



Growth Rate and Antifungal Activities of Acetone Extracts of *Ocimum gratissimum* (Scent Leaf) and *Allium sativum* (Garlic) on Cassava and Banana Peels Formulated Media

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ELO and BOU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UCD and CJE managed the analyses of the study. Author CJE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To study the growth rate and antifungal activities of acetone extracts of *Ocimum gratissimum* (scent leaf) and *Allium sativum* (garlic) on cassava and banana peels formulated media.

Study Design: Nine treatments and control designs were set up in triplicates and incubated at 25°C for 72 h. The nine treatments and control set ups designated as Cassava Glucose Agar, CGA), Banana Glucose Agar, (BGA) and Control (Potato Dextrose Agar, PDA) were used to screen for the growth rate and antifungal activities of plant extracts.

Place and Duration of Study: Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli Nigeria between June, 2019 and August, 2019.

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Methodology: The research study was carried on two formulated media obtained from cassava and banana peels and PDA) using proximate analysis, pour plate technique, agar plug diffusion technique and agar well diffusion technique.

Results: The results revealed that the cassava and banana peels contained nutrients that can meet the nutritional conditions for fungi cultivation. The PDA statistically ($P < 0.05$) had higher mean radial growth (32.33 mm) and growth rate (0.449 h^{-1}) than BGA (14.33 mm; 0.199 h^{-1}) followed by CGA (14.16 mm; 0.197 h^{-1}) in most of the test fungal isolates. The scent leaf acetone extract and nystatin antifungal had higher zones of inhibition (25.00 mm) than garlic acetone extract (24.00 mm) on the tested fungal isolates. There was no statistical significance at $P < 0.05$ on the inhibition zones of the extracts and Nystatin on the formulated media and PDA showing that the extracts possess comparable antifungal activities to the Nystatin.

Conclusion: Thus, our formulated media were comparable to PDA with regards to antifungal activity of the acetone extracts and nystatin and could be utilized as an alternative and cheap ideal reference media for mycological assays.

Keywords: *Cassava glucose agar; banana glucose agar; extracts; susceptibility testing; antifungal activity.*

1. INTRODUCTION

The agricultural-based industries generate significant quantities of organic wastes including peels from banana and oranges as well as straw from cereals. Rather than allowing these wastes to become solid municipal wastes, it is necessary to convert them to useful end products. It is now realized that these wastes could be utilized as cheap raw materials for some industries or as cheap substrates for microbiological processes. The food processing industry generates a large amount of wastes annually including crop residues like peels, husks, cobs, and shells. Such wastes are rich in sugar and are easily assimilated by microorganisms; this makes the wastes suitable materials for growth of microorganisms. Inability to salvage and reuse such materials economically results in the unnecessary waste and depletion of natural resources [1].

In developing countries, there is a growing interest regarding the utilization of organic wastes generated by the food processing sector and through other human endeavours. This has led to a new policy geared towards complete utilization of raw materials so that little or no residue is left to pose pollution problems. The need to develop alternative mycological media in addition to the commonly used mycological media has become imperative as the conventional media used are neither readily available nor cheap in most developing countries like Nigeria [2].

Cassava peels represent 5 -15% of the root and are obtained after the tubers have been peeled mechanically. They usually contain high amounts of cyanogenic glucosides and need to be processed or sun dried in order to reduce its

cyanogenic potential before it can be used. Alternatively, banana peels have high sugar, protein and fibre contents which make them also potentially ideal for culturing microorganisms. Banana peels have so many uses which includes: meat tendering, aphid controlling as well as curing of acne, itches and even hemorrhoids, haemorrhoids. Both materials have potential for antifungal activities.

Fungi constitute one of the largest groups of plants with richest arrays of species. They are a group of eukaryotic spore bearing, achlorophyllous organisms that generally reproduce asexually and sexually. Some are agents of diseases in plants (parasitic), while others are saprophytic. Saprophytic fungi tend to be responsible for most of the disintegration of organic materials and some of them render food material toxic [3]. Fungi grow on diverse habitat in nature and are cosmopolitan, requiring several specific elements for growth and reproduction. In the laboratory, fungi are isolated on specific culture media for cultivation, preservation, macroscopic examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi. These media influence growth, colony, morphology, pigmentation and sporulation with the factor of their compositions, pH, temperature, light, water availability and surrounding atmospheric gas mixture. Generally, growth media for fungi contain carbon and nitrogen sources, and most fungi require several specific elements for growth and reproduction [4,5].

