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Formulation and cultivation of microorganism using alternative media from agricultural wastes

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Abstract
The research was designed to formulate culture media with agro wastes, culture bacteria and fungi therein and to determine the proximate and phytochemicals of the agricultural wastes. Yam peels, pawpaw peels, plantain peels and egg shell were collected from vendors, washed and sundried for 2-3 weeks then ground into powder. Specific quantities of samples were prepared and mixed with agar agar. The formulated media were inoculated with bacteria and fungi. Results indicated that formulated media supported the growth of microorganisms. The proximate and mineral composition analyses of the media included ash content of 5.94% for pawpaw peel, 3.98% (plantain peel), 4.83% (egg shell) and 4.97% (yam peels). Moisture content for the various peels were 3.98%. 2.98% 1.87% and 3.67% respectively for yam peels, plantain peels, pawpaw peel and egg shell. Carbohydrates, proteins, fats, minerals and vitamin C were shown to be present in formulated media. Phytochemicals results/anti nutrients (in mg/100g) showed that yam peel contained the following; alkaloids (3.50), saponins (2.87), flavonoids (2.70), tannins (41.05), oxalates (2.98), steroids (2.30) and reducing sugar. Plantain peels contained alkaloids (3.20), saponin (2.20), flavonoids (2.0), cardiac glycoside, phytate and reducing sugar (2.98) respectively. Egg shell phytochemical composition included alkaloids (1.2), saponins (1.86), flavonoids (1.6), and oxalates (0.82). Formulated media from agricultural waste supported the growth of bacteria and fungi and could be useful for microbial culture for diagnostic and research purposes.

Keywords: Alternative media, agricultural wastes, plantain peels

Introduction
Culture medium is any substrate that can support the growth of microorganisms outside the normal host (Roger *et al.*, 1977) [8]. Different media for the growth and isolation of organisms have been reported from different substrates (Basu *et al.*, 2015) [3]. Some vegetables and fruits have been used to cultivate both fungi and bacteria, such as gooseberry (Sathiya *et al.*, 2013) [9] carrot, tomato, cabbage and pumpkin (Deivanayaki and Iruthayaraji, 2012) [4]. While others including cowpea, green gram and black gram as starch and protein substitutes to reduce the cost of microbial culture (Ravimannan *et al.*, 2014) [7].

Diverse microorganisms exist and so suitable culture media is a necessity to cultivate and study them. Different microorganisms require different environmental conditions and nutrients for culture/growth. Commercial culture media are costly and most times unavailable when needed. It is therefore paramount to formulate more culture media.

There are different types of culture media depending on the nutritional requirements of the organisms. Microorganisms require macro elements including carbon, nitrogen, sulphur, phosphorus, hydrogen and oxygen for the synthesis of lipids, proteins, carbohydrate and nucleic acids. Magnesium, potassium, calcium, and iron are other macro elements that exist as cations in the cell, these in addition to microelements and growth factors are all required by microorganisms for growth (Basu *et al.*, 2015) [3], which must be provided in culture media for microbial culture.

One major setback encountered by the third world agricultural companies is the management of waste. Open air burning is the major way waste from agricultural companies are treated in Nigeria. It is worthy to note that this type of waste treatment is hazardous to health. The improper way agricultural waste are disposed of call for attention. In some cases this kind of wastes are dumped into the environment and becomes a breeding ground for rodents and insects. Rodents and insects are vectors of diseases which not only cause nuisance to people but also break community life and growth. The need to divert these agricultural waste to useful materials which may boost economy and natural resources cannot be overstated.

Yam (*Discorea rotundata*) peels are low in crude fiber and rich in mineral matter, carbohydrates and certain amount of vita mins. The yam peels are also richer in fat, ash, protein and fiber than the tuber tissues.

Egg shell is a semipermeable membrane that is bumpy and grainy in texture which helps to coat the inner membrane of the egg. It consist mainly of calcium carbonate ($CaCO_3$), and research shows that calcium ions are involved in the maintenance of cell structure, motility, transport and cell differentiation processes such as sporulation, heterocyst formation and fruiting body development. Amongst most eukaryotic cells, ranging from fungi to mammals, the ca^{2+} ion serves as a universal messenger, transmitting signals from the cell surface to the interior of the cell. In other words these waste can serve as important substrates for growing microorganisms due to their constituents.

Plantain (*Musa paradisiaca*) serves as a major thestaple food in Nigeria and many other parts of Africa. Plantains are consumed as unripe, overripe and at other stages. Peels make up about 40% and is the most substantial waste derived from plantain processing in Nigeria. The peels are discarded while in other countries, are used as fertilizers. The other part of the plantain is useful for food, local beer production and medicine.

