



Optimization of Mycological Media Using Agro Waste for the Production of Antimicrobial Substance

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Authors' contributions

This work was carried out in collaboration among all authors. Author EOO carried out the laboratory analysis. Author CUU supervised and read the manuscript. Author IHI designed the study and performed the statistical analysis. Author LCA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This present study was conducted to optimize mycological media using agro waste for the production of antimicrobial substance.

Place and Duration of Study: Agro waste (sugarcane and sweet potato, sugarcane and jack fruit) collected within Anambra state between February- August 2019.

Methodology: Sugarcane and sweet potato (AMSSP), sugarcane and jack fruit (AMSJ) were peeled and the peels were air-dried and then ground into powdered form. 10 g each of the agro waste samples was weighed into 400 ml of distilled water in 1000 ml Erlenmeyer flask and allowed for 7 days, after which the mixture was filtered.

Then 200 ml of the filtrate was used.

The experimental conditions were optimized by using agro wastes (20/80 and 50/50 concentrations) as a culture medium, altering the temperature (30°C and 37°C), pH (5, 6, 7, 8, and 9), as well as the carbon and nitrogen source (glucose and NaNO₃). The fungi used were *Aspergillus niger*, *Aspergillus fischeri*, *Aspergillus aculeatus* and *Aspergillus fumigatus*.

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Results: Various agro wastes medium AMSSP and AMSJ were formulated as mycological media and the growth and nutritional conditions were optimized to ascertain antimicrobial substance production using some fungal isolates. Based on different concentrations *Aspergillus fumigatus* showed a promising zone of inhibition on AMSSP at a concentration of 20/80 while in AMSJ the concentration the 50/50 showed a maximum zone of inhibition on *Aspergillus fumigatus* ascertaining the presence of antimicrobial substance. AMSSP was able to produce maximum antimicrobial substance when supplemented with 1.0% glucose, 1.0% NaNO₃ at pH 7 and at temperature of 30 ± 2°C.

Conclusion: Agro wastes from AMSSP as well as from AMSJ contain nutrients that may support fungal growth. Maximum antimicrobial substance production is enhanced when supplemented with 1.0% of the carbon and nitrogen source at a pH of 7 and at a temperature of 30 ± 2°C.

Keywords: Agro waste; antimicrobial agent; optimization; zones of inhibition.

1. INTRODUCTION

Large amount of wastes are generated every year from the industrial processing of agricultural raw materials and individuals homes [1]. Most of these wastes are used as animal feed or burned as alternative for elimination. However, such wastes usually have a composition rich in sugars, minerals and proteins, and therefore, making them useful for other processes directly or indirectly. The presence of carbon sources, nutrients and moisture in these wastes provides conditions suitable for the development of microorganisms and this open up great possibilities for their reuse. The economical aspect is based on the fact that such wastes may be used as low-cost raw materials for the production of other value-added compounds, with the expectancy of reducing production costs [1]. The need to developed alternative media to various culture media has become imperative as the conventional media used are either not readily available or relatively expensive in most developing countries like Nigeria and other developing counties of the world.

Fungi are among the remarkable microorganisms that produce wide range of natural products often called secondary metabolites. In many cases, the beneficial implications of these secondary metabolites to the produced fungal organisms are unknown [2]. However, interest in some of these natural products is considerable, as many secondary metabolites are of medical, industrial and agricultural importance [2]. Some of these natural products are deleterious (example mycotoxins) while other are beneficial (example antibiotics) to human [2]. Although many studies have shown that biosynthesis of these natural products are usually associated with cell differentiation or development. This implies that most secondary metabolites are produced by

fungi that exhibit filamentous growth with relatively complex morphology [2]. Some of these natural products (antibiotics) produced by *Penicillin species*, *Aspergillus species* and other species of fungi, are important antimicrobial agents, lovastatin and mevastatin produced by *Aspergillus terreus* and *Penicillin citrinum* are known as cholesterol lowering agents. Some other secondary metabolites produced from fungi have immunosuppressant activity. A typical example is the non-ribosomal peptide (NRP) cyclosporine produced by *Tolypocladium niveum* and widely used to avoid organ-rejection in transplant surgery [3].

The cost of all the mycological media for biosynthesis of these valuable fungal secondary metabolites is rising at fast pace and many are losing their original quality due to high cost of the constituting ingredients.

