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ORIGINAL ARTICLE

GROUNDNUT SUBSTITUTE MEDIA FOR BACTERIOLOGICAL CULTURE

Saroj Ramesh[1], Hemina Desai[2], Praveg Gupta[3]

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ABSTRACT: BACKGROUND: In the developing countries manual methods are still used for the cultivation of the bacteria and many a times difficulties are involved in procuring suitable components for preparation of various nutrient media. The need has therefore arisen, to investigate the problem of exploitation of indigenous material such as groundnuts to substitute commercial preparations used in the various nutrient media for bacteriological culture. **AIMS & OBJECTIVES:** In the present study, we investigated the problem of incorporation of groundnut as an indigenous protein source, to substitute the use of commercially available nutrients in various simple and selective media. **SETTINGS & DESIGN:** We have used manual methods for preparation of the culture media using both groundnut powder and commercial preparations as a source of protein. Further the various bacteriological parameters were studied comparing the both. **MATERIAL & METHODS:** In present study, the raw groundnut powder was used in the preparation of various media and these media have been further tested for various parameters like – bacterial growth, motility, as a medium for maintenance of stock cultures and for carrying out antibiotic sensitivity tests. **RESULTS & CONCLUSIONS:** The results of groundnut substitute media for bacteriological culture was comparable to that of other commercially available media and it can be employed for routine bacteriological testing of the clinical samples.

KEYWORDS: groundnuts, bacteriological culture; substitute media

INTRODUCTION: The development of bacteriology as a subject of scientific study dates back to days of Louis Pasteur. He and his colleagues settled the fundamental question of microbial existence and developed a technique which made it possible to cultivate bacteria and study them in laboratory. One of the pressing problems of developing countries is the difficulties involved in procuring suitable components for preparation of various nutrient media.

This restricts the investigations of this nature, only to resource full laboratories. The need has therefore arisen, to investigate the problem of exploitation of indigenous material as a substitute of commercial preparations used in the various nutrient media.

With above consideration in view, it was thought worthwhile, investigating the problem of incorporation of ground nut as an indigenous protein source, to substitute the use of commercially available nutrients in various simple and selective media.

In present study, the groundnut media have been further tested for their efficacy as a culture medium and their utility to study other tests like motility and antibiotic sensitivity, and also as a medium for maintenance of stock cultures. The results of investigations carried on these lines, have been presented and discussed in this article.

MATERIAL AND METHODS: This study was carried in 3 parts (A) Preliminary analysis (B) Preparation of media and (C) Comparative study.

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(A) Preliminary analysis: Preliminary analysis was carried out to make a comparison of chemical composition of conventional media with those having incorporated substitute - groundnut. For this purpose, commercial preparation and groundnut powder were subjected to chemical analysis to estimate their moisture, ash, proteins, fat, carbohydrate and fiber contents. Details of the results of this analysis are shown in table 1.

(B) Methods of preparation of Media: The different simple and selective media were prepared incorporating groundnut powder. The procedure used was as follows:

1. **Preparation of basal groundnut broth(groundnut basal medium):**
   a) **Composition:**

Raw groundnut	500 gm
Water	5000 ml
Sodium carbonate 0.8% soln.	2500 ml
Pig pancreatic extract	50 ml
Chloroform	50 ml
Conc. Hydrochloric acid	40 ml

   b) **Procedure:** Raw groundnuts were blended with required amount of water, sodium carbonate, pancreatic extract and chloroform were added to this mixture. The medium was filtered with double gauze and incubated at 37° C for 6 hours, stirring intermittently. When digestion was complete, the conc. hydrochloric acid was added. The medium was then steamed at 100° C for 30 minutes in water bath and filtered by filter paper for several times, till the clear filtrate was obtained. The pH was adjusted to 7.6 by addition of 0.1 N NaOH. It was then autoclaved at 121°C and 15 lbs. for 15 minutes.

2. **Groundnut glucose broth:** One gram of glucose was added to 100 ml of basal groundnut broth, prepared as above. The medium was sterilized in a steam sterilizer at 100° C for 30 minutes for 3 successive days.

3. **Preparation of groundnut peptone water:** 5 gm of NaCl was added to each 1 litre of groundnut basal medium. The pH was adjusted to 7.6 and then medium was filtered. Then it was autoclaved at 121° C and 15 lb. for 15 minutes.

4. **Groundnut alkaline peptone water:** The procedure adopted was same as for groundnut peptone water except final pH was adjusted to 8.2.

5. **Groundnut agar:** This was prepared by addition of 2.5% agar to groundnut broth. This medium is sterilized by autoclave and distributed in the petri dishes or in test tubes in the form of slopes.