The aim of this research was to formulate culture media using these agricultural waste, grow bacteria and fungi with the formulated culture media, and determine the proximate and phytochemicals of the agricultural wastes.

Materials and Methods

Collection of Samples

The plant samples used for culture media formulation were yam peels, plantain peels, egg shells, pawpaw seeds, pawpaw peel and sweet potato peel. The wastes were collected from vendors in Effurun and Abraka, Delta State. The various samples were washed in tap water and sundried for two –three weeks, and grounded to powder then kept in sterile containers until use.

Formulation of media

One gram of each agrowaste was measured into clean conical flask and mixed with 100 ml distilled water. The mixture was soaked for 5 minutes, shaken and filtered with a muslin cloth. Varying quantities of agar agar (0.35g, 0.5g and 1g) of powder were added and sterilized after dissolving.

The various formulated media were poured into sterile petri dishes and observed for a period to solidify. The formulated media containing 1g of agar agar solidified whilst the others containing smaller quantities did not solidify so subsequent agar culture media were prepared using 1g of agar agar for 100 mls of distilled water for the various waste

Inoculation of test organisms into formulated media

Bacterial isolates used included *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus* sp., *Bacillus* sp. and *Klebsiella pneumoniae*. The fungi included *Candida albicans*, *Rhizopus* spp., Aspergillus sp. and *Penicillium* sp. All these isolates were collected from Microbiology laboratory, Delta State Universiy Abraka.

In another experiment, one gram of sodium chloride, glucose, sucrose and *Cambarus* sp (crayfish) were added to the various agricultural waste. The formulated media were - 5g Yam peel powder +2g agar agar +100 ml distilled water - 5g Sweet potato peel powder +2g agar agar +100 ml distilled water

- 6g plantain peel powder +2g agar agar +110ml distilled water
- 6g Egg shell peel powder +2g agar agar +110ml distilled water
- Yam peel powder + Glucose, plantain peel powder +Glucose, Sweet potato peel (powder) +Glucose, Eggshell +glucose.
- Yam peel powder + NaCl, Plantain peel powder +NaCl, Sweet potato peel (powder) + NaCl, Egs + NaCl.

Yam peel powder +*Cambarus sp*, Plantain peel powder + *Cambarus sp*, Sweet potato peel powder + *Cambarus sp*, Egs + *Cambarus sp*, and each sample were sterilized at 121psi for 15 mins inoculated with the various organisms. Fungi (*Penicillium* sp., *Aspergillus* and *Rhizopus* sp.) were incubated at room temperature for 14 days and bacteria and *Candida albicans* for 24-48 hours.

Proximate Analysis and Mineral Composition of formulated media

Standard procedures were used to perform the proximate composition of the various agricultural waste. Moisture content was determined by drying in oven at 103±2 °C for 4 h to constant weight. Ash content was assessed by incinerating dried samples of agro waste at 550°C for 4 h. Protein content was calculated from nitrogen contents (N x 6.25) obtained by the Kjeldahl method. Lipid content was determined by the Soxhlet extraction method using hexane as solvent. Crude fibre content was estimated from the loss in weight of the crucible and its content on ignition. Carbohydrate content was determined by Clegg anthrone method. Vitamin C content was determined by iodometric titration method. Minerals of agro waste such as iron (Fe), sodium (Na), calcium (Ca), magnesium (Mg), manganese (Mn) and zinc (Zn) were determined using atomic absorption spectrophotometer burner (AAS) as described by AOAC (2000) [2].

Phytochemical Analysis Screening and Quantification

Cardiac glycosides, saponins, alkaloids, flavonoids, phytates, oxalates tannins and anthraquinones were determined as described by Soforowa (1993) and Evans (2006) [5].

Table 1: Determination of solidification time of various alternative Media

Agricultural Waste	Quantity of media	Varying quantity of agar agar	Time	Status
Plantain Peel	1g +100 ml H_2O	1g	30 mins.	Solidified
Pawpaw peel	1g +100 ml H_2O	1g	30 mins.	Solidified
Pawpaw seed	1g +100 ml H_2O	1g	30 mins.	Solidified
Yam peel	1g +100 ml H_2O	1g	30 mins.	Solidified
Potato Dextrose broth	1g +100 ml H_2O	1g	54 mins.	Solidified
Sweet potato peel	1g +100 ml H_2O	1g	30 mins.	Solidified
Egg Shell	1g +100 ml H_2O	1g	30 mins.	Solidified

