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PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOL EXTRACTS FROM *MUCUNA PRURIENS* HUSKS AGAINST SOME FOODBORNE MICROORGANISMS

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Abstract: *Recently, plant by-products are being explored as safe and sustainable sources of bioactive compounds. This is the first study to screen phytochemicals and evaluate the antimicrobial activity of extracts from Mucuna pruriens husk for potential application as food preservative. Aqueous and ethanol extract from Mucuna pruriens seed husk were examined for presence of phytochemicals. The antimicrobial activities of the extracts against the predominant food spoilage bacterial and fungal isolates were evaluated by agar well diffusion method. Flavonoids, phenol, glycosides, steroids, saponins and anthraquinones were present in both aqueous and ethanol extracts while tannins and phlobatannins were absent. The test isolates except Candida sp. FY22 were sensitive to at least one extract. Aqueous extracts showed stronger and wider spectrum of antimicrobial activity with 100 mg/ml of the extract demonstrating highest activity index of 1.15 compared to gentamycin (10 µg) against Pseudomonas sp. BB22. This study confirms the potential of extracts from Mucuna pruriens husk as a natural source food preservative.*

Keywords*: Mucuna pruriens, Phytochemicals, Antimicrobial, Husk*

1. Introduction

Food spoilage and safety remain among the most serious economic and public health concerns globally [1]. It is estimated that more than half of the world's harvested and traditionally processed crops are lost to microbial deteriorations [2]. Up to 1.5 billion cases of morbidity and more than three million deaths occur in children every year as a result of food and water contamination. Conventional methods and synthetic additives employed to promote shelf-life stability and eliminate microbial hazards in foods are not affordable and with safety concerns to consumers. In addition, most of the target microorganisms have developed resistance to these strategies [3]. This rationalizes the recent exploration of natural sources, including plants, animals and microorganisms for novel antimicrobials that meet the criteria of safety, availability, affordability, eco-friendliness and efficacy for food applications.

Compounds derived from different plant parts have been used since ancient times for flavoring, coloring and preserving foods without reports of harm to consumers [3, 4]. Preparations from plants have been used orally for centuries in traditional medicine to treat infectious diseases. Antimicrobial activity of plant materials is due to the presence of some chemical compounds, including; alkaloids, flavonoids, phenolics, quinones, saponins and tannins [3].

Mucuna pruriens, also known as *yerepe* in Yoruba is a twinning annual plant that belongs to the family Fabaceae [5]. It is widespread in tropical and subtropical regions of the world, thriving under warm and moist conditions [6]. Its leguminous fruit is a longitudinal pod that contains about 4 to 6 shiny black or brown seeds [7]. The husk of the pod is densely covered with pale-brown or grey trichomes that cause irritating blisters if they come in contact with the skin. *Mucuna pruriens* has gained prominence as a valuable plant in the pharmaceutical and food industries. The seed was reported to have hypocholesterolemic, hyploglycemic, antiparkinson, antioxidant, laxative, aphrodisiac, immunostimulatory, anthelmintic and antimicrobial effects [8]. In addition, it is rich in polyunsaturated fatty acids, dietary minerals and highly digestible proteins [8]. Hence, the high demand on the seed of *Mucuna pruriens*. *Mucuna prurience* seed husk is a by-product that is largely underutilized and typically considered as wastes. Some studies have shown that plant husks can have similar or even higher proportions of bioactive compounds than the regularly used parts [3]. They could be abundant and readily available sources of phytochemicals that have several functionalities such as antimicrobial activity. High concentration of phenol and antimicrobial activity against *Staphylococcus aureus* was reported in aqueous extract of walnut green husk [9]. Coconut husk extract inhibited the growth of some biofilm-forming bacteria such as *Pseudomonas* sp., *Alteromonas* sp. and *Gallionella* sp. [10]. However, there is no report on the value of *Mucuna pruriens* seed husk as a potential source of bioactive compounds. Therefore the objectives of this study are to screen for the phytochemicals and determine the antimicrobial properties of different extracts from *Mucuna pruriens* seed husk against isolated food pathogenic and spoilage microorganisms.

