Original Article

Use of Cowpea and Pigeon pea as Nutritional Ingredients in Culture Media

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ABSTRACT

Background: Dehydrated commercial culture media are hygroscopic and expensive. Cheap, locally available plant seeds such as cowpea *(Vigna unguiculata)* and pigeon pea *(Cajanus cajan)* could be used in the design and formulation of microbial culture media in order to reduce the cost.

Objective: To make use of locally cheap seeds as a basic nutrient medium for the isolation of different microorganisms.

Materials and methods: Solid culture media from locally available plants were designed to include three types, (i) simple; (ii) enriched with the addition of human blood; (iii) differential with lactose and phenol red as a pH indicator, and formulated to contain cowpea and pigeon pea flours in combination in a concentrations of 2%. The name DANIEL & SHAMSOUN (D & S) was used for the designed media. Twenty bacterial species and *Candida albicans* were inoculated for the observation of the growth response.

Results: On D & S simple medium all the organisms grew typically except, *Corynebacterium diphtheriae* which did not grow and *Streptococcus pyogenes* and *Neisseria meningitidis* which revealed atypical colonies. On D & S human blood agar medium, all the organisms grew typically, but the β- hemolysis of some of the β-haemolytic species was not detected and some species revealed green pigmented colonies and green pigmentation on the medium. On D & S differential medium, all lactose-fermenting species revealed typical, yellow colonies and all non-lactose-fermenting species revealed typical, pink-red colonies, except, *Vibrio cholerae, Bacillus cereus* and *Candida albicans* which revealed typical, yellow colonies.

Conclusion: The flours of cowpea and pigeon pea are good sources of protein, carbohydrates and minerals, so they can be used in the preparation of different types of culture media for the isolation of different species of bacteria and *Candida albicans* as shown in this study.

Key words: Cowpea, pigeon pea, nutrient medium, DANIEL & SHAMSOUN.

To grow microorganisms in the laboratory, whether on a small or large scale, a nutrient environment or culture medium is required which will satisfactory supply all the factors demanded by the organism for multiplication [1]. The majorities of the commonly used culture media are now available commercially as dehydrated products, in either powder or tablet form. Most of the dehydrated products are hygroscopic and expensive [2]. Cheap, locally available plant seeds such as cowpea and pigeon pea can be thought of in the design and formulation of microbial culture media in order to reduce the cost (60.12 SDG vs. 130 SDG for 500 grams of local seeds and nutrient agar, respectively) if proved to give satisfactory growth of the common pathogenic microorganisms.

Cowpea (*Vigna unguiculata*), [Fig.1], cultivars grown for the dry seeds are also known as black-eye pea, black-eye bean, southern bean, China pea, Kaffir pea and marble pea.

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Fig.1**:** *Vigna unguiculata* seeds

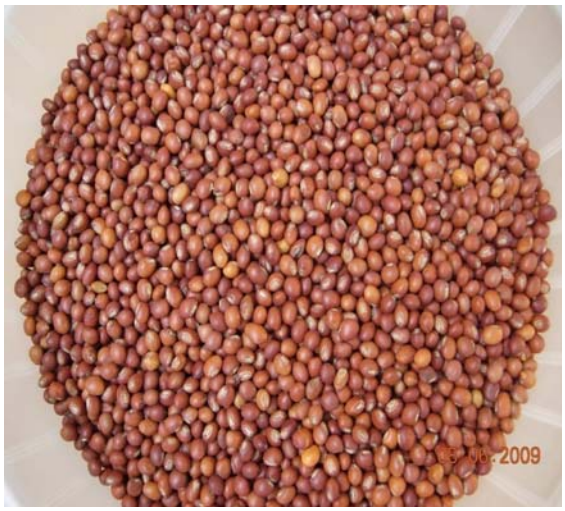

Fig.2: *Cajanus cajan* seeds

The dry pulse contains approximately: water 11.0 per cent; protein 23.4 per cent; fat 1.3 per cent; carbohydrate 56.8 per cent; fibre 3.9 per cent; ash 3.6 per cent [3].

Pigeon pea (*Cajanus cajan*), [Fig.2], other common names include red gram, Congo pea, and no-eye pea.

Dry ripe pigeon peas contain: water 10.1 per cent; protein 19.2 per cent; fat 1.5 per cent; carbohydrate 57.3 per cent; fibre 8.1 per cent; ash 3.8 per cent [3].