The feasibility of developing alternative media for cultivation of fungi apart from the commercial

brands like Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) has been studied by different researchers. Akharaiyi and Abiola [6] reported a maximum yield of *T. viride*, *A. flavus* and *A. fumigatus* upon cultivation on yam glucose agar and plantain glucose agar. They also showed that these wastes can be used as an alternative culture media for growing fungi and some of these fungi actually grow better on these agro wastes than on the conventional media. Anbu et al. [7] conducted a study using fruit peels waste materials such as pineapple, mango, jack fruit, green banana, yellow banana, sweet lime and pomegranate to formulate growth media. The fungi isolated from the media were *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium chrysogenum*. *A. niger* growth was recorded in the medium containing pineapple, mango, jack fruit and green banana. As a result, there is growing interest in alternative media formulation since the conventional media are either expensive or not easily available in most emerging countries of the world like Nigeria. Thus, this study was undertaken to screen for antifungal activities of acetone extracts of *Ocimum gratissimum* (scent leaf) and *Allium sativum* (garlic) on agro wastes formulated media with the sole purpose of comparing the activities on agro wastes formulated media with that of Potato Dextrose Agar (PDA) medium.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The samples collected were cassava peel (CP) and banana peel (BP). The cassava and banana peels were collected from fruit and local dealers in Chukwuemeka Odumegwu Ojukwu University (COOU), Uli Market, Ihiala Local Government Area, Anambra State Nigeria. The samples were collected with a clean dry polyethylene bag, brought to Microbiology Laboratory COOU, washed under running tap water and then sun dried for 7 days. The dried samples were blended into smooth powders and stored in sterile air tight plastic containers before use.

2.2 Proximate Analysis of Cassava Peel and Banana Peels

The proximate compositions of the samples were determined using the standard methods of AOAC [8]: the moisture content by air oven (Gallenkamp, Germany) method at 105°C for 3 h; the ash content by a muffle furnace (Life

Assistance Scientific, UK) at 550°C for 4 h, the carbohydrate content by anthrone method, protein content by micro-Kjeldahl method and the crude fibre content by an analytical multimeter (DSS – 11A, China).

2.3 Composition of Culture Medium from Agro Wastes

Twenty grams of each powdery sample (CP and BP) were weighed and mixed with 200 mL of boiling water and allowed to cool. Each mixture was filtered using muslin cloth and a filter paper. Thirty-eight grams of glucose (supplement for the dextrose in PDA) and 5 g of Agar Agar (gelling agent) were added to 150 mL of each sample filtrate. The mixtures were then sterilized by autoclaving at 121°C for 15 min. After cooling to about 45°C, 1 mL of 0.005% chloramphenicol was added in order to inhibit bacterial growth [3].

2.4 Fungal Isolation

2.4.1 Source of test organisms

The test organisms used for the experiment were isolated from soil samples from different farmlands, contaminated water, air, cow dung and pig dung around Uli and different Abattoirs in Ihiala L.G.A. The samples were collected aseptically with a sterile spatula, placed in sterile polyethylene bag and 1 L plastic container, transported to Microbiology Laboratory COOU.

2.4.2 Serial dilution and sample inoculation

One gram of the soil samples, cow dung, pig and 1mL of the contaminated water were measured into different sterile tubes containing 9 mL of distilled water to produce a stock suspension (10^{-1}). The stock suspension was shaken vigorously for five seconds to dislodge the organisms. After this, ten-fold serial dilution was carried out up to 10^{-5} dilution and sterile plates of the PDA inoculated using standard pour plate technique. Air samples were inoculated by exposing sterile PDA plates for 30 min and the culture plates were incubated at laboratory temperature of 25°C for 72h [6,9,10]. After incubation, nine pure cultures were obtained by sub-culturing in Bijou bottles containing sterile PDA medium and kept at 4°C refrigeration temperature for further microbiological examination.

2.5 Description and Identification of Fungal Cultures

A small amount of aerial growth of each fungus was removed using mounted needle and transferred to a drop of cotton blue lactophenol on a clean slide. The hyphae were teased apart with the needle and a cover slip was placed over the preparation taking care to prevent air bubbles. The preparation was viewed under a digital camera compound microscope (Stereo OF0533, China) for observation of features such as nature of hyphae, fruiting structures, spore types and spore attachment. The descriptions of the nine fungal isolates were compared with those of known taxon described in the identification key of fungal atlas [3,10,11].