2. Materials and methods

2.1 *Plant materials*

Dry pods of *Mucuna pruriens* were hand-picked from the matured plants in Eguare Quarters, Ebele, Igueben Local Government Area, Edo state, Nigeria in October, 2015. The plant was identified in Biological Sciences Department, Samuel Adegboyega University, Ogwa, Edo state. Husk was obtained by breaking the pod and separating the seed.

2.2 *Preparation of aqueous and ethanol extracts of husk*

The husk of *Mucuna pruriens* was sun-dried, crushed and milled into fine powder. Ten grams each of *Mucuna pruriens* husk powder was weighed with an analytical balance (Setra model BL200S) and added to 200 ml of distilled water and 98% ethanol in separate conical flasks at ambient temperature (28-30 ^{o}C) for 48 hours. The solutions were decanted and the supernatant were filtered using clean muslin cloths. The filtrates were concentrated to dryness by evaporating the solvents in the water bath at 45 ^{0}C [11].

2.3 *Isolation and characterization of test microbial strains*

Enumeration and isolation of microorganisms from deteriorating bread, fish and meat samples were carried out using the following media: Nutrient agar for bacteria, Brilliant Green Bile agar for coliforms and Yeast Peptone Dextrose (YPD) agar (supplemented with streptomycin) for yeasts. Distinct isolates were selected after incubation and characterization using morphological and biochemical methods [12, 13]. Probable

identification of isolates was done by reference to standard descriptions [14, 15].

2.4 *Phytochemical screening of extracts*

Aqueous and ethanol extracts of *Mucuna pruriens* seed husks were tested for the presence of alkaloids, anthraquinones, flavonoids, glycosides, phenols, phlobatannins, saponins, steroids and tannins using standard methods [16].

2.5 *Antimicrobial activity assay of extracts*

Antimicrobial properties of the aqueous and ethanol extracts were determined by agar well diffusion assay. Test bacteria and yeasts were inoculated in sterile nutrient and YPD broth respectively and incubated for 18 hours to obtain fresh cultures. Cell pellets were obtained from the broth cultures after centrifugation (12000 g for 15 min) and suspended in sterile distilled water to a turbidity that is equivalent to 0.5 McFarland standards. A total of 100 µl of suspensions of bacteria and yeasts were spread on already solidified Mueller-Hinton and YPD agar respectively. For both extracts 5 mg/mL and 10 mg/mL were prepared in sterile distilled water. Wells (6 mm diameter) were bored in the agar and 200 µl of extracts were dispensed into them. The plates were kept in the refrigerator for 2 hours for diffusion of the extracts into the agar and incubated at 37 ^{0}C for 24 hours. The plates were examined for zones of inhibition around the wells and the zones were measured in diameters [4].

2.6 *Antibiotic sensitivity assay*

The antibiotic sensitivity patterns of bacterial isolates were determined using the agar overlay disc diffusion method of National Committee for Clinical Laboratory Standards [17]. Mueller-Hinton agar plates were seeded by spread plate method with 100 µl of 18 hours old standardized bacterial suspension and left to dry at 30 ^{0}C for 15 mins. Disc containing different concentration of antibiotics were placed on seeded agar with the aid of sterile forceps under aseptic conditions. The antibiotics disc were; Ceftazidime (30 µg), Efuroxime (30 µg), Gentamicin (10 µg), Cefixime (5 µg), Ofloxacin (5 µg), Augmentin (30 µg), Nitrofurantin (30 µg) and Ciprofloxacin (5 µg). The plates were incubated at 37℃ for 24 hours. The resultant visible zones of inhibition were measured in millimeters (mm). Zones lesser than 14 mm were regarded as resistant (R), those ranging from 14 to 17 mm were indicated as intermediate (I) and zones greater than 17 mm were indicated as susceptible (S) [18].