Some of the media are no longer in existence and others contain reagents that are mutagenic and carcinogenic. To tackle this problem, some new microbiological media should be designed which are efficient as well as cost effective. The component of the media should be environmental friendly. These may be achieved by using agricultural waste as raw materials for mycological media. Utilization of agricultural waste as a substrate for fungal cultures for the production of valuable natural products has been reported which includes cellulose production by some fungi cultured on pineapple waste [4], carotenoid production using *Blakesla trispora* on agricultural waste [5], enzyme production by *Aspergillus niger* on agricultural waste [6], energy source for production of lipase by *Aspergillus fumigatus* on agricultural waste [7] and other valuable natural products by filamentous fungi on agricultural wastes [8].

Basically, every fungus requires carbon, nitrogen and energy source to grow and survive. The agricultural waste may meet these requirements and work as fungal growth medium for biosynthesis of secondary metabolites and can replace expensive, scarce, carcinogenic and mutagenic media in markets.

2. MATERIALS AND METHODS

2.1 Formulation of Medium

Agro waste medium (AMSSP): Sugarcane and sweet potato were peeled and the peels were air-dried and then ground into powdered form. Twenty grams comprising 10 g each of the sugarcane and potato samples was weighed into 400 ml of distilled water in 1000 ml Erlenmeyer flask and allowed for 7 days, after which the mixture was filtered.

Then 200 ml of the filtrate was used.

2.1.1 Agro waste medium (AMSJ)

Sugarcane and Jack fruit peels were air-dried and ground into powdered form. Twenty grams comprising 10 g each of the sugarcane and jack fruits was weighed into 400 ml of distilled water in 1000 ml Erlenmeyer flask and allowed for 7 days, after which the mixture was filtered. Then 200 ml of the filtrate was used.

2.2 Optimization of Carbon source, Nitrogen source, pH and Temperature for Production of the Antimicrobial Substance

The effect of 2% of carbon sources (glucose, sucrose), 2% nitrogen source (NaNO_3 , $(\text{NH}_4)_2\text{SO}_4$) was investigated by supplementing the agro waste media with the above carbon and nitrogen sources at pH 7. The optimum pH (5, 6, 7, 8, 9) and temperature (30°C, 37°C) for antimicrobial substance production using the optimum carbon and nitrogen sources was also investigated [9,10].

2.3 Isolation of Fungal Organisms

One tenth milliliter (0.1 ml) inoculum each was aseptically collected from 1:1000 dilution and plated on Sabourand Dextrose Agar (SDA) containing 0.05% chloramphenicol, using spread plating method. The inoculated plates were incubated invertedly at room temperature

(30±2°C) for 3-5 days after which the colonies from the culture were sub cultured on Sabourand Dextrose Agar (SDA) and PDA supplemented with 0.05% chloramphenicol to obtain pure culture [11].

2.4 Identification of Fungal Isolates

The fungi that were isolated were purified, characterized and identified based on the macroscopic, microscopic and molecular characteristics [12].

2.5 Isolation of Bacterial Test Organisms (*Staphylococcus aureus*, *Escherichia coli*)

The test isolates *Staphylococcus aureus* and *Escherichia coli* were collected from Microbiology Laboratory, Chukwuemeka Odumegwu Ojukwu University. The test isolates were further confirmed using MacConkey agar and Indole test for *E. coli* and Mannitol salt agar and coagulase test for *S. aureus*. The confirmed isolates were used for the study [13].

2.6 Screening of the Antimicrobial Substance on Bacterial Organisms

This was carried out using agar-well diffusion techniques as described by [14]. The broth culture of the test bacterial Isolates was prepared and incubated at 37°C for 24 h. This was swabbed on sterile poured plates containing MHA and was allowed for 1 h. Then wells were made on the seeded plates using sterile cork borer (5 mm). Then 100 ul of each component was carefully deposited into the wells and incubated at 37°C for 48 h, after which the diameter zones of inhibition were observed and recorded.

2.7 Statistical Analysis

The data that were generated from this study were presented inform of mean ± standard deviation, also in tables and figures. The statistical significance of the data generated were ascertained using one-way Analysis of variance (ANOVA) whereas pair wise comparison was carried out using student "t" test [15].

