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Original Research Article

Evaluation of Okra (*Abelmoschus esculentus*) Seeds Formulated as an Alternative Culture Media for Selected Test Bacteria

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Abstract: The increasing cost of conventional microbiological culture media have prompted the mining for alternative, cost-effective and locally formulated options. This study evaluated the potential of okra (*Abelmoschus esculentus*) seeds formulation as an alternative culture media for the growth of selected pathogenic bacteria. The following test bacteria *Klebsiella pneumoniae, Shigella flexneri, Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were characterized via Gram staining and biochemical tests. Proximate analysis of the okra seeds, formulation of okra seed agar and broth were carried out. Total colony count on okra agar and population density (0-72 hours) in the okra and nutrient broth was also ascertained. The proximate analysis okra seeds revealed moisture content (8.5%), ash content (0.5%), crude lipids or fat (1.58%), crude fiber (30.9%), crude protein (15.6%), carbohydrate (4.92%), and calorific content (220.113Kcal). Okra seed agar media was formulated by dissolving 4g of okra seed powder, 0.5g of NaCl, and 1.5g of agar (solidifying agent) into 100ml of distilled, while okra seed broth was formulated without the addition of agar. Total colony count (CFU/mL) of the test bacteria on okra agar includes *Klebsiella pneumoniae* (2.7×10^{9}) , *Shigella flexneri*, (2.9×10^{8}) , *Proteus mirabilis* (2.6×10^{9}) , *Pseudomonas aeruginosa* (1.3×10^{8}) and *Staphylococcus aureus* (3.1×10^{7}). Highest population density (CFU/mL) after 24hours incubation recorded in okra broth was demonstrated by *Klebsiella pneumoniae* (3.7×10^{8}), *Shigella flexneri*, (3.2×10^{8}) , *Pseudomonas aeruginosa* (3.8×10^{8}) and *Staphylococcus aureus* (4.0×10^{8}); while *Proteus mirabilis* (3.2×10^{8}) recorded the highest in nutrient broth. Findings demonstrated that the okra seed formulations supported significant bacterial growth and can be deployed as an alternative culture media for the isolation of some pathogenic bacteria.

Keywords: Okra Seed Agar, Okra Seed Broth, Alternative Culture Media.

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INTRODUCTION

Okra (*Abelmoschus esculentus*) is cultivated in Africa,Asia, southern Europe and America (Durazzo *et al.,* 2018). It is medical as it contains folic acid, vitamin C, amino acids, phytochemicals, thiamine, carotene and polyphenolic compounds (Ansari *et al*., 2005; Roy *et al*., 2014; Hu *et al*., 2014; Petropoulos *et al*., 2017). The leaves Besides this, okra leaves consists of natural antioxidants, polysaccharides, polyphenols, glycosides, alkaloids and volatile oils (Guebebia *et al*., 2023; Ayushi *et al*., 2016). Plants secondary metabolites hugely contain polyphenols such as tannins, phenols, flavonoids and phenolic acids (Chiocchio *et al*., 2021). As a result some database have opined that okra embodies a major hurb of flavonoids and phenolic compounds with many vital biological activities such as anti-inflammatory antioxidant, antibacterial, immunomodulatory, anti-diabetic, anticancer, anti-fatigue and organ-protective activities, that are relevant to pharmaceutical and food preparations (Islam, 2019; Kanfon *et al*., 2018). Therefore, okra is seen as a contributor to health via dietary consumption of vegetables (Guebebia *et al*., 2023). Culture media are fundamental components in microbiology, providing the necessary nutrients and environment for microorganisms to grow, be isolated, and be studied in controlled laboratory conditions (Atlas, 2010). Traditional culture media, such as nutrient agar, are widely deployed to cultivating pathogenic bacteria, which are significant for medical research, diagnostics, and disease control (Atmanto *et al*., 2002). However, conventional media can be expensive and are often derived from animal sources, raising concerns about cost, sustainability, and ethical considerations (Tortora *et al*., 2010). These challenges are especially pronounced in

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low-resource settings, where the cost of standard media and limited access can hinder microbiological studies and diagnostics (Wertheim *et al*., 2021). *Abelmoschus esculentus* (okra) is an extensively available crop in many parts of the world, known for its dietary worth and the mucilaginous properties of its seeds and pods (Adelakun *et al*., 2009). Okra seeds contain polysaccharides, proteins, and minerals that could potentially support bacterial growth, making them a viable alternative to standard culture media (Owheruo *et al*., 2023).

