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Original Article

ANTIMICROBIAL ACTIVITY OF MEDICINAL COMPOUNDS PRODUCED BY FUNGAL DEGRADATION OF SOYBEAN OIL USING LIGNOCELLULOSE BIOMASS AS SURFACE

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ABSTRACT

Methyl esters were produced by fungal degradation of soybean oil using banana leaves as surface. Analysis of the products also revealed medicinal compounds (Benzyl Benzoate obtained from the banana leaves used as surface for the fungal degradation of the soybean oil and 1, 2-Benzene dicarboxylic acid dioctyl ester obtained from the soybean oil impurities).Microorganism activity test was carried out on the medicinal compounds to confirm their antibacterial and antifungal activity. The result showed that both had antibacterial and antifungal activity as shown by the zones of inhibition and as a result can find useful applications in food processing and preservation as well as agriculture as pest/disease control agents.

KEYWORDS: Antimicrobial; medicinal compounds; fungal degradation; soybean oil; methyl esters.

INTRODUCTION

Essential oils are made up of hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, aldehydes, ketones, phenols, ethers, esters, lactones, and phenol ethers). These compounds can pass bio membranes by free diffusion and as a result, exhibit a good reactivity when applied via skin, mucosal surfaces, inhalation, and ingestion (Djilani and Dicko, 2012).

Soybean oil is an important oil with numerous increasing applications in the modern day world. It is classified as a linolenic acid oil since it contains the more highly unsaturated linolenic acid. Other oils include castor oil (a hydroxyl-acid oil) which contains glycerides of ribonucleic acid. Also worthy of note is the coconut oil, which unlike most vegetable oils, is solid at room temperature due to its high proportion of saturated fatty acids (92%) particularly lauric acid. Due to its almost homogenous composition, coconut oil has a fairly sharp melting point, unlike other fats and oils which melts over a range.

Soybean oils are used for food products such as shortenings, salad and cooking oils, and

margarines, large quantities serve feed and industrial needs. The latter applications include chemicals such as plasticizers, which add pliability to plastics and other substances (Israel, Sunday, Mangsong and Ebong, 2016); stabilizers, which help other substances resist chemical change; emulsifiers, which enable the mixing of normally immiscible liquids; surfactants, which reduce the surface tension of liquids and are commonly used in detergents; and esters, nylons, and resins, which are basic ingredients in many industrial products. Besides detergents and plastics, products that contain chemicals derived from vegetable oils include lubricants, coatings, corrosion inhibitors, adhesives, cleaners, cosmetics, water repellents, and fuels. (Markley, 1961).

Antimicrobial compounds are widely used for prevention of food contamination from pathogenic bacteria (De and Agarwala, 2014). The assessment of natural and non-toxic antimicrobial substances to replace synthetic chemicals is becoming more relevant as the result can help limit the overuse of antibiotics to treat diseases and inhibit the growth of multi-drug resistant bacteria strains.

This study seeks to investigate the microbial activity of some medicinal compounds present in soybean oil crude extract in order to ascertain its use in medicinal applications.

MATERIALS AND METHOD

1. Sample collection and treatment

The Lignocellulose was extracted from banana leaves and the soybean oil purchased from Grand Cereal Plc, Jos. The banana leaves were collected from a group of banana trees planted by Akpabuyo Local Government Council banana plantation in Cross River State. The leaves were fresh and matured at the time of collection. The leaves were first sun-dried for one week and later oven-dried at 37^0C for four hours and ground using a pestle and mortar, and then sieved with a mesh size of < 250um to fine particles.

The processed lignocellulosebiomas (40g) was weighed and mixed with 250ml of distilled water in a flat bottom flask.The mixture was kept for 21days for degradation to take place. After 21days, the slurry was dried in a fume cupboard for 48 hours and soxhlet extracted. The residue obtained (cleaned lignocellulose) was dried in a fume cupboard for 48hours and then stored in a stoppered bottle, to be used as surface for fungal degradation.

2. Fungal degradation of the lignocellulose sample

About 4.0 g of the cleaned lignocellulose was accurately weighed and mixed in 25 ml distilled water containing 0.16g of yeast (a fungus) in a round bottomed flask. The set up was kept for twenty-one days for fungal degradation to take place. At the end of 21 days, the content of the flask (the slurry) was concentrated by evaporation to dryness in a fumed cupboard. The residue was then subjected to soxhlet extraction using absolute methanol to extract the bioliquid. The bioliquid mixture was kept in a fume cupboard to expel the methanol and then weighed to a constant weight. The lignocellulose residue was also air-dried and weighed to constant weight and labelled **LR**.