2.7 *Determination of activity index of selected extract*

The activity index (AI) of the extract that showed the widest spectrum of antibacterial activity with reference to selected antibiotics towards which all test isolates showed zones of inhibition were calculated as described by Erhabor *et al.* [19].

$$(AI) = \frac{\text{Zone of inhibition of extract}}{\text{Zone of inhibition of antibiotics}}$$

3. Results and discussion

3.1 *Microbial analysis*

Enumeration of the microbial load revealed that the deteriorating food samples were heavily contaminated with bacteria and fungi (Table 1). Fish sample had the highest total bacteria count (8.2175 Log_{10} CFU/g) while bread sample had the lowest total bacteria count (7.2765 Log_{10}

CFU/g). The total coliform counts range from 6.0000 to 8.8325 Log_{10} CFU/g. Yeast was not cultured from meat sample but bread and fish samples had yeast counts of 8.0000 and 7.1139 Log_{10} CFU/g respectively. In developing countries, food handling practices, especially the processing of fresh fish and meat are carried out with poor implementation of Good Manufacturing and Hygienic Practices, thereby exposing them to several sources of microbial contamination. These foods have high water activity and supply complete range of nutrients for microbial growth; hence, they are prone to spoilage [20].

Table 1

Microbial counts in deteriorating food samples

Food sample	Total bacteria (Log_{10} CFU/g)	Coliform (Log_{10} CFU/g)	Yeast (Log_{10} CFU/g)
Bread	7.2765	8.1367	8.0000
Fish	8.2175	8.8325	7.1139
Meat	7.2625	6.0000	NG

Key: NG: No growth, CFU/g: Colony forming units per gram

Table 2

Probable identity of selected bacteria and yeast isolates from deteriorating food samples

Probable identity	Food source
Pseudomonas sp. BB21	Bread
Pseudomonas sp. BB22	Bread
Lactobacillus sp. BM21	Bread
Listeria sp. FM21	Fish
Aeromonas sp.FN21	Fish
Pseudomonas sp. MM1	Meat
Bacillus subtilis MN1	Meat
Saccharomyces cerevisiae BY21	Bread
Kluyveromyces africanus BY22	Bread
Candida sp. FY22	Fish

Based on morphological examination and biochemical tests, the bacteria were probably identified as species of *Aeromonas, Bacillus, Lactobacillus, Listeria* and *Pseudomonas.* The yeasts are *Candida* sp., *Kluyveromyces africanus* and *Saccharomyces cerevisiae* (Table 2). The bacteria identified in this study have been implicated in the spoilage of fresh meat and fish and their respective products [2]. Yeasts are important food spoilage microorganisms, including foods with low water activity such as bread [22].

3.2 *Phytochemical analysis*

Table 3 shows the phytochemical constituents of the aqueous and ethanol extracts from husk of *Mucuna pruriens.* Four and five chemicals were identified in the aqueous and ethanol extracts respectively. In both extracts, phenols, glycosides and saponins were present while tannins and phlobatannins were absent. Flavonoids and anthraquinones were detected in ethanol extract. Husks of seeds which constitute about 45% of plant biomass have been noted to be more concentrated with phytochemicals such as

flavonoids, phenols and tannins compared to their respective seeds [3, 23]. As far as we know, this is the first report on the phytochemicals in *Mucuna pruriens* seed husk extracts. However, studies on the aqueous and ethanol extracts of *Mucuna pruriens* seeds and leaves also revealed the presence of similar phytochemicals [24, 25]. In agreement with ours, studies that explored phytochemicals in husks from other plants reported high concentration of phenol in *Juglans regia* (walnut) and *Cocos nucifera* (coconut) husk extracts [9, 10].