6. **Groundnut blood agar:** for preparation of this medium 10 ml of sheep's blood was added to every 100 ml of sterile groundnut agar just before it solidified. Medium was distributed in the petri dishes.

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7. **Groundnut Mac Conkey:**
   a) **Composition:**

Sodium taurocholate(commercial)	5 gm
Groundnut broth	1 liter
Agar	20 gm
2 % neutral red in 50 % ethanol	3.5 ml
10 % lactose aqueous soln.	100 ml

   b) **Procedure:** Sodium taurocholate was dissolved in water by heating. Agar was then added to it and dissolved. If necessary, clearing was done by filtration. The pH was adjusted to 7.5. Lactose and neutral red were then added. Thorough mixing was done. Sterilization was done by heating in autoclave with "free steam" at 100 °C for 1 hour then at 115°C for 15 minutes.

(C) Comparative study: Methods for comparative study of conventional and groundnut media included growth kinetic and other culture characteristic of various organisms from culture. Subcultures of these organisms isolated from human infections were made and the stock cultures maintained in the laboratory. The organisms studied were: (1) Staphylococcus – aureus, albus and citreus (2) Streptococcus – beta hemolyticus, viridans (3) Salmonella – typhi, paratyphi A and paratyphi B (4) Escherichia coli (5) Proteus vulgaris (6) Pseudomonas aeruginosa (7) Shigella – shigae, sonnei and flexneri (8) Vibrio cholerae – inaba type, ogawa type (9) Klebsiellapneumoniae and (10) Bacillus subtilis.

The details of the type of study carried out with each of the substitute medium and conventional medium, using different organisms are shown in table 2.

1. **Quantitative study of Growth:** This was carried out by periodic total counts from growth obtained on different substitute and conventional liquid media at the intervals of 2, 4, 8, 12, 16, 20, 24 and 28 hours respectively after inoculation of standard amount of inoculum obtained from stock culture. The total count of bacteria was estimated by the technique of photoelectric colorimeter.[1] A comparative growth study for Staphylococcus Aureus was made using groundnut glucose broth and conventional glucose broth; while growth study of E. Coli was made using groundnut peptone water and conventional peptone water. The growth at different time intervals was noted.

2. **Cultural Characteristics:** To study the various cultural characteristics, organisms (as shown in table 2) were cultivated on substitute and conventional media. The cultural characters of growth obtained on both were then compared after incubation of 24 hours at 37° C. The various parameters studied were gross appearance of culture, motility, colony character and pigment production, hemolysis and also study of antibiotic sensitivity. The utility of the substitute media as a stock culture and isolation of organisms from the clinical samples like urine and stool was also studied.

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OBSERVATIONS:

1. Growth kinetic study: The rate of growth of E. Coli and Staph. Aureus in groundnut medium at various time intervals of incubation after inoculation was measured in the terms of turbidity by the photoelectric colorimeter. The comparison is shown in table 3, 4 and graph – fig1, 2respectively for E.coli and Staph. aureus.

2. Culture characteristics:

A) liquidMedia:

I. With inoculation of staphylococcus species; uniform turbidity was observed after 24 hour incubation at 37° C and it was comparable with the conventional broth.

II. With beta hemolytic streptococci, granular deposits were noted after 24 hour incubation at 37° C which were comparable with that of conventional glucose broth.

III. With V. cholerae, surface pellicle was formed after 48 hours of incubation at 37° C in both conventional and groundnut alkaline peptone water. The cholera red reaction was though positive in both conventional and groundnut peptone water it was difficult to interpret in the groundnut media due to its pink color.

B) Motility: Motility was well observed with 4 hour culture in the conventional and groundnut peptone water. Darting motility of V. cholerae was also equally appreciated.

C) Colony character and pigment production: The colonies of Staphylococcus aureus were relatively larger in the groundnut agar with occasional areas of clearing around it. The pigment production was quite well marked and more intense in the groundnut agar.

D) Hemolysis: The zones of hemolysis observed were equal with the conventional and groundnut media. In alpha hemolysis green discoloration was more intense in the groundnut media.

DISCUSSION: Use of indigenous materials (e.g. groundnuts) as a substitute of nutrient in the preparation of various bacteriological culture media was attempted by various authors previously [2, 3]. Also, the easy availability and cheaper cost of these materials was one of the reasons for studying its utility as a source of nitrogen in the preparation of various nutrient media. The results of study of growth of various organisms on groundnut media are fairly comparable with those of conventional media.