METHODOLOGY

Processing of Okra Seeds and Proximate Analysis of Okra Seeds Powder

Fresh and matured okra seeds (*Abelmoschus esculentus*) were collected from Amassoma and Tombia markets. Seeds were washed thoroughly under running tap water, rinsed with distilled water, and air-dried. To ensure sterility, seeds were surface-sterilized using 70% ethanol for 2 minutes followed by washing with sterile distilled water. Seeds were then oven-dried at 50°C for 24 hours to remove residual moisture and subsequently ground into a fine powder using a sterile laboratory blender, as adapted from similar procedures in plant-based media preparation (Ajiboye and Ahmad, 2025). Proximate analysis was done by estimating the water activity, mineral composition, protein content, fat and oil content, total carbohydrates and calorific value in okra seed powder (Akubor and Ogu, 2012; AOAC, 2005).

Characterization and Identification of Test Bacteria

A total of five pathogenic bacteria were selected including Gram's negative *Klebsiella pneumoniae, Shigella flexneri, Pseudomonas aeruginosa, Proteus mirabilis* and one Gram's positive bacteria *Staphylococcus aureus.* The physiological characterization of the isolates was carried out via Gram staining to confirm the isolates to be used. (Cheesbrough, 2006). Various biochemical tests such as indole test, catalase test, citrate utilization, oxidase, coagulase test and Kligler iron agar test were performed for identification of isolates (Shin *et al.,* 2020).

Optimization and Formulation of Okra Agar

The okra seed powder was measured in concentrations of 0.75g, 3g, 4g and 5g and were added into clean conical flasks with 50ml of water. Okra agar was then formulated by adding 1.5g of agar-agar to each conical flask, 0.5g of sodium chloride (Nacl) was added to each conical flask and in each conical flask the distilled water was made up to 100ml and was mixed to dissolve. The media contained in the conical flasks were covered properly with a masking tape to avoid spillage and was placed in the autoclave for sterilization at 121°C for 15minutes under 15 psi pressure. The media were mixed and was poured aseptically into sterile petri dishes and allowed to solidify (Anibijuwon *et al.,* 2017).

Formulation of Okra Broth

Okra broth was formulated by adding 3g Okra seeds powder and 0.5g of Nacl to 100ml of distilled water in the colonial flask. 5ml of this prepared medium was dispensed into the bijou bottles, which was autoclaved at 121°c under 15 minutes at 15psi (Anibijuwon *et al.,* 2017).

Inoculation and Incubation and Evaluation of Growth of Test Bacteria

Sterile petri dishes were prepared with the formulated okra seeds media. Each plate was inoculated with a loopful of selected test bacteria from stock preparation using the streak plate technique. The inoculated plates were incubated at 37°C for 18 hours. Bacterial growth was monitored by counting colonies and assessing morphological characteristics and then sterilized in the autoclave. Growth on the formulated okra agar media were assessed and compared to growth on nutrient agar plates. The evaluation was based on: Colonial morphology (color, shape, size, texture), Colony count (CFU/mL where applicable) and Growth rate (qualitative scoring: growth or no growth seen) (Osawaru *et al.,* 2013).

Serial Dilution of Test Bacteria and Total Colony Count

Two colonies each of *Klebsiella pneumoniae, Shigella flexneri, Proteus mirabilis Pseudomonas aeruginosa* and *Staphylococcus aureus* were inoculated into 5ml of nutrient broths and also 5ml of okra broth and incubated at 37^{0}c for 24hours. A 6-fold serial dilution was carried out and 0.1ml of each serially diluted isolate was inoculated in triplicates onto nutrient agar and okra agar plates. It was then evenly spread with a hockey stick on the surface of the nutrient agar and okra agar plates and incubated aerobically at 37^{0}C for 24hours (APHA,1998). The total numbers of viable colonial growth on the plates were counted on okra agar and compared with nutrient agar. For convenience the results were given as CFU/ml (colony-forming units per milliliter) and this was calculated using the formula: CFU/ml = (no. Of colonies × dilution factor)/volume of culture plate (Zhang *et al*., 2024).