Table 3

Phytochemical constituents of *Mucuna pruriens* husk extracts

Phytochemical constituent	Aqueous extract	Ethanol extract
Tannins	-ve	-ve
Flavonoids	-ve	+ve
Phenols	+ve	+ve
Glycosides	+ve	+ve
Steroids	+ve	-ve
Phlobatannins	-ve	-ve
Saponins	+ve	+ve
Anthraquinones	-ve	+ve

Key: +ve: Present, -ve: Absent

3.3 *Antimicrobial activity*

Table 4 shows the antimicrobial activity of aqueous and ethanol extracts from *Mucuna pruriens* against the food spoilage bacteria and yeast isolates. The spectra of antimicrobial activity of both extracts increased with concentration, however, aqueous extract demonstrated wider spectrum than the ethanol extract. All test isolates were resistant to 50 mg/ml of ethanol extract while 4 test isolates were susceptible to the 100 mg/ml of the same extract. 50 mg/ml of aqueous extracts inhibited 6 isolates with zones of inhibition ranging from 5-10 mm while 100 mg/ml of the extract demonstrated stronger and wider spectrum of activity, inhibiting 9 test isolates with zones of inhibition of 5-15 mm.

Table 4

Antimicrobial activity of *Mucuna pruriens* husk extracts

Test microorganisms	Diameter of inhibition zones (mm)			
	Aqueous extract (50 mg/ml)	Aqueous extract (100 mg/ml)	Ethanol extract (50mg/ml)	Ethanol extract (100mg/ml)
Pseudomonas sp. BB21	-ve	10	-ve	5
Pseudomonas sp. BB22	-ve	15	-ve	10
Lactobacillus sp. BM21	10	15	-ve	5
Listeria sp. FM21	5	5	-ve	1
Aeromonas sp.FN21	5	10	-ve	-ve
Pseudomonas sp. MM1	5	5	-ve	1
Bacillus subtilis MN1	-ve	5	-ve	-ve
S. cerevisiae BY21	10	15	-ve	-ve
K. africanus BY22	5	15	-ve	-ve
Candida sp. FY22	-ve	-ve	-ve	-ve

Key: -ve: No inhibition

Lactobacillus sp. BM21 and *Saccharomyces cerevisiae* BY21 were more sensitive to the aqueous extract while *Pseudomonas* sp. BB22 was the most sensitive test isolate to ethanol extract. *Candida* sp. FY22 was resistant to all the extracts. The results obtained in this study present almost similar antimicrobial capacity with *Juglans regia* husk aqueous extract, inhibiting Gram positive and negative bacteria but unable to inhibit *Candida albicans* and *Cryptococcus neoformans* [9].

Table 5

Antibiotic sensitivity pattern of test microorganisms

Test isolate	Gram reaction	Diameter of zone of inhibition (mm)													
		PEF	**CN**	**APX**	**Z**	**AM**	**R**	**CPX**	**S**	**SXT**	**E**	**OFX**	**AU**	**CH**	**SP**
Pseudomonas sp. BB21	-ve	17 (S)	19 (S)	NA	NA	-ve (R)	NA	21 (S)	-ve (R)	-ve (R)	NA	25 (S)	-ve (R)	17 (I)	24 (S)
Pseudomonas sp. BB22	-ve	23 (S)	13 (I)	NA	NA	15 (I)	NA	27 (S)	-ve (R)	-ve (R)	NA	15 (I)	-ve (R)	17 (I)	23 (S)
Lactobacillus sp. BM21	+ve	19 (S)	17 (S)	-ve (R)	-ve (R)	-ve (R)	8 (R)	25 (S)	-ve (R)	-ve (R)	-ve (R)	NA	NA	NA	NA
Listeria sp. FM21	+ve	23 (S)	23 (S)	-ve (R)	-ve (R)	-ve (R)	-ve (R)	26 (S)	17 (S)	13 (R)	-ve (R)	NA	NA	NA	NA
Aeromonas sp.FN21	-ve	21 (S)	13 (I)	NA	NA	-ve (R)	NA	23 (S)	-ve (R)	-ve (R)	NA	27 (S)	-ve (R)	15 (I)	17 (S)
Bacillus subtilis MN1	+ve	15 (I)	15 (S)	-ve (R)	-ve (R)	-ve (R)	-ve (R)	23 (S)	-ve (R)	-ve (R)	-ve (R)	NA	NA	NA	NA