MATERIALS AND METHODS

Collection and preparation of the seeds flour:

Mature dried seeds were bought from Omdurman market in Khartoum State -Sudan. These were white coat cowpeas and red-brown coat pigeon peas. The seeds were sorted to remove stones and bad ones and then washed first with tap water three times and finally with distilled water. The washed seeds were dried in day sunlight and finely grounded into fine flour with a mill and stored in plastic bottles at room temperature ready for use.

Solid culture media were designed to include three types, simple, enriched with the addition of human blood, and differential with the addition of lactose and phenol red as a pH indicator.

All types of culture media were formulated to contain sodium chloride and agar (Bacteriological) in concentrations of 0.5% (5 g/l) and 1.2% (12 g/l), respectively [4, 5]. The flours of cowpea and pigeon pea were used in a combination in a concentration of 2% (20 g/l). The simple culture medium was enriched with the addition of 5% (v/v) human blood to prepare human blood agar medium, because it was not possible to obtain sheep blood. The differential culture medium was formulated to contain lactose in a concentration of 1% (10 g/l) and phenol red as a pH indicator in a concentration of 0.08 g/l [4].

Culture media Identification:

Culture media were identified as DANIEL & SHAMSOUN simple medium, blood agar medium, and differential medium.

Culture media preparation and sterilization:

Cowpea and pigeon pea flours were suspended in distilled water, mixed well until a uniform suspension obtained. The suspension was heated just for 2 minutes (uncooked) over gauze and flame and was allowed to cool to 50-55 °C then filtered through a tea strainer.

Sodium chloride was suspended in distilled water, mixed well, and then the agar powder was added and mixed well. The suspension brought to the boil over gauze and flame to dissolve the agar completely. After cooling the cowpea and pigeon pea flours suspension was added to the sodium chloride - agar suspension

and mixed well, then the pH value of the media was measured using a pH meter and adjusted to 7.2 or 7.4 ± 0.2 by the addition of 4% sodium hydroxide.

Concerning the differential medium, the lactose and the phenol red indicator suspended in distilled water separately, mixed well until completely dissolved and added to the complete medium. The culture media were sterilized by autoclaving at 121°C (15 p.s.i.) for 15 minutes.

After cooling to 45-50 °C, the simple culture medium enriched with the addition of 5% (v/v) human blood [5].

Source of bacterial strains and *Candida albicans*:

Twenty bacterial species and *Candida albicans* were used for the observation of the growth response in different types of designed and formulated culture media, and were collected from The Microbiology Laboratory, Faculty of Medical Laboratory Sciences, The National Ribat University. All bacterial strains, except *Staphylococcus aureus* and *Escherichia coli* were teaching strains without known reference institution and strain number, but they were well characterized and identified before use. All strains were inoculated in a standard technique on the different culture media.

Control culture media and growth scoring:

All tested organisms were inoculated on ordinary nutrient agar, human blood agar, and MacConkey agar media as a positive control. The colonial morphology on the inoculated ordinary culture media and the designed media was compared and the growth rate was scored as typical, atypical and no growth.

RESULTS

In this study most of the designed culture media (simple, enriched and differential) supported the growth of the microorganisms under study with variation in the growth performance relative to their growth in the control culture media as follows:

DANIEL & SHAMSOUN Simple Medium:

D and S support the growth of all the microorganisms giving typical colonies [Fig.3] except, *Corynebacterium diphtheriae* which did not grow and *Streptococcus pyogenes* and *Neisseria meningitidis* which revealed atypical colonies compared to the colonial morphology on the ordinary blood agar medium.

DANIEL & SHAMSOUN Blood Agar Medium:

D and S support the growth of all the microorganisms revealing typical colonies. *Staphylococcus aureus* [Fig.4], *Vibrio cholerae*, *Bacillus cereus* and *Clostridium tetani* produced beta-haemolysis and *Streptococcus pneumoniae* produced alpha-hemolysis. However, there was green pigmentation on the medium produced by *Staphylococcus epidermidis*, *Enterococcus faecalis*, *and Streptococcus pyogenes* and green-pigmented colonies were revealed by *Escherichia coli*, *Salmonella typhi*, *Salmonella Paratyphi B*, *Serratia marcescens*, *Vibrio cholerae*, and *Listeria monocytogenes*.