Temperature and pH Analysis

The setting temperature of the okra agar and nutrient agar was checked using a digital thermometer. The thermometer was placed onto the poured plate to determine the temperature. The pH of the okra medium was analysed using a pH meter which was standardized by using an acidic buffer solution of pH 4, neutral of pH 7 and basic of pH 10. Then the pH meter was used to measure the pH of the Okra medium and compared to traditional nutrient agar (Smith *et al.,* 2021).

Absorbance and Population Density

The absorbance was measured at 625nm using spectrophotometer to ascertain the population density of bacteria isolates in okra broth and nutrient broth.

Measurements were done at time intervals of 0hours, 6 hours, 12hours, 24hours, 48hours and 72hours (Zhang *et al.,* 2024). The population density was ascertained using formula for CFU/ml (conversion of absorbance to CFU/ml):

2×10^8 X absorbance + 4×10^6

RESULT

Table 4.1 below shows the composition of ingredients for the preparation of okra culture media, and either 3g, 4g or 5g of the okra powder can be used with 0.5g of Nacl and 1.5g of agar in 100ml of distilled water.

Table 4.2 shows the presence or absence of bacteria growth on the different concentrations (in percentage %) of okra powder. Okra powder concentrations of 3%, 4% and 5% had growth seen and 0.75g demonstrated no bacteria growth compared to nutrient agar which had growth seen also for all the selected test bacteria.

Table 4.3 shows the biochemical reaction of test isolates, the test showed that all isolates are catalase positive, all except *Shigella flexneri* is indole positive, all except *Staphylococcus aureus* are coagulase negative, all except *Pseudomonas aeruginosa* is oxidase negative. *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are citrate positive but *Shigella flexneri* and *Staphylococcus aureus* are negative, *Staphylococcus* aureus, *Proteus mirabilis* and *Klebsiella pneumoniae* are urease positive while *Shigella flexneri* and *Pseudomonas aeruginosa* are negative. *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* produced acid yellow slant and butt with gas production but only *Proteus mirabilis* produced H_2S.

Table 4.4 depicts that the highest total colony count on okra agar was exhibited by *Klebsiella pneumoniae* ($2.7 x 10^9$). *Pseudomonas aeruginosa* ($1.3 x 10^8$) and *Proteus mirabilis* ($2.6 x 10^9$) exhibited similar total colony count on nutrient and okra agar.

Table 4.5 reveals that the highest population density (CFU/mL) after 24hours incubation recorded in okra broth was demonstrated by *Klebsiella pneumoniae* ($3.7 x 10^8$), *Shigella flexneri*, ($3.2 x 10^8$), *Pseudomonas aeruginosa* ($3.8 x 10^8$) and *Staphylococcus aureus* ($4.0 x 10^8$); while *Proteus mirabilis* ($3.2 x 10^8$) recorded the highest in nutrient broth.

The table 4.6 below shows the constituents and quantities in percentage that was derived from the analysis of okra seed powder in the laboratory. Total calorific content gotten is 220.113kxal/100g.

Table 4.1: Preparation of Okra Agar with Ingredients

S/N	Ingredients	Quantity
1.0	Okra powder	3g, 4g, 5g
2.0	Sodium chloride	0.5g
3.0	Agar	1.5g
4.0	Water	100ml

Table 4.2: Evaluation of Growth of the Selected Test Bacteria

Selected test bacteria	Okra agar 0.75%	Okra agar 3%	Okra agar 4%	Okra agar 5%	Nutrient agar
Klebs	No growth	Growth seen	Growth seen	Growth seen	Growth seen
Shig	No growth	Growth seen	Growth seen	Growth seen	Growth seen
Pseudo	No growth	Growth seen	Growth seen	Growth seen	Growth seen
PRO	No growth	Growth seen	Growth seen	Growth seen	Growth seen
StaphA	No growth	Growth seen	Growth seen	Growth seen	Growth seen

Key: *Shig*; *Shigella flexneri* Pseudo: *Pseudomonas aeruginosa;* StaphA: *Staphylococcus aureusKlebs*: *Klebsiella pneumoniae*; PRO: *Proteus mirabilis.*