2.6 Growth Screening of the Test Fungi

The test organisms from the pure culture plates were introduced into the prepared sterile agar plates using agar plug diffusion technique. A 9 mm sterile cork borer was used to introduce the agar plugs of the nine fungal isolates that were previously cultured in PDA medium for 7 days to both the formulated media (Cassava Glucose Agar and Banana Glucose Agar) and PDA (positive control) and then incubated at 25 °C for 72 h. After incubation, the mean radial growth of each of the test fungus was measured in (mm) and recorded. The growth rate was compared between both formulated media and PDA (control medium) [3].

2.7 Plant Extracts Preparations

The plants used were *Ocimum gratissimum* (scent leaf) and *Allium sativum* (garlic) bought at Afo Egbu Market, Uli, Anambra State. They were thoroughly washed with running tap water and dried at laboratory temperature of 25°C for 14 days and then ground into smooth powder with electric blender. Ten grams of both powders were weighed and placed into separate 250 mL conical flasks containing 100 mL of acetone for 24 h. After 24 h, they were double filtered with a muslin cloth and Whatman No. 1 filter paper and left to allow acetone to evaporate. The crude extracts were finally stored at 4°C refrigeration temperature for further analysis [6].

2.8 Antifungal Activity Using Composed Media and PDA

The antifungal activities were determined using agar well diffusion technique by adopting the

method of Akharaiyi and Abiola [6]. Nine Petri dishes of the formulated cassava glucose agar (CGA) and banana glucose agar (BGA) in triplicates were evenly seeded with the nine test organisms and were allowed to stand for 1 h. Thereafter, a 9 mm sterile cork borer was used to create 4 wells on each of the inoculated plates. Zero point two millilitre of the crude extracts, 0.01% nystatin (positive control) and sterile distilled water (negative control) were aseptically dispensed into each of the respective wells with their labels on them and left for 30 min to allow proper diffusion of the antimicrobial agents. The plates were then incubated at laboratory temperature of 25°C for 72 h and the zone of inhibition was measured in mm.

2.9 Analysis of Biodata

The results were expressed as mean \pm standard deviation (SD) and were subjected to one factor analysis of variance (ANOVA) with Dunnett comparative test using GraphPad Prism software version 7.00 to determine their significant levels at 95% confidence interval. Values were considered statistically significant if $P < 0.05$.

3. RESULTS

3.1 Proximate Analysis

The nutrient content of the agro-waste materials is presented in Table 1. The results showed that cassava and banana peeled samples had identical carbohydrate and protein contents of 26.00 mg/100g and 9.16 mg/100 g, respectively. Cassava peeled sample had higher moisture and ash contents of 13.86% and 2.80% while banana peeled sample had higher crude fibre content of 12.93 mg/ 100 g.

3.2 Isolated Organisms

The fungal isolates from this study were *Aspergillus flavus*, *Fusarium* sp., *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans*, *Cladosporium* sp., *Geotrichum candidum* and *Rhizopus stolonifer* and result is presented in Table 2. *Aspergillus* genera were the major fungal isolates characterized and described.

3.3 Growth Rate Profile

The result of the mean radial growth (MRG) of the test fungi on the formulated media and PDA

is shown in Table 3. From the Table, *Rhizopus stolonifer* had the highest radial growth of 14.16 mm on CGA, *Aspergillus terreus* had the highest radial growth of 14.33 mm on BGA while *Rhizopus stolonifer* had the highest radial growth of 32.33 mm on PDA medium. Furthermore, the highest (32.33 mm) and lowest (8.33 mm) MRG were recorded by *Rhizopus stolonifer* and *Rhizopus* sp. on PDA and BGA media, respectively. In general, the MRG on the control medium (PDA) was higher in all the test fungal isolates except *Aspergillus fumigatus* where CGA had a slightly higher MRG (11.16 mm) than PDA (10 mm). Similarly, Fig. 1 illustrated the growth rates (GR) of the test fungi on the formulated media and PDA. The result showed similar trends with *Rhizopus stolonifer* having the highest (0.449 h^{-1}) on PDA medium while and *Rhizopus* sp. had the lowest (0.116 h^{-1}) GR on BGA medium, respectively. Comparatively, PDA had higher MRG and GR than CGA followed by BGA in most of the test fungal isolates. Statistically, the MRG and GR between PDA and the formulated media was significant at $P < 0.05$ using ANOVA with Dunnet test ($P < 0.05$).