Nutrient broth	1g +100 ml H_2O	1g	30 mins.	Solidified
Potato Dextrose broth	1g +100 ml H_2O	1g	30 mins.	Solidified
Plantain Peel	1g +100 ml H_2O	0.5 g	50 mins.	Unsolidified
Pawpaw peel	1g +100 ml H_2O	0.5 g	1h 30 mins.	Unsolidified
Pawpaw seed	1g +100 ml H_2O	0.5 g	1h 30 mins.	Unsolidified
Yam peel	1g +100 ml H_2O	0.5 g	1h 30 mins.	Unsolidified
Potato dextrose broth	1g +100 ml H_2O	0.5 g	1h 30 mins.	Unsolidified
Sweet potato peel	1g +100 ml H_2O	0.5 g	1h 30 mins.	Unsolidified
Egg Shell	1g +100 ml H_2O	0.5 g	1h 30 mins.	Unsolidified
Nutrient broth	1g +100 ml H_2O	0.5 g	1h 30 mins.	Unsolidified
Potato Dextrose broth	1g +100 ml H_2O	0.5 g	1h 30 mins.	Unsolidified
Plantain Peel	1g +100 ml H_2O	0.5g	1h 30 mins.	Unsolidified
Pawpaw peel	1g +100 ml H_2O	0.3g	2 hours	Unsolidified
Pawpaw seed	1g +100 ml H_2O	0.3g	2 hours	Unsolidified
Yam peel	1g +100 ml H_2O	0.3g	2 hours	Unsolidified
Potato Dextrose broth	1g +100 ml H_2O	0.3g	2 hours	Unsolidified
Sweet potato peel	1g +100 ml H_2O	0.3g	2 hours	Unsolidified
Egg Shell	1g +100 ml H_2O	0.3g	2 hours	Unsolidified
Nutrient broth	1g +100 ml H_2O	0.3g	2 hours	Unsolidified
Potato Dextrose broth	1g +100 ml H_2O	0.3g	2 hours	Unsolidified

Table 2: Qualitative growth results of microorganisms on formulated media

Organisms	Yam	YS	YG	YC	P	PS	PG	PC	PL	PLS	PLG	PLC	E	ES	EG	EC
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+and green colour	+	+	+	+	-	+ and green colour	-	+
Escherichia coli	+	+	+	+	+	+	+	-	+	+	+	+		-	+	
Bacillus sp	+	+	+	+	+	+	-	-		+	+	+		+	+	+
Staphylococcus aureus	+	+	+	+		+	Flat colonies	-	+	+	-	+		+	+	
Streptococcus sp	+	+	+	+	+		Dry growth	-	+	+	-	+		+	+	+
Klebsiella pneumoniae	+	+	+	+	+	+	+	-	+	+	+	-		+	+	+
Proteus mirabilis	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Candida albicans	+	+	+	+		+	+	-	-	-	-	-	+	-	+	-
Rhizopus sp	+	+	+	+		+	+	-	-	-	-	-	+	+	+	-
Penicillium sp	+	+	+	+		+	+	-	-	-	-	-	+	-	+	-
Aspergillus sp	+	+	+	+		+	+	-	-	-	-	-	+	+	+	-

Key: YS=Yam peel +NaCl, YG=Yam peel + glucose, YC= Yam peel +*Cambarus* sp
P=sweet potato peel, PS=sweet potato peel +NaCl, PS= sweet potato peel + glucose, PC = sweet potato peel +cambarus sp
PLS=Plantain peel +NaCl, PLS= Plantain peel + glucose, PLC = Plantain peel + *Cambarus* sp
E- Egg shell, ES= Egg shell +NaCl, EG= Egg shell + glucose, EC= Egg shell + *Cambarus* sp

Table 3: Proximate and mineral composition analysis the various agro wastes

S/No	Parameter	Yam peels	Plantain peel	Pawpaw peel	Egg shell
1	Ash content (%)	4.02	3.98	5.94	4.83
2	Fat (%)	3.17	1.33	2.07	0.08
3	Moisture (%)	3.98	2.98	1.87	3.67
4	Carbohydrate (%)	49.48	48.94	54.79	50.03
5	Protein (%)	21.44	10.32	10.3	1.98
6	Crude fibre (%)	8.94	7.84	9.43	1.03
7	Manganese ppm	0.48	0.59	ND	0.84
8	Magnesium ppm	0.87	1.22	8.47	1.34
9	Zinc ppm	1.46	1.94	ND	1.87
10	Iron ppm	1.36	2.09	ND	2.08
11	Calcium ppm	2.49	3.37	ND	3.39
12	Sodium ppm	1.03	2.18	ND	2.08
13	Vitamin C ppm	2.09	3.02	ND	3.77