3. Fungal degradation of soybean oil on the degraded lignocellulose

The lignocellulose residue (3.6g) obtained from the second stage of the work above (**LR**) was placed in a flat bottomed flask. Soybean oil (0.4g) was weighed and added to the lignocellulose residue in the flask to give 4g of substrate. Exactly 40ml of n-hexane was added to the lignocellulose-oil mixture to help spread the oil evenly on the surface of the lignocellulose residue where the fungus will attack and degrade the oil. The hexane was later evaporated off in a fume cupboard. About 0.16g of yeast was weighed and dissolved in 25ml distilled water and the yeast solution was added to the lignocellulose – oil mixture in the flask and the mixture was kept for 21 days for biodegradation to occur. After 21 days, the fungal slurry was evaporated to dryness and the residue subjected to soxhlet extraction using absolute methanol to obtain the bioliquid.

4. Separation of the insoluble components of the bioliquid by precipitation

About 1.0g of the concentrated bioliquid from the degraded lignocellulose slurry (**bioliquid A**) was dissolved in a mixture of 1 ml methanol and 40 ml n-hexane in a 250 ml beaker and kept in a refrigerator for 24 hours. This procedure was repeated for the degraded soybean oil(**bioliquid B**). At the end of 24 hours, it was observed that each bio-liquids separated into two components; an insoluble component which settled at the bottom of the beaker and a soluble component which remained in the solution. A separating funnel was used to separate the insoluble

component from the soluble component.A portion of the soluble and insoluble component was subjected to GC- MS analysis.

5. Microbial Activity Test

The soluble bio-liquids were further subjected to microbial test (susceptibility test and Minimum Inhibition Concentration test (MIC).

Susceptibility Test

i. About 1g of the soluble component of bioliquid from lignocellulose and soybean oil wereix. separately obtained using the digital balance and then dissolved in 10ml n-hexane to get the stock solution of 1000mg.

ii. 1:9 dilution ratio was carried out to obtain 100mg of the drug i.e. 1ml of stock solution was added to 9ml of n-hexane

iii. Dilution ratios of 1:1 was carried out to obtain 50mg/ml of the soluble component of the bioliquid from lignocellulose degradation (the drug).

iv. Dilution ratios of 1:21/2 was carried out to obtain 40mg/ml of the drug.

v. Dilution ratios of 1:4 was carried out to obtain 20mg/ml of the drug and

vi. Dilution ratios of 1:9 was carried out to obtain 10mg/ml of the drug.

In each case, 1ml of the stock (100mg) was added to the corresponding amount of n-hexane.

vii. Later, 1ml each of 24 hours broth culture of the clinical isolates *Escherichia Coli, staphylococcus aureus, Candida albicans, Aspergillus fumigates* and *salmonella typhi* was placed in each of the five sterile petridishes, and previously sterilized Mueller Hinton agar held at 45-55^{0}C was poured into each petridish. The plates were then allowed to stand for 30-45 minutes to solidify.

viii. After solidification of the plates, wells were bored on the surface of the solidified agar at distinct locations representing the various dilutions or concentrations of the drug using a sterile cork borer.

Finally, with the help of sterile dropping pipettes, 0.2ml of each dilution (concentration) of the drug was carefully transferred into each well and the plates covered. The prepared plates were then incubated at 37^{0}C for 24 hours, and at the end of incubation, the diameters of the zones of clearance (inhibition) observed were measured in millimeters (mm) and recorded.

Minimum Inhibition Concentration (MIC) Test

The use of dilution technique was employed in obtaining various concentrations (dilutions) of the bioliquids A and B. To determine MBC (Minimum Bactericidal Concentration), the tube preceding the MIC tube was selected.The experiment was repeated for each of the clinical isolates namely *Escherichia coli, Staphylococcus aureus, Candida albicans, Aspergillus fumigates* and *Salmonella typhi.*

RESULTS AND DISCUSION

The results of the GC-MS analysis of the bio-liquid B is presented in table 1. The result reveals compounds with their corresponding retention time, molecular weight, molecular formula and structure.

Table 1: Product of Analysis of degraded Soybean Oil (bioliquid B)

RETENTION TIME (MIN)	MOLECULAR WEIGHT	MOLECULAR FOMULAR	STRUCTURE	NAME
21.342	270	$C_{17}H_{34}O_2$	0 0	Haxadecanoic acid Methyl ester
23.435	294	$C_{19}H_{34}O_2$	0 0	9, 12-Octadecadie-noic acid, Methyl ester
23.475	292	$C_{19}H_{36}O_2$	0 0	9- Octadecenoic acid, Methyl ester

23.708	298	$C_{19}H_{38}O_2$		Octadecanoic acid. Methyl ester
19.925	212	$C_{14}H_{12}O_2$		Benzyl benzoate

Sensitivity Test of Microorganisms in Different Concentrations of Extracts

The results of the sensitivity test of microorganisms namely *Staphylococcus aureus, Escherichia coli, Salmonella typhi*, *Bacillus subtilis, Candida albicans*and *Aspergillusfamigatus* are presented in Table 2. The results show the effect of different concentration of extracts on the microorganisms.