3.4 Susceptibility Patterns

Figs. 2, 3 and 4 illustrated the susceptibility patterns of the test fungal isolates to scent leaf acetone extract, garlic acetone extract and positive control agent nystatin antifungal drug. From the Fig. 2, scent leaf acetone extract inhibited the growth of all the test fungal isolates in the formulated media CGA and BGA (10.00 – 25.00 mm) except *Aspergillus niger* (0.00 mm) and *Aspergillus fumigatus* (0.00 mm) in BGA. All the test fungal isolates except *Aspergillus terreus* (0.00 mm), *Candida albicans* (0.00 mm), *Cladosporium* sp. (0.00 mm), *Geotrichum candidum* (0.00 mm) and *Rhizopus stolonifer* (0.00 mm) were susceptible (9.00 – 18.00 mm) to scent leaf acetone extract in PDA. The highest zone of inhibition (25.00 mm) was recorded by *Rhizopus stolonifer* in the BGA. The garlic acetone extract inhibited all the test fungal isolates (11.00 – 20.00 mm) in CGA, except *Geotrichum candidum* (0.00 mm). All the test fungal isolates except *Candida albicans* (0.00 mm) were susceptible (10.00 – 24.00 mm) to the garlic acetone extract in BGA and PDA with the highest inhibition zone (24.00 mm) recorded by *Fusarium* sp. in PDA (Fig. 3). Also, from Fig. 4, nystatin inhibited (11.00 – 25.00 mm) all the test fungal isolates in CGA, BGA and PDA while there was no inhibition (0.00 – 0.00 mm) of the negative control (water) on all the media. There

was no statistical significance at $P < 0.05$ on the inhibition zones of the extracts and nystatin on the formulated media and PDA using ANOVA with Dunnet test ($P > 0.05$).

4. DISCUSSION

One of the most important challenges faced by both advanced and emerging countries is the management of wastes. Consequently, there is an increasing attention on how these generated wastes especially from agro allied industries could be transformed into valuable and suitable forms. This joined with the extremely high cost of the conventional media has made it necessary to create newer media using cheap agro waste materials. In this study, the results showed that all the formulated media supported the growth of fungi as previously described above though at varying degrees and this is in conformity with the findings of Adesemoye and Adedire [12], Laleye et al. [13], Itelima et al. [3] and Umedum and Enejekwute [14] who reported the use of alternative culture media for growing fungi.

The result in Table 1 showed that the agro - wastes contained nutrients such as protein, carbohydrate, crude fibre, and ash contents. The advanced cultivation of the tested fungal isolates on the formulated media implies that the wastes (peels) which were used in formulating the media possessed the necessary nutrients for fungal growth. A larger proportion of microbial biomass is made of proteins which are needed for the growth of microorganisms because of their nitrogen and amino acid contents [15]. Carbohydrates are also needed for fungal growth as they provide greater amounts of carbon contents which act as building blocks. The proximate analysis of the agro wastes as presented in Table 1 showed differences in their proximate composition which led to variation in the concentration of media components and also played a role in the overall outcome of the formulated media in good support of fungal growth. The ability of the agro-allied wastes to support good growth of the fungi showed that they not only contained the right nutrients but also probably contained them in the right proportions.

The results in Table 3 and Fig. 1 revealed that CGA had a better MRG and GR compared to BGA. The control media (PDA) had a better MRG and GR than the formulated media (CGA and BGA) in most of the tested fungal isolates. This

observation contradicts the research studies of Adesemoye and Adedire [12], Itelima et al. [3], Akharaiyi and Abiola [6] and Ruth et al. [16] which stated that all their tested fungal isolates grew in their formulated media in a comparable manner to the conventional media.

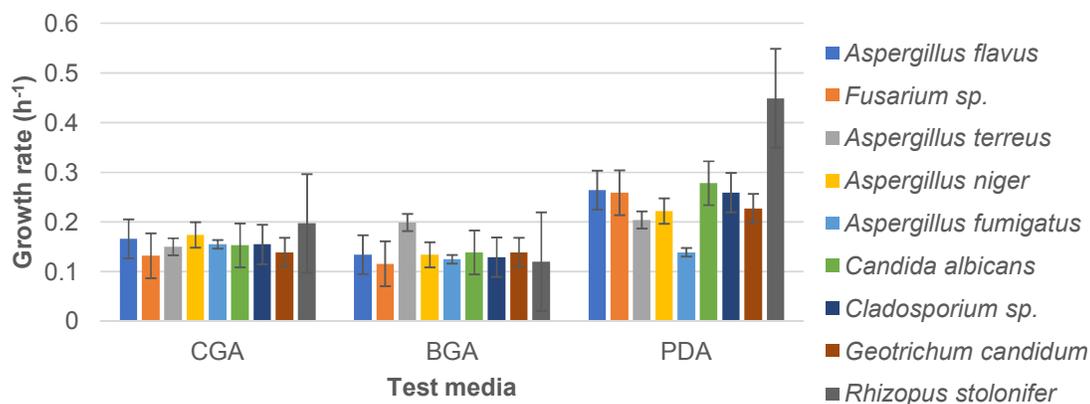


Fig. 1. Growth rates (GR) of the test fungi on the formulated media and PDA

Key: CGA= Cassava glucose agar; BGA= Banana glucose agar; PDA= Potato dextrose agar; Error bar = Standard error in mean; Dunnet test ($P < 0.05$)