3. RESULTS

The extracts from *Aspergillus* isolates exhibited significant inhibitory activities against the

bacterial isolates (Table 1). The inhibitory activity of extract from *A. fumigatus* was significantly ($P<0.05$) most compared to that of other species. It was also observed that the extracts from the fungal species inhibited *E. coli* significantly ($P<0.05$) more than *S. aureus*. Also, the extract from those *Aspergillus* species grown in agro waste medium sugarcane and sweet potatoes (AMSSP) significantly ($P<0.05$) inhibited the bacterial isolates more than those species grown in agro waste medium sugarcane and jackfruit (AMSJ).

The inhibitory activities of the extracts from the *Aspergillus* species grown AMSSP and AMSJ against *E. coli* and *S. aureus* were significantly ($P< 0.05$) at different pH (Table 2). The study revealed that the inhibitory activities increased significantly ($P< 0.05$) from pH 6.0 to 7.0 and decreased significantly ($P< 0.05$) above neutral pH (7.0).

The inhibitory activities of *Aspergillus* species extract against *E. coli* and *S. aureus* at different temperature is shown in Table 3. The study revealed that the inhibitory activity of the extracts were significantly ($P< 0.05$) pronounced at $30\pm 2^{\circ}\text{C}$ than 37°C , and AMSSP were more pronounced than the extracts from those species grown in AMSJ.

4. DISCUSSION

As a result of search for cost effective and environmental friendly media that can support fungal growth and antimicrobial substance

production, the use of agro waste as raw material for the formulation of mycological media has been achieved.

The growth and nutritional condition of the media were optimized to enhance the production of antimicrobial substance. The fungal isolates used were *Aspergillus niger*, *Aspergillus fischeri*, *Aspergillus aculeatus* and *Aspergillus fumigatus*. The agro waste medium AMSSP and AMSJ were optimized based on concentration, pH, temperature and nutritional content of the medium.

The maximum zone of inhibition which *Aspergillus fumigatus* exhibited at the concentration of 20/80 (%) of AMSSP could be due to the potency of the fungi as well as the nutritional ratio of the agro waste medium.

Also the zone of inhibition exhibited by *Aspergillus fumigatus* at the concentration of 50/50 (%) of AMSJ could be attributed also to the nutritional ratio of the medium.

The zones of inhibition of the antimicrobial agent secreted by the isolates when supplemented with carbon sources on the test bacterial organism (*S. aureus*, and *E. coli*) revealed that agro waste medium (AMSJ and AMSSP) supplemented with 1.0% carbon source (Glucose) produced a tested zone of inhibition on the test organisms than others 0.1%, 0.5% and 2% respectively. This is in line with [16] review that nutritional requirements are necessary for cultivation and optimization of fungal growth for increased metabolites production.

Table 1. Diameter zones of inhibition of antimicrobial agent secreted by the isolates in agro waste medium sugarcane and sweet potatoes (AMSSP) against *S. aureus* and *E. coli* and isolates in agro waste medium sugarcane and jack fruits (AMSJ) against *S. aureus* and *E. coli*

Concentration (%)	Isolate	Agro waste medium			
		AMSSP		AMSJ	
	<i>Aspergillus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
20/80	<i>niger</i>	20.33 \pm 1.15	21.67 \pm 1.15	10.67 \pm 0.58	14.67 \pm 0.58
	<i>fischeri</i>	18.33 \pm 0.58	19.67 \pm 1.15	11.67 \pm 0.58	13.67 \pm 0.58
	<i>aculeatus</i>	18.67 \pm 0.58	18.67 \pm 0.58	17.33 \pm 0.58	14.00 \pm 0.00
	<i>fumigatus</i>	23.67 \pm 1.15	26.33 \pm 1.15	15.33 \pm 0.58	18.67 \pm 0.58
50/50	<i>niger</i>	10.67 \pm 0.58	13.00 \pm 0.00	20.67 \pm 0.58	23.33 \pm 0.58
	<i>fischeri</i>	10.33 \pm 0.58	13.67 \pm 0.58	17.67 \pm 0.58	23.00 \pm 1.00
	<i>aculeatus</i>	11.67 \pm 0.58	13.33 \pm 0.58	17.33 \pm 0.58	24.00 \pm 0.58
	<i>fumigatus</i>	15.33 \pm 0.58	19.33 \pm 1.15	22.33 \pm 1.15	26.00 \pm 0.58

Keys: AMSSP: Agro waste medium sugarcane and sweet potatoes,
S. aureus: *Staphylococcus aureus*, *E. coli*: *Escherichia coli*
 AMSJ: Agro waste medium sugarcane and jack fruits