The technique of preparing groundnut peptone is complicated as shown in the study of SubbaRao and NarsimhaRao et al.[3] But it can be simple as per use of raw powder of groundnut seeds as shown in the study of Sivrajan et al.[2] In the present study the simple method of raw powder of groundnut seeds was used. In the present study 6.5% groundnut broth was used as compared to 10 % concentration used by Sivrajan et al[2] but the growth pattern of bacteria was still comparable with the conventional media.

A preliminary analysis was undertaken to estimate the proximate principles of raw groundnut and commercially prepared nutrient. (table 1) the protein content was 30.8% in our study

which was fairly comparable 26.7% with another series reported by Arkroyd et al.[4] The variability of the protein contents can be explained as different varieties of groundnuts vary in their chemical composition.[5]

For the growth kinetic study; photoelectric colorimeter was used in the present study. The photoelectric colorimeter uses light transmission and optical density for measuring turbidity of the broth culture which is proportional to bacterial density throughout the positive phases of growth[6] The bacterial cell density is measured by photoelectric colorimeter and this gives the measure of growth.

The only disadvantage of this method is that; precipitates from decomposition of constituents of nutrient media or accumulation of toxic substances by bacterial growth may also contribute to part of turbidity. This however being negligible, turbidimetric measurements gives a fair measure of growth. It is generally held that during the logarithmic phase all the bacteria are alive and all are actively dividing. If the assumption was correct, then all the organisms present during this phase should be visible and the total count would be essentially identical with the viable count.

This may be true when organisms are growing under optimal conditions and are followed for a few generations only. However, ordinary broth culture has shown that the total number of organisms generally exceeds the number of viable organisms even during the logarithmic phase.[7]This was also an additional reason why method of photoelectric colorimeter was preferred for our study.

In the growth curve (fig 1 & 2) of the various bacteria studied, lag phase is essentially same for the conventional and groundnut media while logarithmic phase show steep rise in the conventional media as compared to groundnut media. The height of stationary phase was better observed in conventional then groundnut media.

In culture characteristics results were similar for both conventional and groundnut media in the liquid media like glucose broth, peptone water and nutrient broth. The groundnut media is slightly pinkish in color so the cholera red reaction was difficult to interpret at times.

Results of motility testing's were similar in the conventional and groundnut media and this was also observed by Subba Rao & Narsimha Rao et al.[3]

In colony characteristics pseudomonas aeruginosa and staphylococcal colonies were much bigger in the groundnut media and also zone of clearing was observed. The pink background of the groundnut medium appears to be of value in the appreciation of pigment produced by various bacterial colonies. Hemolysis was observed with the hemolytic strains of the bacteria and it was comparable with the conventional media.

Though the antibiotic sensitivity test depends on the many factors like pH of medium, stability of drug, size of inoculum etc. groundnut medium was found equally useful when compared with conventional media. Similar observations were made by other authors also.[2,3]

Thus groundnuts can be utilized successfully as a substitute for the nutrition in the various bacteriological culture media.

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Analysis	Groundnuts (%)	Commercial Preparation (%)
Moisture	2.66	15.2
Total ash	1.02	19.3
protein(Nx6.25)	30.8	52.97
Fat	41.96	0
Carbohydrate, fibre etc.	23.56	12.53

Table 1: Results of preliminary analysis of raw groundnuts & commercial preparation

No.	Type of study	Organism used	Type of substitute medium	Conventional medium for comparison
1	Comparison of quantitative growth at different time intervals in both ubstitute and conventional media	Staphylococcus aureus E. coli	Groundnut glucose broth groundnut peptone water	Glucose broth peptone water
2	Cultural characteristics			
A	Liquid media	Staph.aureus Staph.albus Staph.citreus Streptococci(beta hemolytic) V. cholerae(inaba)	Groundnut broth groundnut glucose broth alk. Groundnut peptone water	Nutrient broth glucose broth alk. Peptone water
B	Motility	E. coli S.typhi S.paratyphi A S.paratyphi B B. subtilis V. cholerae	Groundnut peptone water alk. Groundnut peptone water	Peptone water alk. Peptone water
C	Colony character and pigment production	Staph. Aureus Staph.albus Staph. citreus Ps. aeruginosa	Groundnut agar	Nutrient agar