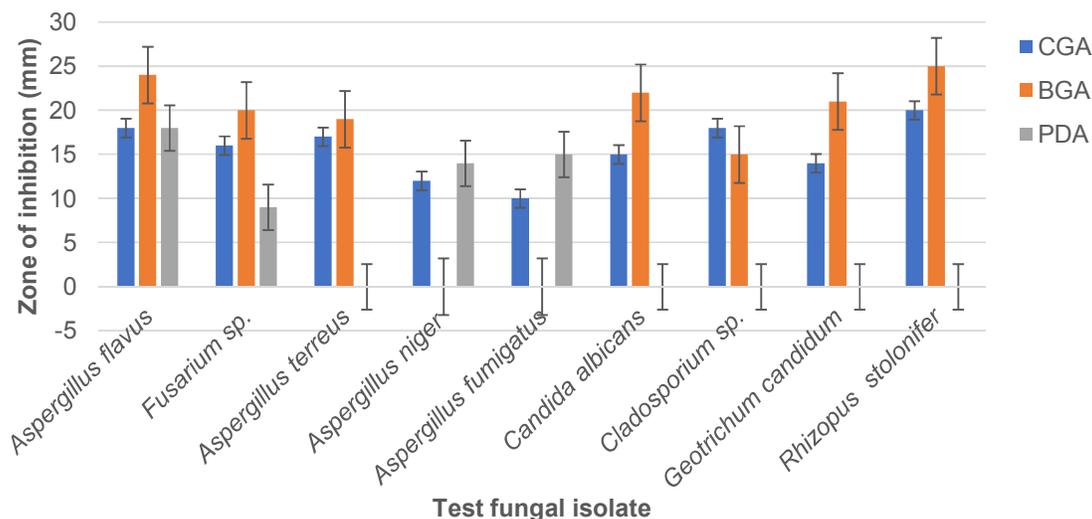


Fig. 2. Susceptibility patterns of the test fungal isolates to scent leaf acetone extract

Key: CGA= Cassava glucose agar; BGA= Banana glucose agar; PDA= Potato dextrose agar; Error bar = Standard error in mean; Dunnet test ($P > 0.05$)

Table 1. Nutrient content of agro-waste materials

Wastes	Carbohydrate content (mg/ 100g)	Crude protein (mg/ 100g)	Crude fibre (mg/ 100g)	Moisture content(%)	Ash content (%)
Cassava peel	26.00	9.16	4.41	13.86	2.80
Banana peel	26.00	9.16	12.93	7.46	1.15