Table 4.3: Gram reaction and biochemical tests

Selected test bacteria	Gram Staining Reaction	Oxidase	Indole	Citrate	Urease	Catalase	Cogaulase	KIA	A/A	K/A	H2S	Gas
Klebsiella pneumoniae	Gram -ve bacilli	-	-	+	+	+	-		+	-	-	+
Shigella flexneri	Gram -ve bacilli	-	+	-	-	+	-		-	+	-	-
Pseudonas aeruginosa	Gram -ve bacilli	+	-	+	-	+	-		+	-	-	+
Proteus mirabilis	Gram -ve bacilli	_	_	+	+	+	-		+	+	+	+
Staphylococcus aureus	Gram +ve cocci	_	_	-	+	+	+		+	-	-	-

Key: H2S; hydrogen sulphide, PRO; Proteus mirabilis, -ve; negative, +Ve; positive, KIA; Kligler iron agar, A/A: Acid slant/Acid butt, K/A: Alkaline Slant/Acid butt

Table 4.4: Mean total colony count on nutrient and okra agar

S/N	Test bacteria	Nutrient agar (CFU/mL)	Okra Agar (CFU/mL)
1	*Klebsiella pneumoniae*	$3.7x10^5$	$2.7x10^9$
2	*Shigella flexneri*	$2.2x10^9$	$2.9x10^8$
3	*Pseudomonas aeruginosa*	$1.3x10^8$	$1.3x10^8$
4	*Proteus mirabilis*	$2.6x10^9$	$2.6x10^9$
5	*Staphylococcus aureus*	$1.5x10^9$	$3.1x10^7$

Table 4.5: Mean population density (CFU/mL) of test bacteria in nutrient and okra broths

Test bacteria	Broth	0 hours	6 hours	12 hours	24hours	48 hours	72 hours
Klebsiella pneumoniae	Nutrient	$1.2x10^8$	$1.5x10^8$	$2.1x10^8$	$2.7x10^8$	$2.7x10^8$	$3.2x10^8$
Klebsiella pneumoniae	Okra	$3.6x10^8$	$3.2x10^8$	$3.2x10^8$	$3.7x10^8$	$3.7x10^8$	$4.1x10^8$
Shigella flerneri	Nutrient	$1.3x10^8$	$1.3x10^8$	$1.7x10^8$	$2.5x10^8$	$3.1x10^8$	$3.5x10^8$
Shigella flerneri	Okra	$3.8x10^8$	$3.4x10^8$	$3.3x10^8$	$3.2x10^8$	$3.2x10^8$	$3.3x10^8$
Pseudomonas aeruginosa	Nutrient	$8.7x10^7$	$7.9x10^7$	$1.4x10^8$	$2.3x10^8$	$1.1x10^8$	$2.3x10^8$
Pseudomonas aeruginosa	Okra	$3.5x10^8$	$3.5x10^8$	$3.6x10^8$	$3.8x10^8$	$3.2x10^8$	$3.6x10^8$
Proteus mirabilis	Nutrient	$1.2x10^8$	$1.4x10^8$	$1.9x10^8$	$3.2x10^8$	$3.6x10^8$	$4.0x10^8$
Proteus mirabilis	Okra	$3.3x10^8$	$3.5x10^8$	$3.5x10^8$	$2.8x10^8$	$3.4x10^8$	$2.7x10^8$
Staphylococcus aureus	Nutrient	$2.8x10^7$	$1.7x10^8$	$2.0x10^8$	$2.2x10^8$	$2.5x10^8$	$3.3x10^8$
Staphylococcus aureus	Okra	$3.7x10^8$	$3.9x10^8$	$3.9x10^8$	$4.0x10^8$	$4.0x10^8$	$4.0x10^8$

Table 4.6: Proximate Analysis of Okra Seeds

Constituents	Percentage (%)
Moisture content	8.5
Ash content	0.5
Crude fat	1.58
Crude fiber	30.90
Crude protein	15.60
Carbohydrates	42.92
Total calorfic content	220.113kcal/100g