DANIEL & SHAMSOUN Differential Medium:

Lactose-fermenting species (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Escherichia coli*) showed typical, yellow (acidic reaction) colonies. Non-lactose-fermenting species (*Salmonella typhi*, *Salmonella paratyphi* B, *Shigella dysenteriae*, *Proteus mirabilis*, *Serratia marcescens*, *Providencia rettgeri*, and *Pseudomonas aeruginosa*) showed typical,

pink-red colonies, however, *Vibrio cholerae*, *Bacillus cereus* and *Candida albicans* gave typical, yellow colonies.

DISCUSSION

Cowpea and pigeon pea are species of legumes grown locally in Sudan and they are available all over the year. They are cheap, good sources of protein, carbohydrates and minerals.

In this study, the simple culture medium prepared from the combination of cowpea and

Table (1): Growth response of 20 bacterial strains and *Candida albicans* on D & S culture media

Strain	D&S Simple Medium	D&S Human Blood Agar Medium	D&S Differential Medium
Staphylococcus aureus	T	T	T – Y
Staphylococcus epidermidis	T	T – GB	T – Y
Enterococcus faecalis	T	T – GB	T – Y
Streptococcus pyogenes	AT	T – X – GB	†
Streptococcus pneumoniae	T	T	†
Neisseria meningitidis	AT	T	†
Escherichia coli	T	T – GC	T – Y
Klebsiella pneumoniae	T	T	T – Y
Salmonella typhi	T	T – GC	T – P/R
Salmonella paratyphi B	T	T – GC	T – P/R
Shigella dysenteriae	T	T	T – P/R
Proteus mirabilis	T	T	T – P/R
Serratia marcescens	T	T – GC	T – P/R
Providencia rettgeri	T	T	T – P/R
Pseudomonas aeruginosa	T	T	T – P/R
Vibrio cholera	T	T – GC	T – Y
Bacillus cereus	T	T	T – Y
Listeria monocytogenes	T	T – X – GC	†
Corynebacterium diphtheriae	NG	T *	†
Clostridium tetani	T	T	†
Candida albicans	T	T	T – Y

T, typical colony; AT, atypical colony; NG, no growth; X, beta-hemolysis was not detected; GP, green-pigmentation on the medium; GC, green-pigmented colony; Y, yellow colour (acidic reaction); P/R, pink/red colour (alkaline reaction); *, after 48 hours; †, not inoculated on this medium.

pigeon pea flours in a concentration of 2% (DANIEL & SHAMSOUN Simple Medium), allowed the growth of almost all the tested organisms typically.

The characteristic features of some organisms, such as pigmentation, swarming and smell were detected and observed, compared to the colonial morphology on the ordinary nutrient agar medium though it is well known that the fastidious organisms such as *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Clostridium tetani* and *Candida albicans* do not grow on the nutrient agar medium.

Two bacteria, *Streptococcus pyogenes* and *Neisseria meningitidis*, showed atypical colonies, compared to the colonial morphology on ordinary blood agar medium, and *Corynebacterium diphtheriae* failed to grow on this medium.

On culture medium prepared from the combination of cowpea and pigeon pea flours in a concentration of 2% and enriched with the addition of 5% (v/v) human blood (DANIEL & SHAMSOUN Blood Agar