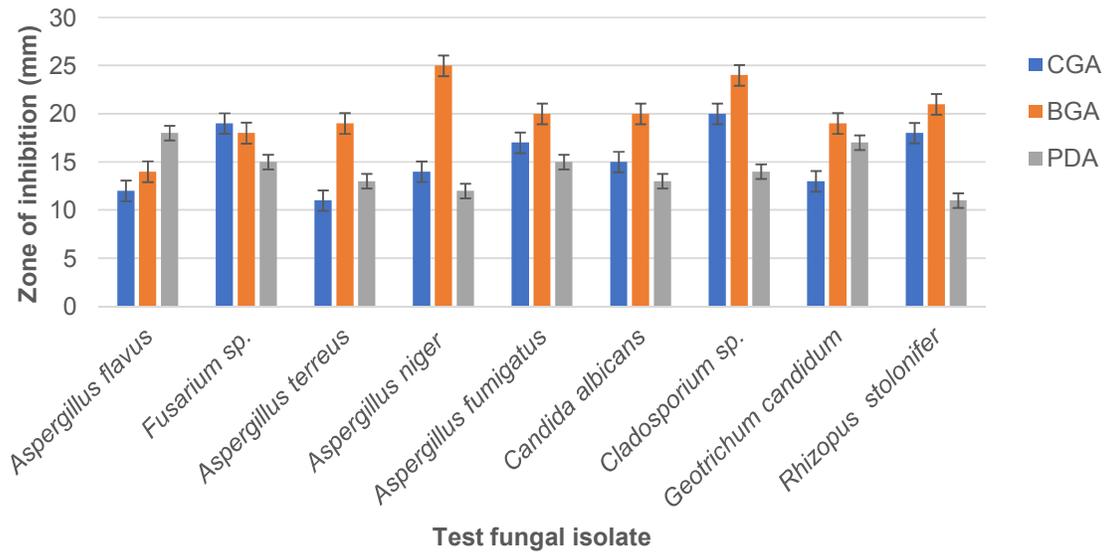


Fig. 3. Susceptibility patterns of the test fungal isolates to garlic acetone extract
 Key: CGA= Cassava glucose agar; BGA= Banana glucose agar; PDA= Potato dextrose agar; Error bar = Standard error in mean; Dunnet test ($P > 0.05$)

Table 2. Morphological description of the isolated fungal isolates

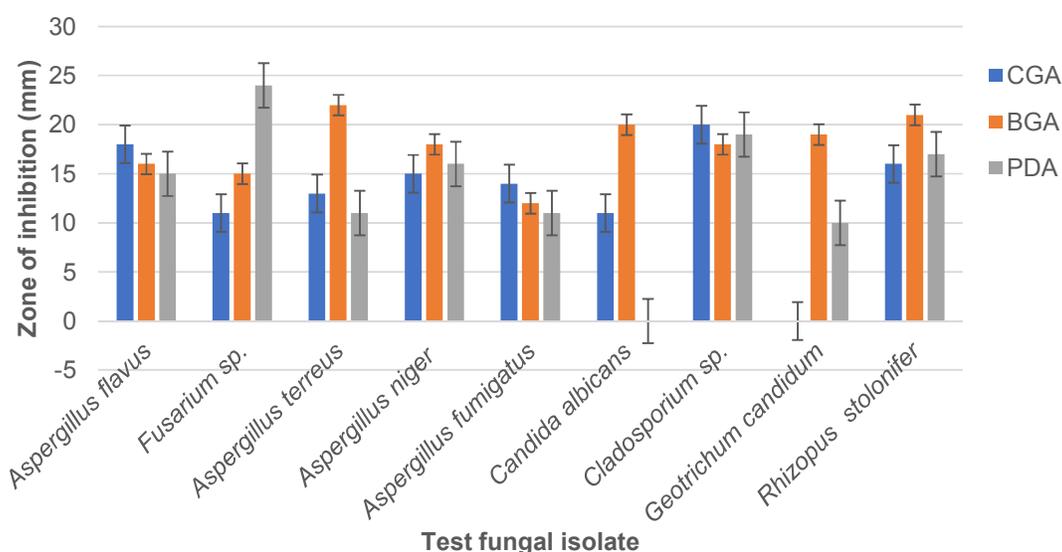
S/N	Macroscopic appearance	Microscopic appearance	Identity of isolate
1	Colonies are yellow green on the surface, pale or yellowish on the reverse, downy to powdery texture with a rapid growth.	Conidial heads mostly radiate, with conidial masses splitting into blocky columns at maturity. Conidiophores with roughened walls, especially near the vesicle; Philades uniseriate and biseriate. Some strains produce brownish sclerotia	<i>Aspergillus flavus</i>
2	Colonies growing slowly; surface usually orange to deep apricot due to confluent conidial slime; aerial mycelium sometimes floccose and whitish	Hyphae separate, hyaline; phialides long or short, cylindrical, simple or branched, with a scarcely discernible collarette at the apex. Microconidia unicellular, sometimes bicellular, hyaline, ovoid to ellipsoid in slimy head or in chains. Macroconidia curved, multicellular, with a foot cell at the base.	<i>Fusarium sp.</i>
3	Colonies are typically pseudo-like and cinnamon-buff to sand-brown in colour with a yellow to deep dirty brown reverse.	Conidia heads in the form of compact columns, conidia in chains, round, smooth walled, brown; conidiophores hyaline,	<i>Aspergillus terreus</i>