Table 2. Diameter zones of inhibition of the antimicrobial agents secreted by the isolates in AMSSP supplemented with 1.0% Glucose and NaNO₃ in different pH against *S. aureus* and *E. coli*

Isolate	<i>S. aureus</i>					<i>E. coli</i>				
	5.0	6.0	7.0	8.0	9.0	5.0	6.0	7.0	8.0	9.0
ANCBS	14.00±0.00	16.00±0.00	21.00±1.00	17.33±0.58	16.67±0.58	16.00±0.00	17.00±1.00	25.00±1.00	21.00±0.00	18.00±1.00
AFNRRL	15.33±0.58	16.33±1.15	22.67±0.58	18.00±1.00	17.33±0.58	17.33±0.58	19.67±0.58	27.33±0.58	23.33±0.58	19.33±0.58
AAATCC	16.67±1.15	18.33±0.58	24.00±1.00	19.67±1.15	17.33±0.58	19.00±1.00	22.00±1.00	31.00±1.00	25.67±1.15	21.00±1.00
AFAF	19.67±0.58	21.00±1.00	26.33±0.58	21.00±0.00	18.67±0.58	22.00±0.00	25.00±1.00	34.67±0.58	27.00±1.00	22.67±1.15

Table 3. Diameter zones of inhibition of the antimicrobial agents secreted by the isolates in AMSSP and AMSJ supplemented with 1.0% glucose and NaNO₃ at pH 7 at different temperature against *S. aureus* and *E. coli*

Isolate	AMSSP				AMSJ			
	<i>S. aureus</i>		<i>E. coli</i>		<i>S. aureus</i>		<i>E. coli</i>	
	30±2°C	37°C	30±2°C	37°C	30±2°C	37°C	30±2°C	37°C
ANCBS	21.33±0.58	18.00±1.00	28.00±1.00	19.67±1.15	18.67±0.58	14.33±0.58	22.00±1.00	19.33±0.58
AFNRRL	23.00±1.00	19.33±0.58	32.33±0.58	21.00±1.00	19.33±1.15	16.67±0.58	23.00±1.00	18.67±0.58
AAATCC	25.67±1.15	21.00±0.00	35.67±1.15	23.33±1.15	23.00±1.00	19.67±0.58	28.33±1.15	24.67±0.58
AFAF	28.33±0.58	22.33±0.58	38.33±0.58	24.67±0.58	25.67±0.58	22.33±0.58	31.00±1.00	24.67±0.58

The diameter zone of inhibition of the antimicrobial agents secreted by the isolates when supplemented with carbon and nitrogen source in AMSJ and AMSSP, revealed that 1.0% of glucose, 1.0% NaNO₃ produced a maximum zone of inhibition on the various test bacterial organism than 0.1%, 0.5% and 2% respectively as collaborated by [16] that basic nutritional needs of fungi and yeast could be met when supplied with an aerobic environment, glucose, ammonium salt, inorganic growth factors to enhance their survival. Though, 1.0% glucose and 1.0% NaNO₃ produces best zone of inhibition.

The diameter zone of inhibition of the antimicrobial agents secreted by the fungal isolates in AMSSP supplemented with 1.0% glucose and NaNO₃ against *S. aureus* and *E. coli*, which showed that *A. fumigatus* exhibited maximum zone of inhibitions on the bacterial test organism at a pH of 7.0.

The zone of inhibition of the antimicrobial agents secreted by the isolates in AMSSP and AMSJ when supplemented with 1.0% glucose and NaNO₃ at pH 7 and at different temperature against *S. aureus* and *E. coli*, revealed that *A. fumigatus* produced a maximum zone of inhibition against the test organism at a temperature of 30±2°C which is in collaboration with the finding of [17] who reported that fungi grow best at a temperature range from 15 – 37°C.

5. CONCLUSION

This research revealed that the agro waste from sugarcane and sweet potatoes (AMSSP) as well as from sugarcane and jackfruit (AMSJ) waste contain nutrients that may support fungal growth for the production of the antimicrobial substance. The study also revealed that maximum production of the antimicrobial substance is enhanced when supplemented with 1.0% of the carbon and nitrogen source at a pH of 7 and at a temperature of 30 ± 2°C.

Since the cost of most mycological media for the biosynthesis of these valuable fungi antimicrobial substance is rising at a fast pace and many are losing their original quality due to adulteration. Therefore, new mycological media should be designed by using agricultural waste as raw material.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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