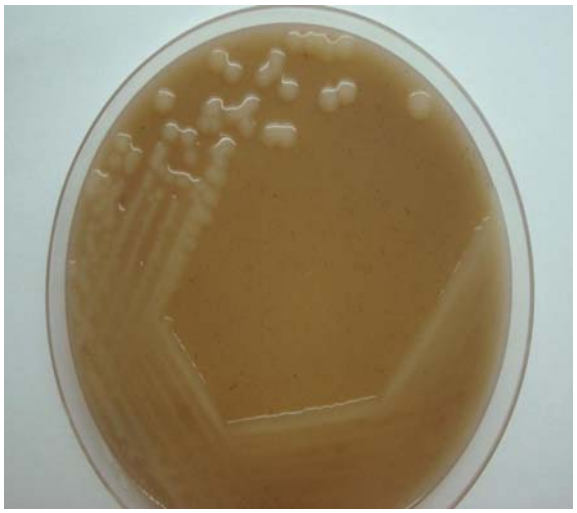

Fig.3: *Klebsiella pneumoniae* on D & S Simple Medium

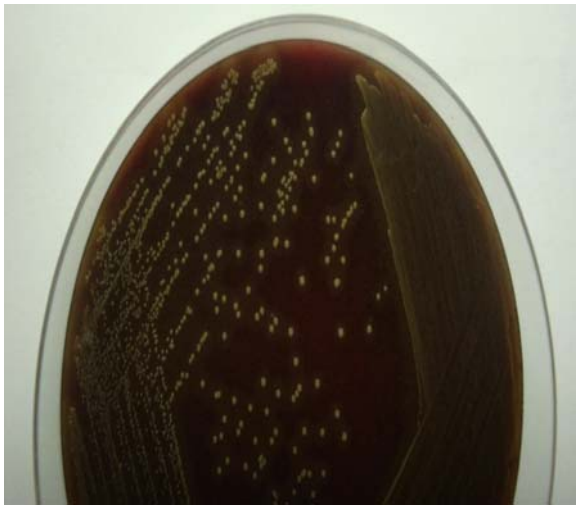

Fig.4: *Staphylococcus aureus* on D & S Human Blood Agar Medium.

Medium), only the beta-hemolysis of *Staphylococcus aureus*, *Vibrio cholerae*, *Bacillus cereus* and *Clostridium tetani* was detected. *Streptococcus pneumoniae* showed very good alpha-hemolysis with greenish-brown pigmentation. However, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Streptococcus pyogenes* showed green-pigmentation on the medium, and *Escherichia coli*, *Salmonella* typhi, *Salmonella* paratyphi B, *Serratia marcescens*, *Vibrio cholerae* and *Listeria monocytogenes* revealed green-pigmented colonies. However, to differentiate which can simply be done by Gram stain and the green-pigmented colonies is not difficult catalase test.

All the tested organisms showed typical colonial morphology on the enriched culture medium after 18-24 hours, except *Corynebacterium diphtheriae* which gave typical colonies after 48 hours, compared to the colonial morphology on the ordinary blood agar medium.

The differential culture medium was prepared from the combination of cowpea and pigeon pea flours in a concentration of 2%, and formulated with 1% lactose and phenol red as a pH indicator (DANIEL & SHAMSOUN Differential Medium), similar to the ordinary MacConkey or CLED differential culture media.

All known lactose-fermenting species revealed typical, yellow (acidic reaction) colonies and all known non-lactose-fermenting species revealed typical, pink-red (alkaline reaction) colonies, except *Vibrio cholerae*, *Candida albicans* and *Bacillus cereus* which showed typical ,yellow colonies. However those organisms do not present a problem in the routinely cultured specimens, as stool culture for *Vibrio cholerae* is not indicated routinely, *Candida albicans* can be identified by the indirect Gram-stained smear (Gram – positive yeast cells) and *Bacillus cereus* can be identified easily by its colonial morphology (large 2 - 5 mm, granular colonies).

Swarming of *Proteus mirabilis* and blue pigmentation of *pseudomonas aeruginosa* were observed.

In the literature there were no previous studies evaluating these local legumes for preparation of culture media. However other local plants were evaluated by other workers in Sudan. These include *Corchorus olitorius*, *Hibiscus sabdariffa*, *prosopis chilensis* and *Adansonia digitata*.

Growth responses on culture media prepared from cowpea and pigeon pea were excellent and satisfactory compared to the growth responses on culture media prepared from the previously mentioned plants, which can be

explained by the high contents of proteins and carbohydrates found in cowpea and pigeon pea compared to the low content in the other plants. Future slight manipulation of these media expected to end up with a very useful, cheap readily available locally prepared media. Plates of the designed culture media have been distributed to microbiology laboratory of The Ribat Hospital and Khartoum State Laboratories Directorate for trial on clinical specimens and the researchers received very good reports regarding the performance of the media.

CONCLUSION

The best preparation of the tested plants was the combination of cowpea and pigeon pea at a concentration of 2% plus lactose. The best result revealed by the combination in the isolation of the enterobacteriaceae which is comparable to the media in general use.

Acknowledgements

The authors would like to acknowledge the following people and institution for their support and assistance: Mr. Abdullah Basher, Mr. Mustafa Mahmoud Shiekh Idrees, and the staff members of Faculty of Veterinary Medicine, U of K, for their permission to carry out some of the laboratory procedures.

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