S/N	Macroscopic appearance	Microscopic appearance	Identity of isolate
		smooth walled; phialides biseriate, limited mainly to the upper part of the vesicle surface.	
4	Colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads.	Conidia heads radiate, splitting into loosely structured columns in age; conidia brown, rough, rough walled, in chains; conidiophores with smooth walls; phialides biseriate, covering the entire surface of the vesicle.	<i>Aspergillus niger</i>
5	Colonies are typically blue-green with a suede-like surface, rapid growth and downy to powdery texture	Conidial head in the form of compact columns; conidiophores smooth-walled, often tinted greenish; phialides uniseriate, concentrated on the upper surface of the vesicle; conidia round, with finely roughened walls, in chains	<i>Aspergillus fumigatus</i>
6	Colonies are white to cream-coloured smooth, glabrous, yeast-like.	Spherical to subspherical budding blastoconidia, 2-7 x 3-8 µm in size.	<i>Candida albicans</i>
7	Colonies are slow growing to rapid, mostly olive brown to blackish-brown on the surface and reverse with a velvety texture	Hyphae septate, brown; conidiophores brown, often septate; blastoconidia brown, in a very fragile branching chains, bicellular and shield shaped at the base of the chains, unicellular and ellipsoidal to round at the tip, prominent black scars are visible at the point of attachment	<i>Cladosporium</i> sp.
8	Colonies are fast growing, flat, white to cream, dry and finely suede-like with no reverse pigment.	Hyphae septate, hyaline; conidiophores absent; arthroconidia rectangular, not alternating, liberated by the fission of double walls; blastoconidia absent.	<i>Geotrichum candidum</i>
9	Colonies are very fast growing, about 5-8 mm high, white cottony at first becoming brownish grey to blackish-grey on the surface and pale on the reverse.	Hyphae broad, not or scarcely septate, rhizoids and stolons present; sporangiospores brown, solitary or in tufts on the stolons, diverging from the point at which the rhizoids form; sporangia rather round; sporangiospores ovoid.	<i>Rhizopus stolonifer</i>

Table 3. Mean radial growth of the test fungi on the formulated media and PDA

Isolate	CGA (mm)	BGA (mm)	PDA (mm)
<i>Aspergillus flavus</i>	12.00 ± 2.65	9.66 ± 0.94	19.00 ± 1.92
<i>Fusarium</i> sp.	9.50 ± 0.76	8.33 ± 1.00	18.67 ± 1.24
<i>Aspergillus terreus</i>	10.83 ± 2.11	14.33 ± 1.70	14.67 ± 1.24
<i>Aspergillus niger</i>	12.50 ± 2.36	9.66 ± 2.42	16.00 ± 2.16
<i>Aspergillus fumigatus</i>	11.16 ± 2.11	9.00 ± 0.57	10.00 ± 1.63
<i>Candida albicans</i>	11.00 ± 2.08	10.00 ± 1.29	20.00 ± 2.16
<i>Cladosporium</i> sp.	11.16 ± 1.68	9.33 ± 1.37	18.67 ± 1.25
<i>Geotrichum candidum</i>	10.00 ± 1.63	10.00 ± 1.29	16.33 ± 1.89
<i>Rhizopus stolonifer</i>	14.16 ± 2.41	8.66 ± 0.48	32.33 ± 1.70

CGA = Cassava glucose agar; BGA = Banana glucose agar; PDA = Potato dextrose agar

**Fig. 4. Susceptibility patterns of the test fungal isolate to Nystatin antifungal drug**

Key: CGA = Cassava glucose agar; BGA = Banana glucose agar; PDA = Potato dextrose agar; Error bar = Standard error in mean; Dunnet test ($P > 0.05$)

Furthermore, the scent leaf acetone extract had the highest inhibition on *Rhizopus stolonifer* isolate (25.00 mm) while it had the least inhibition (0.00 mm) on *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Candida albicans*, *Cladosporium* sp., *Geotrichum candidum* and *Rhizopus stolonifer* in both the formulated media and PDA as revealed in Fig. 2. The result in Fig. 3 also revealed that *Fusarium* sp. was the most inhibited isolate (24.00 mm) by the garlic acetone extract while *Geotrichum candidum* and *Candida albicans* were the least inhibited (0.00 mm) in both the formulated media and PDA. The result in Fig. 4 further revealed that nystatin had a good inhibition (25.00 mm) on *Aspergillus niger* isolate while it had the least inhibition (11.00 mm) on *Aspergillus terreus* and *Rhizopus stolonifer*,

respectively. There was no inhibition (0.00 mm) of the tested fungal isolates by the negative control (water) in both the formulated media and PDA. The reason for inhibition by the antimicrobial extracts could be due to the presence of bioactive ingredients such as flavonoid, saponins, tannins et cetera as previous study by Uba et al. [17] reported that active phytochemicals such as flavonoid, saponins, tannins et cetera found in garlic extract contributed to its antimicrobial activity. The observation of no statistical significance ($P > 0.05$) on the inhibition zones of the extracts and the nystatin against the tested fungal isolates implied that the extracts have potent antifungal activities comparable to the known antifungal drug (nystatin). Hence, they could be sources of

antifungal remedies especially in these days of multidrug resistance with known antimicrobials. It is also laudable to note that our formulated media were comparable to PDA in terms of susceptibility testing suggesting the possibilities of our formulated media (CGA and BGA) being used as ideal reference testing media for antimicrobial testing and other mycological assays. These unique features of our formulated media are in line with the valid criteria established by Akharaiyi and Abiola [6] which stated that an ideal medium for reference testing should be totally defined, reproducible, free of antagonists or boosters of antimicrobial action, well buffered to maintain pH and available in both liquid and solid formulations.