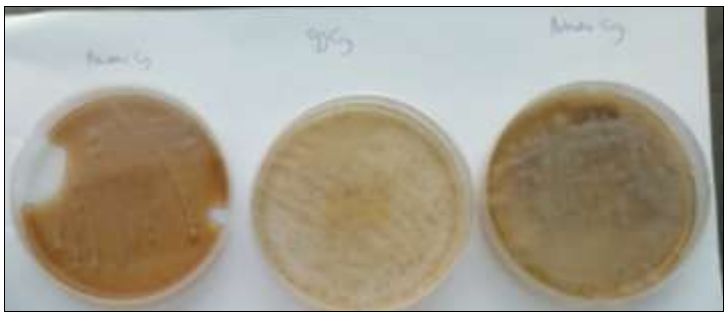

Fig 1: *Streptococcus* sp. on the various agar supplemented by *Cambarus* sp

Fig 2: Rhizopus sp. growth at 48hours after incubation

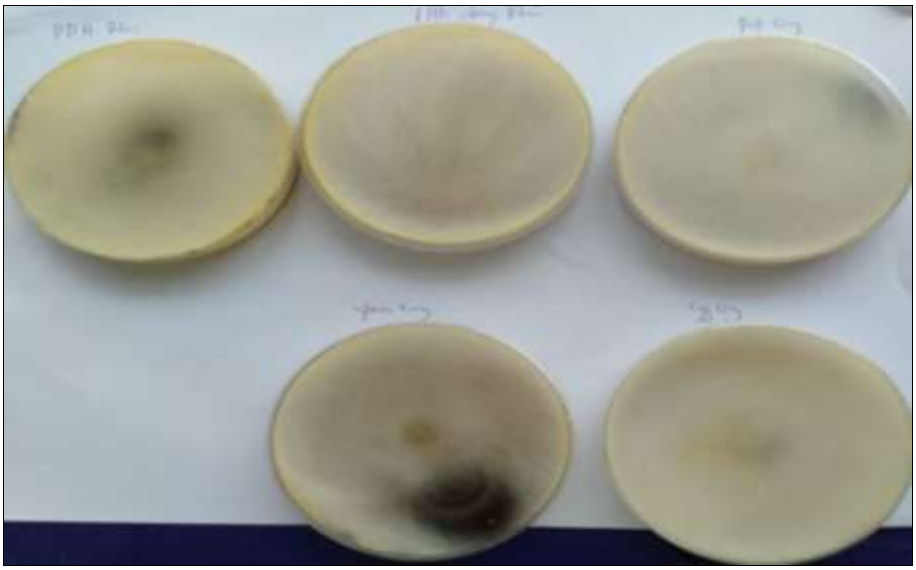

Fig 3: Rhizopus sp. at 11th day of incubation with various agar supplemented with *Cambarus* sp.

Fig 4: *Rhizopus* sp at the 14th day of incubation on the various agar supplemented with *Cambarus* sp fillings

Table 4: Phytochemical Screening and Quantification of the various agricultural wastes

S/no	Parameter	Yam peels mg/100g	Plantain peel mg/100g	Egg shell mg/100g
1	Alkaloids Qualitative	++	++	+
	Quantitative	3.5	3.2	1.2
2	Saponin Qualitative	++	+	-
	Quantitative	2.87	2.2	1.86
3	Flavonoids Qualitative	++	+	+
	Quantitative	2.70	2.0	1.60
4	Tannin Qualitative	+	-	-
	Quantitative	41.03	-	-
5	Cardiac glycoside Qualitative	-	+	+
	Quantitative	-	-	-
6	Anthraquinone Qualitative	-	-	-
	Quantitative	-	-	-
7	Phytate Qualitative	-	+	-
	Quantitative	-	2.98	-
8	Oxalates	+	-	+
	Quantitative	2.98	-	0.82
9	Reducing sugar	+	2.5	-
				-
10	Terpenoids		+	-
				-
11	Steroids	++		
		2.3		

Results and Discussion

Table 1 presents the solidification time of various alternative media in this study. The various alternative and commercial media contained the same quantity each (1g) but different quantities of agar agar. The agar agar used varied from 0.3g to 1g. Both formulated culture media and the commercial media (nutrient broth and potato dextrose broth) solidified at the same time (30 minutes). However, those containing lesser quantities of agar agar did not solidify though allowed to stand for more than one hour. Similar study carried out by Uthayasooriyan *et al.* (2016) where 3.0g of sample with varying weight of agar agar were studied. One of the experimental results showed that the media formulated with 3.0g of sample and 3g of agar agar solidification time was 24 minutes (for processed soy flour and thinai (Setaria italia) while the shortest time was 14minutes for chicken pea. The essence of media formulation is to reduce cost during research and also to see if waste could be useful in other aspects especially in the growth of microorganisms for diagnostic, industrial and other products for human benefits which could invariably, reduce the cost of production at the long run.