DISCUSSION

The okra agar formulation consisted of NaCl (0.5g), agar (1.5g) and varying concentrations of okra powder (3g,4g and 5g) in 100ml of distilled water and all preparations solidified. In a related culture media formulation research,1gm each of yam peels, plantain peels, egg shells, pawpaw seeds, pawpaw peel and sweet potato peel were grinded and one gram was mixed in 100 ml distilled water, including varying concentrations of agar (0.35gm, 0.5gm and 1gm) but only the formulated media with 1gm agar solidified (Patience and Obaro, 2023). In another similar study, 3gm each of rice, chickpea, corn, dhal, thinai, natural soy flour and processed soy flour was dissolved in 100ml distilled water, then 1 gm of agar was added, and all formulations solidified. This may be because of concentration as the formulated culture media solidified in all experiments when the agar concentration was 1gm and above (Mekala *et al*., 2016). In a study to ascertain the growth rate of test bacteria, *Klebsiella sp*., showed significantly ($p>0.05$) high growth rate in chickpea than in rice in rice. *Pseudomonas sp*., showed significantly ($p>0.05$) high growth rate in natural soy flour than in in rice. Staphylococcus sp., showed significantly ($p>0.05$) high growth rate in Nutrient agar next to chickpea and showed less growth rate in thinai. *E.coli* showed significantly ($p>0.05$) high growth rate in chickpea than in rice. *Klebsiella sp*. showed significantly ($p>0.05$) high growth rate in chickpea than nutrient agar. *Pseudomonas sp*. showed comparatively high growth rate in natural soy flour than nutrient agar (Mekala *et al*., 2016). In this study, *Klebsiella pneumoniae* also had a higher total colony count per mL on okra agar ($2.7x10^9$) compared to nutrient agar ($3.7x10^5$). *Pseudomonas aeruginosa* ($1.3x10^8$) and *Proteus mirabilis* ($2.6x10^9$) both had the same total colony counts on okra agar and nutrient agar. However, *Staphylococcus aureus* exhibited a lower total colony count on okra agar ($3.1x10^7$) compared to nutrient agar ($1.5x10^9$). After 4hrs incubation, *Klebsiella pneumoniae* had a higher population density (CFU/mL) in okra broth ($3.7x10^8$) than nutrient broth ($2.7x10^8$). *Shigella flerneri* ($3.2x10^8$), *Pseudomonas aeruginosa* ($3.8x10^8$) and *Staphyloccoccus aureus* ($4.0x10^8$) also had higher mean population density in okra broth than nutrient broth.A study by Youssef *et al*., (2015) suggested that, there could be potential influence from plant- based media colonies on biochemical tests but Okra agar colonies showed no significant influence on biochemical tests. Okra agar sets at temperature 28°c and pH 6.37 (neutral) gave beautiful growth on all selected test bacteria and compared to Shareef (2015) which pH of medium was not specific. The Shelf life of Okra agar after kept in sterile and dry bottles away from direct sunlight stayed up to 30days and was still viable for use. Moreover, the presence of essential nutrients in okra

seeds, such as proteins, fat, ash, moisture, fiber and carbohydrates likely contributes to their suitability for bacterial cultivation similar to Shareef (2019) Formulation of alternative culture media from natural plant protein sources for cultivation of different bacteria and fungi.

CONCLUSION

The study demonstrates that okra seeds formulated culture media can potentially support the growth of selected test bacteria, offering a viable alternative to conventional nutrient agar. The media's performance in terms of bacterial growth and colonial morphology is comparable to standard traditional media, highlighting its potential for widespread application in microbiological practices. The use of okra seeds, a locally available and cost-effective resource, aligns with sustainable and accessible approaches to scientific research.

Recommendations

Based on the findings, the following recommendations are proposed:

Further Research: Investigate the efficacy of okra seeds media in cultivating a broader range of microorganisms, including fungi and fastidious bacteria, to assess its versatility.

Optimization Studies: Explore the optimization of okra seed media formulations by adjusting parameters such as pH, nutrient concentration, and sterilization methods to enhance performance.

Comparative Analyses: Conduct comparative studies between okra seed media and other plant-based media to evaluate relative effectiveness and identify the most suitable alternatives for specific applications.

Implementation in Education: Promote the use of okra seed media in educational settings to provide cost-effective and accessible resources for teaching microbiological techniques.

Scale-Up Production: Assess the feasibility of large-scale production and commercialization of okra seed media to facilitate broader adoption in clinical and research laboratories.

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