Key: AM:- 30 µg Amoxacillin, APX:- 30 µg Ampiclox, AU:- 25 µg Augmentin, CH:- 30 µg Chloramphenicol, CN:- 10 µg Gentamycin, CPX:- 10 µg Ciprofloxacin, OFX:- 30 µg Tarivid, PEF:- 10 µg Pefloxacin, R:- 25 µg Rocephin, S:- 30 µg Streptomycin, SP:- 10 µg Sparfloxacin, SXT:- 30 µg Septrin, Z:- 20 µg Zinnacef**,**
-ve: No inhibition, NA: Not applicable, Resistant (R), Intermediate (I), Susceptible (S)

Table 6

Antimicrobial activity index of 100 mg/ml aqueous extract from *Mucuna pruriens* husk against selected antibiotics

Test isolate	PEF	CN	CPX
Pseudomonas sp. BB21	0.59	0.53	0.48
Pseudomonas sp. BB22	0.65	1.15	0.56
Lactobacillus sp. BM21	0.79	0.88	0.60
Listeria sp. FM21	0.22	0.22	0.19
Aeromonas sp.FN21	0.48	0.77	0.44
Bacillus subtilis MN1	0.33	0.33	0.22

CN: 10 µg Gentamycin, CPX: 10 µg Ciprofloxacin, PEF: 10 µg Pefloxacin

Most reports support stronger antimicrobial activity when extraction is done using organic solvents. However, considering the extraction conditions and

plant material, there are few reports of comparatively better extraction and higher antimicrobial activity with aqueous extracts. Gomaa *et al.* [26] reported the release of more phytochemicals and wider spectrum of antimicrobial activity in aqueous extracts from seeds of *Jatropha curcas, Simmondsia chinensis* and *Datura metel* compared to their respective ethanol extracts. Similarly, Ali *et al.* [27] reported that aqueous extract of *Vitex doniana* stem back demonstrated higher antibacterial activity than the methanolic extract. In a similar manner, aqueous extracts from stem bark, stem wood and root of *Ternimalia brownii* exhibited the strongest activity against both bacteria and fungi compared to petroleum ether, dicloromethane and methanol extracts [28].

The antibiotic sensitivity and resistance pattern of the test isolates to different types of antibiotics is presented in Table 5. All test isolates were sensitive to pefloxacin, gentamycin and ciprofloxacin. The Gram positive test isolates were resistant to ampiclox, erythromycin and zinnacef while the Gram negative isolates were resistant to augmentin. The activity indexes of 100 mg/ml aqueous extract from *Mucuna pruriens* husk compared to gentamycin (10 µg), ciprofloxacin (10 µg) and pefloxacin (10 µg) are presented in Table 6. The highest activity index value (1.15) was shown against gentamycin for *Pseudomonas* sp. BB22 while the least activity index value (0.19) was recorded against ciprofloxacin for *Listeria* sp. FM21. Relative to standard antibiotics, the activity index of the 100 mg/ml aqueous extract is considered to be significant against the test isolates. The ranges of activity index reported in this study have also been reported for extracts from other plant materials [19, 30].

4. Conclusion

The present study has revealed the presence of valuable bioactive compounds in *Mucuna pruriens* husk extracts. In addition, the extracts exhibited strong antimicrobial activities against food spoilage and pathogenic bacteria and fungi. This by-product that was hitherto discarded as wastes could be an affordable, eco-friendly and sustainable sources of food additives for preservation purposes.

5. References

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