Sureda A, Rasekhian M, Raciti R, Silva AS, et al. Almonds (Prunus dulcis Mill. DA webb): A source of nutrients and health-promoting compounds. Nutrients. 2020;12(3):672. doi: 10.3390/nu12030672.
5. Batool M, Ranjha MM, Roobab U, Manzoor MF, Farooq U, Nadeem HR, et al. Nutritional value, phytochemical potential, and therapeutic benefits of pumpkin (Cucurbita sp.). Plants (Basel). 2022 May 24;11(11):1394. doi: 10.3390/plants11111394.
6. Bhuvaneswari S, Manonmani AM, Geetha I. Cost-effective medium for the production of mosquito pupicidal lipopeptide from Bacillus subtilis subsp. subtilis (VCRC B471). J Vector Borne Dis. 2015;52(1):58–62.
7. Deivanayaki M, Antony IP. 20123199022, English, Journal article, Bangladesh, 2222-5234 2220-6655, 2, (5), Dhaka, International Journal of Biosciences (IJB), (47–51), Shamokal Publications, Alternative vegetable nutrient source for microbial growth. (2012).
8. Berde CV, Berde VB. Vegetable waste as alternative microbiological media for laboratory and industry. World J Pharm Pharm Sci. 2015;4(5):1488–1494.
9. Santos FP, Magalhães DC, Nascimento JD, Ramos GL. Use of products of vegetable origin and waste from hortofruticulture for alternative culture media. Food Sci Technol. 2021;42:e00621. doi: 10.1590/fst.00621.
10. Jukanti AK, Gaur PM, Gowda CL, Chibbar RN. Nutritional quality and health benefits of chickpea (Cicer arietinum L.): A review. Br J Nutr. 2012:108 Suppl 1:S11–S26. doi: 10.1017/S0007114512000797.
11. Monis J, Panuganti R, Chitnis K. Millets based alternative sustainable cost-effective culture media for microbial growth. Plant Science Today. 2024;11(2):99–104. doi: 10.14719/pst.2789.
12. Mourad EF, Sarhan MS, Daanaa HS, Abdou M, Morsi AT, Abdelfadeel MR, et al. Plant materials are sustainable substrates supporting new technologies of plant-only-based culture media for in vitro culturing of the plant microbiota. Microbes Environ. 2018;33(1):40–49. doi: 10.1264/jsme2.ME17135.
13. Ravimannan N, Arulanantham R, Pathmanathan S, Niranjan K. Alternative culture media for fungal growth using different formulation of protein sources. Ann Biol Res. 2014;5(1):36–39.
14. Pareek S. Nutritional and biochemical composition of banana (Musa spp.) cultivars. Nutritional Composition of Fruit Cultivars. 2016:49–81. doi: 10.1016/B978-0-12-408117-8.00003-9.
15. Jadhav P, Sonne M, Kadam A, Patil S, Dahigaonkar K, Oberoi JK. Formulation of cost effective alternative bacterial culture media using fruit and vegetables waste. Int J Curr Res Rev 2018;10(2):6–15. doi: 10.7324/IJCRR.2018.1022.

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16. Ramesh S, Desai H, Gupta P. Groundnut substitute media for bacteriological culture. J Evol Med Dent Sci. 2014;3(41):10306–10315. doi: 10.14260/jemds/2014/3338.
17. Shareef SA. Formulation of alternative culture media from natural plant protein sources for cultivation of different bacteria and fungi. Zanco J Pure Appl Sci. 2019;31(4):61–69. doi: 10.21271/zjpas.31.4.7.
18. Uthayasooriyan M, Pathmanathan S, Ravimannan N, Sathyaruban S. Formulation of alternative culture media for bacterial and fungal growth. Der Pharmacia Lettre. 2016;8 (1):431–436.
19. Youssef HH, Hamza MA, Fayez M, Mourad EF, Saleh MY, Sarhan MS, et al. Plant-based culture media: Efficiently support culturing rhizobacteria and correctly mirror their in-situ diversity. J Adv Res. 2016;7(2):305–316. doi: 10.1016/j.jare.2015.07.005.
20. Acuña JJ, Marileo LG, Araya MA, Rilling JI, Larama GA, Mora ML, et al. In situ cultivation approach to increase the culturable bacterial diversity in the rhizobiome of plants. J Soil Sci Plant Nutr. 2020;20:1411–1426. doi: 10.1007/s42729-020-00222-0.
21. Sarhan MS, Mourad EF, Hamza MA, Youssef HH, Scherwinski AC, El-Tahan M, et al. Plant powder teabags: A novel and practical approach to resolve culturability and diversity of rhizobacteria. Physiologia Plantarum. 2016 Aug;157(4):403–1
22. Huang CM, Chen WC, Lin SH, Wang YN, Shen FT. Exploration of root-associated bacteria from the medicinal plant Platycodon grandiflorum. Microbes Environ. 2019;34(4):413–420. doi: 10.1264/jsme2.ME19030.

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