The results show that *Staphylococcus aureus*is more susceptible to the extract, followed by *Escherichia coli, Salomonellatyphi*and *Bacillus subtilis*in that order. This inference is as a result of the largest zone diameter exhibited by the bacteria in different concentrations of the extract. The results also show that *Candida albicans*is more susceptible to the extract due to larger zone diameter in different extract concentrations.

Table 2: Sensitivity test of microorganisms in different concentration of extracts

Name of Isolates		Concentration of Extract (µG/ML)					
		100	50	25	12.5	6.25	10.0
	Zone of Diameter (MM)						
Staphylococcus aureus		20.98	32.06	19.48	7.97	32.96	15.38
Escherichia coli		15.06	10.00	15.97	6.00	32.98	19.63
Salmonella typhi		11.36	19.00	26.99	17.97	24.30	21.00
Bacillus subtilis		6.06	6.03	19.53	25.06	28.00	16.00
Candida albicans		5.93	6.00	20.96	28.33	31.20	18.00
Aspergillusfamigatus		17.98	10.93	5.93	23.06	27.10	14.00

Minimum Inhibition Concentration (MIC), Minimum Bacteriocidal Concentration (MBC) And Minimum Fungicidal Concentration (MFC) Test

The results of the Minimum Inhibition Concentration (MIC), Minimum Bacteriocidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) Test are presented in Table 3.

Table 3: Minimum Inhibition Concentration (MIC), Minimum Bacteriocidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) Test

Isolate	***Minimum Inhibition Concentration (µg/ml)***	***Minimum Bacteriocidal Concentration(µg/ml)***	***Minimum Fungicidal Concentration(µg/ml)***
Staphylococcus aureus	*1.57*	*3.13*	-
Escherichia coli	*25.0*	*50*	-
Samonellatyphi	*12.5*	*25.0*	-
Bacillus subtilis	*12.5*	*25*	-
Aspergillusfumigatus	*25.0*	-	*50*
Candida albicans	*25*	-	*50*

The results shows that the Minimum Inhibition Concentration (MIC) is 1.57µg/mland the Minimum Bacteriocidal Concentration (MBC) was obtained by picking the test tube preceding the Minimum Inhibition Concentration (MIC) tube which is 3.13µg/ml. Minimum Fungicidal Concentration (MFC) was obtained by picking the test tube preceding Minimum Inhibition Concentration (MIC) tube which is 50µg/ml.

Microorganism activity test, carried out on the two medicinal compounds, Benzylbenzoate and 1,2-benzene dicarboxylic acid, dioctylester to confirm their antibacterial and antifungal activities showed that both had antibacterial and antifungal activities as shown by the zones of inhibition.

Currently, there is still need for new approaches to diminish or eradicate foodborne pathogens. Despite increased hygiene and advanced food production techniques, illness outbreaks as a consequence of consumption of contaminated foods still poses a serious problem in the world. Bio-preservatives from plants, natural food and essential oils are presently in high demand (Medina, Castro, Romero, Ramírez and Brenes, 2013; Nyong and Ekwenchi, 2017).

It was the aim to compare the bactericidal effect of degraded soybean oil against pathogenic microorganisms like as *Staphylococcus aureus*and*E. coli.*

The degraded soybean oil showed remarkable antifungal activity. This is in agreement with the results presented by (Medina*et al,* 2013). The results show that the better inhibition against *Candida Albicans*by soybeanoils is consistent with findings from previous studies.

The antibacterial and antifungal activity of Benzyl Benzoate was investigated by carrying out susceptibility test, Minimum Inhibition concentration test (MIC) and Minimum Bacteriocidal Concentration (MBC) test respectively using the clinical isolates *E. Coil, Staphylococcus Aureus, Candida Albicans*and*Aspergillus fumigates*. The susceptibility test was carried out to determine whether the bioliquids would inhibit or kill the microorganisms. The (MIC) test was carried out to determine the least or minimum concentration of the bioliquid that could inhibit or kill the microorganism. The minimum bactericidal concentration (MBC) is the concentration in the tube preceding the MIC tube and it is also the minimum concentration that could inhibit or kill the microorganism. The appearance of zones of clearance or inhibition confirmed that both bioliquids A and B had Antibacterial and Antifungal activity. This is probably due to the presence of Benzyl Benzoate and 1,2- Benzene dicarboxylic acid dioctyl ester in the extract.

CONCLUSION

The medicinal compounds discovered in degraded soybean oil is an indication that soybean oil and lignocellulose biomass can find useful applications in food processing and preservation as well as agriculture as pest/disease control agents.

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