5. CONCLUSION

This study revealed that agro wastes (cassava and banana peels) contained nutrients that can meet the nutritional conditions for fungi cultivation. Our formulated media were comparable to PDA with regards to fungal susceptibility testing and thus they can be utilized as an alternative and cheap ideal media for mycological assays. Furthermore, the acetone extracts of *Ocimum gratissimum* (scent leaf) and *Allium sativum* (garlic) exhibited laudable antifungal activities against the isolates used which could be comparable to those of the known nystatin drug. Hence, the extracts could serve as possible cheap sources of antifungal remedies in these days of multiple antimicrobial resistance to conventional antimicrobials.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- 1 Nwabueze T, Otuwa U. Effect of supplementation of African bread fruit (*Treculia africana*) hulls with organic wastes on growth of *Saccharomyces cerevisiae*. Afr J Biotech. 2006;5(16):1494 – 1498.
- 2 Amadi OC, Moneke AN. Use of starch containing tubers for the formulation of culture media for fungal cultivation. Afr J Microbiol Res. 2012;6(21):4527–4532
- 3 Itelima J, Onyimba I, Nyam M, Nwachukwu I. Utilization of food crop wastes for the formulation of laboratory media used for cultivating soil fungi. Int J Microbiol Imm Res. 2014;2(1):10–14.
- 4 Kuhn DM, Ghonnoum MA. Indoor mold, toxigenic fungi and *Stachybotrys chartarum*: Infectious disease perspective. Clin. Microbiol. Rev. 2003;16(1):144–172.
- 5 Kumara KLW, Rawal RD. Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (*Carrica papaya* L). Trop. Agric. Res. Ext. 11:7–12.
- 6 Akharaiyi FC, Abiola MA. Isolation and cultivation of fungi with agrowastes formulated media. Der Pharm Chem. 2016; 8(9):56–62.
- 7 Anbu S, Padma J, Punithavalli K, Saranraj P. Fruits peel waste as a novel media for the growth of economically important fungi. J Pharmacog Phytochem. 2017;6(6):426–428.
- 8 AOAC. Official methods of analysis of the association of official analytical chemistry. 16th edition. Washington, USA: AOAC International; 2000.
- 9 Onome F, Ejale AU. An attempt to formulate culture media for the culture of air-borne fungi using local plant flours. Iss Biol Sci Pharm Res. 2018;6(3):39–45.
- 10 Sah SN, Ghimire T, Yadav SP, Shah PK. Growth pattern of economically important fungi in fruits peel waste media. Int J Grad Res Rev. 2019;5(1):60–66.
- 11 Uba BO, Okoye EL, Anyaeji OJ, Ogbonnaya OC. Potentials of actinomycetes isolated from coastal area of Niger Delta against *Citrus sinensis* (Sweet Orange) and *Lycopersicum esculentum* (Tomato) fungal pathogens. ResRev: A JBiotech. 2019;8(3):4–15.
- 12 Adesemoye AO, Adedire CO. Use of cereals as basal medium for the formulation of alternative culture medium for fungi. W Afr J Microbiol Biotech. 2005; 21:329–336.
- 13 Laleye SA, Tedela PO, Adesua B, Famurewa O. Growth of some microorganisms on media formulated from local raw materials. Res J Microbiol. 2007; 2:545–549.
- 14 Umedum CU, Enejekwute NP. Exploration of fungi growth on media formulated from agro-allied wastes. Trop J Appl Nat Sci. 2017;2(1):69–73.

- 15 Prescott LM, Harley DA. Microbiology. 5th edition. London: McGraw-Hill Publishers; 2002.
- 16 Ruth AO, Gabriel A, Mirrila EB. Basal media formulation using *Canavalia ensiformis* as carbon and nitrogen source for the growth of some fungi species. J Microbiol Biotech F Sci. 2012;1(4):1136–1151.
- 17 Uba BO, Okoye EL, Udejah OP. Antimicrobial activities of *A. sativum*, *Z. officinale* and *O. gratissimum* extracts on plant and fish pathogens. Afr J Edu Sci Tech. 2016;3(2):213–221.

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