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D	Hemolysis	Staph.aureus Strep. hemolyticus Strep.viridans	Groundnut blood agar	Blood agar
E	Antibiotic sensitivity test	Staph. Aureus E. coli	Groundnut agar	Nutrient agar
F	Maintenance of stock culture	S. typhi, S.paratyphi A S.paratyphi B Sh. sonnei Sh.flexneri Sh. shigae E.coli B.proteus Ps. aeruginosa V. cholera(ogawa) V. cholera (inaba) Staph. Aureus Staph. Albus Staph. citreus Kl. pneumonae B.sutilis	Groundnut agar	Nutrient agar
G	Isolation from urine sample isolation from stool sample	S.typhi S. typhi Sh. shigae Sh. sonnei V. cholerae	Groundnut mac Conkey Groundnut mac Conkey	Mac Conkey Mac Conkey

Table 2: Type of studies carried out with groundnut substitute media

Hours of incubation after inoculation	Transmission T for peptone water	Transmission T for groundnut peptone water
2	0	0
4	0.1	0.1
8	0.19	0.12
12	0.3	0.16
16	0.45	0.28
20	0.7	0.45
24	0.7	0.7
28	0.7	0.7

Table 3: Comparison of growth kinetics of E. coli

Hours of incubation after inoculation	Transmission T for glucose broth	Transmission T for groundnut glucose broth
2	0.1	0.1
4	0.4	0.3
8	0.6	0.4

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12	0.7	0.5
16	0.78	0.55
20	0.8	0.6
24	0.8	0.6
28	0.8	0.6

Table 4: Comparison of Staphylococcus aureus growth kinetics

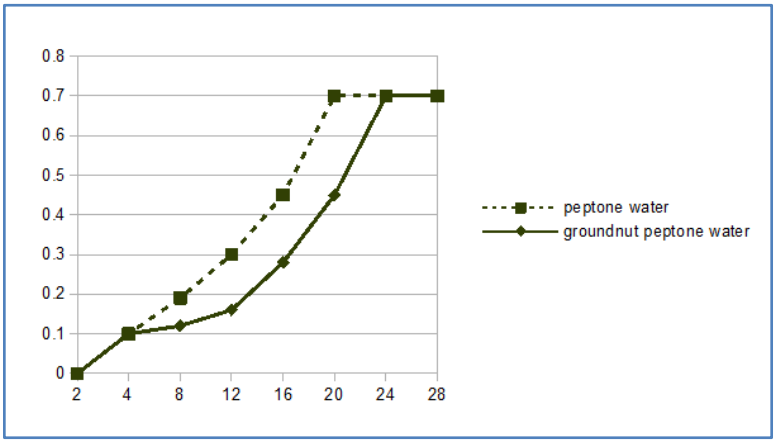

Fig. 1: Growth kinetics of E. coli: Time intervals in Hours(X axis) and transmission (Y axis)

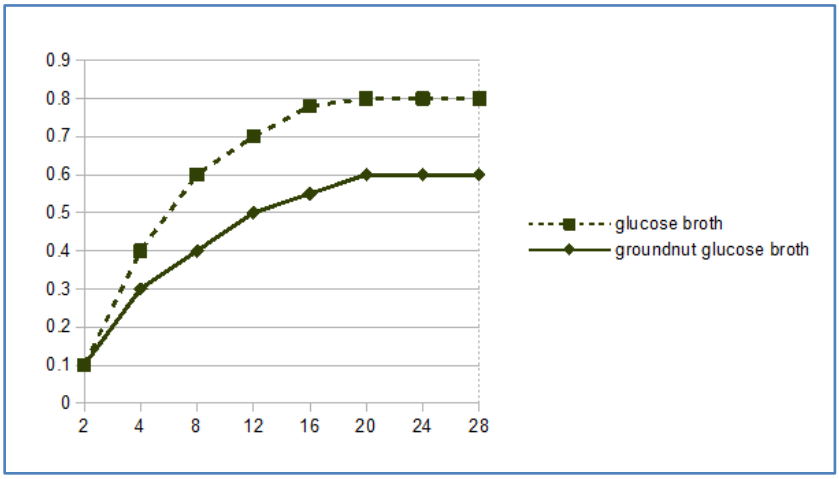

Fig. 2: Growth kinetics of staph. Aureus: Time interval in hours (X axis) and transmission (Y axis)

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AUTHORS:

1. Saroj Ramesh
2. Hemina Desai
3. Praveg Gupta

PARTICULARS OF CONTRIBUTORS:

1. Ex. Professor and HOD, Department of Pathology, BJMC, Ahmedabad.
2. Associate Professor, Department of Pathology, BJMC, Ahmedabad.
3. Assistant Professor, Department of Microbiology, BJMC, Ahmedabad.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Hemina Desai,
#40, RajdhaniBunglows,
Near RamwadiTekra,
Isanpur Road,
Ahmedabad-382443.
Email: hemina@ymail.com

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