Table 2 shows the qualitative growth of microorganism on formulated media. Growth was seen in all the culture media inoculated with bacteria except for *Proteus mirabilis* where only plantain peel supplemented with *Cambarus* sp (crustacean) supported the growth. *Staphylococcus aureus* and *Streptococcus* sp produced flat colonies and dry growth respectively when grown in sweet potato peel and glucose. The various formulated media from agricultural wastes supported the growth of *Rhizopus* sp, *Penicillium* sp and *Aspergillus* species more than *Candida albicans*. Yam peel alone, also in combination with other substances (Sodium chloride, glucose and *Cambarus* sp) supported the growth of all the fungi but plantain peels as presented did not support their growth unlike bacteria.

The proximate and mineral composition analysis of the media is presented in table 3. The ash content was higher for pawpaw peel (5.94%) than plantain pee (3.98%). The least fat content was recorded for egg shell (0.08%) while highest was yam peels (3.17%). Moisture content for the various peels were 3.18%. 2.98, 1.87% and 3.67% respectively for yam peels, plantain peels, pawpaw peel and egg shell. Carbohydrate composition for the different waste were 49.48% (yam peel, 48.94% (plantain peel) 54.79% (paw paw peel and 50.23% for egg shell. Of all other various agricultural waste, Protein composition for yam peels was 21.44% being the highest value recorded and egg shell the least 1.98%. This tallied with previous report on nutritional composition and physiochemical features of unpeeled and peeled yam (Akintola, 2020) [1].

Pawpaw peel had crude fibre of 9.43% while plantain 7.84% and egg shell 1.03%. Mineral composition determined were manganese, magnesium, zinc, iron, calcium and sodium. Yam peel contained 0.48 ppm for magnesium, 1.46 ppm, zinc, 1,36 ppm iron, 2.49 ppm calcium and 1.03 ppm sodium. Plantain peels powder was 0.5 ppm, 1.22 ppm 1.94, 2.09, 3.37 and 2.18 for the various listed minerals. Egg shell contained 0.84, 1.34, 1.87, 2.08, 3.39 and 2.08 ppm. The vitamin C composition for plantain peel and egg shell were comparable. The iron content for the various agar formulated varied but plantain peel contained the highest quantity.

From these results, it can be said that these agricultural wastes were rich in nutrient and may be utilized for the cultivation and growth of microorganisms in the laboratory especially when the cost of commercial culture media are high. Little wonder herbivores depend solely on plant and plant waste for survival.

Mycological experiments and assay carried out in the laboratory for diagnostics and other functions make use of antibiotics to prevent bacterial contamination of fungal plates. In the course of the study, there was no bacterial contamination on fungal plates. This feature in the formulated media is desirable and could be utilized.

Previous studies have shown that phytochemicals present in plant materials have antimicrobial activity. In spite of the chemical composition, the microorganism inoculated grew (figures 1- 4).

Yam peel showed that alkaloids (3.50), saponins (2.87),

flavonoids (2.70), tannins (41.05), oxalates (2.98), steroids (2.3) and reducing sugar were present.
Lawal *et al.* (2014) [6] result on phytochemicals of yam peels were alkaloids (0.03±0.01), tannin (8.19 ± 0.01), Phytate (0.36±0.00) and oxalate (0.028 ± 0.00).
Plantain peel result included the following phytochemicals: alkaloids (3.20), saponin (2.20), flavonoids (2.0), cardiac glycoside, phytate (2.98), reducing sugar (2.98) and terpenoids.
Egg shell contained alkaloids (1.2), saponins (1.86), flavonoids (1.6), cardiac glycoside and oxalates (0.82).

Conclusion
The various alternative media from agricultural wastes studied supported the growth of microorganisms. However, the culture media supplemented with *Cambarus* sp as compared with those supplemented with glucose and sodium chloride had better growth than the other ones studied. Formulated media could be utilized for microbial cultivation and growth in the laboratory thereby reducing cost and harness waste into alternative culture media for growth of microorganisms for diagnostic and research studies.

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Conflict of Interest
Not available

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