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Full Length Research

MICROBIAL PROFILING AND ANTIFUNGAL SCREENING OF SHEA BUTTER, COCONUT OIL AND PALM KERNEL OIL SOLD IN PORT HARCOURT METROPOLIS, NIGERIA

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Edible oils (Shea butter, coconut oil, and Palm kernel oil) are used extensively in Nigeria. These oils are processed to eliminate undesirable constituents. They are in use for domestic and industrial purposes. The study investigated the microbial profile of the oils as well as the antifungal screening. The parameters analyzed were: total heterotrophic bacterial (THB), total heterotrophic fungi (HTF), total *coliforms,* total *Salmonella Shigella*, Minimal Inhibitory concentration screening of selected antifungal. The result revealed total heterotrophic bacterial count of Shea butter as 9.5 x 10^{-4} - 2.1 x 10^{-6}cfu/g, Coconut oil as 7.5 x 10^{-4} - 1.68 x 10^{-6} cfu/ml while Palm kernel oil as 6.0 x 10^{-4} - 1.2 x 10^{-6} cfu/ml. Total *coliforms* were 9.0 x 10^{-2} - 9.9 x 10^{-3} cfu/g and total *Salmonella Shigella* were 1.2 x 10^{-2} - 1.4 x 10^{-4} cfu/ml. The bacteria genera were *Bacillus* sp, *Pseudomonas* sp, Escherichia coli, *Proteus* sp, *Serratia* sp, *Micrococcus* sp, *Klebsiella* sp, *Staphylococcus* sp, *Flavobacteria* sp, *S. marcescens*, *Salmonella* sp and the fungi genera were *Aspergillus* sp, *Penicillin* sp, *Rhizopus* sp, *Fusarium* sp, *Candida* sp, *Mucor* sp, *Paecilomyces* sp, *Cladosporium* sp, *Saccharomyces* sp. The antifungal screen shows Nystatin and Ketoconazole recorded a MIC of 12.25 mg having 71 % and 81.9 % respectively. It is recommended that edible oil be produce with sanitized equipment and be stored in sterilized containers.

Keywords: Edible Oils, Antifungi, *S. marcescens*, *Aspergillus sp*, Inhibitory, Mycoses.

INTRODUCTION

The African continent has boundless botanic species from whose seeds or fruits, edible oils can be extracted. Edible oils are food substances, other than dairy products of the plant, animal or microbial origin that is manufactured for human consumption, wholly or in part from fat or oil (Arunina and Rajamohan, 2013). They are either solid or liquid at room temperature (Robin, 1999). Some edible oils that are considered healthy for human consumption are: coconut oil, palm oil, corn oil, cottonseed oil, olive oil, sunflower oil, peanut oil, soybean oil. The oil is edible because they consist of carboxylic acid with long hydrocarbon chains, (Arunina and Rajamohan, 2013) the carboxylic group provide the site for enzymes action accelerating the metabolism of the food substance and ultimately absorption of the diet. Shea butter, coconut oil and palm kernel oil are the example of edible oil commonly used in various parts

of Nigeria. They are not only used for their cooking and cosmetic benefit but also for their therapeutic and pharmaceutical properties (Hamburger and Hostettmann, 2001).

It is the belief that most rural dwellers in Nigeria depend on traditional medicine for most of their health concerns (Ekpa and Ebana, 2006) but with Shea butter, coconut oil and palm kernel oil both rural and urban dwellers utilize these edible oils. The demand of the oils by the international community is also on the increase (Honofo, et al., 2012). Giving the afore-mentioned incentive, it is imperative that the microbial level of these edible oil be evaluated and statically record be kept. The study is aimed at (i) to determine the microbial quality of edible oil (Shea butter, coconut oil and palm kernel oil) (ii) to ascertain the minimal inhibitory concentration (MIC) of some antifungal against the fungal isolate.

MATERIALS AND METHOD

Collection of Sample

Samples were randomly obtained from various markets around Obio Akpor Local Government area in Port Harcourt metropolis. The samples were Shea butter (SHB), Coconut oil (CNO) and Palm Kernel oil (PKO) and these were transported in sterile bottles to the laboratory for analysis. The samples were designated as follows; Shea butter (SHB1, SHB2, SHB3, SHB4, SHB5, SHB6, SHB7, SHB8, SHB9, SHB10) Coconut oil (CNO1, CNO2, CNO3, CNO4, CNO5, CNO6, CNO7, CNO8, CNO9, CNO10) Palm kernel (PKO1, PKO2, PKO3, PKO4, PKO5, PKO6, PKO7, PKO8, PKO9, PKO10).

Enumeration of Total Bacteria

The pour plate serial dilution method of Pepper and Gerba (2001) and Benson (2002) was adopted. About 1 ml of coconut oil, palm kernel oil and 1g of Shea butter were weighed using nutrient agar for total bacteria, Eosin methylene blue agar (EMB) for isolation of enteric bacteria and MacConkey agar for Coliforms. Using analytical balance and homogenized with 9 ml of sterile peptone water to obtain a stock solution. Thereafter tenfold serial dilution was carried out up to 10^{-5} dilution. 0.1 ml of the appropriate dilution was poured into the plate containing particular medium for the isolation of the organisms; the plates were incubated at 37^0C for 24 hours After incubation the number of discrete colonies were counted and expressed as colony forming units (cfu/ml) of the samples (Ohimain et al., 2013; Okechalu et al., 2011). Pure cultures of the bacteria were streaked on nutrient agar slant and preserved at 4^0C for identification.

Enumeration of Total Fungi

The enumeration of total fungi was done using the spread plate technique. About 0.1 ml of 10^{-3} and 10^{-4} dilution of each sample was spread on the surface of the Potato dextrose agar (PDA) into which 0.1 ml of 8.5 % lactic acid was added and incubated at 28^0C for 4 - 5 days.

Identification of Isolates

All bacterial isolates were characterized and identified based on their cultural, morphological and biochemical characteristics (Gram staining, Catalase, Oxidase, Coagulase, Urease, Indole, Motility, MR/VP test) as described in Bergey's manual of Determinative Bacteriology by Holt et al., (1994) and Scheme of Cheesbrough (2000) and (2004).

The fungi identification was based on the macroscopic and microscopic morphology. Their characteristic hyphal and reproductive structure was observed. For the microscopic morphology a portion of the mold growth on PDA was aseptically placed on a drop of Lacto-phenol cotton blue stain on a clean microscope slide (Larone, 1995).

Minimum Inhibitory Concentration Assay for Fungi

The minimum inhibitory concentration of the antifungal against the test organisms was determined using the broth dilution method (Sahn and Washington,1990). The inoculum were prepared by making a direct broth suspension of isolated colonies. About 1 ml of the isolated colonies was added to 1 ml of PDA, it was adjusted to achieve a turbidity equivalent of 0.5 McFarland turbidity standard. Serial dilution of the different concentrations (100 %, 50 %, 25 %, 12.5%) of the antifungal agent (Nystatin, Griseofulvin and Ketoconazole) was added to the PDA growth medium in separate test tubes and mixed thoroughly by vortexing. The tubes were then inoculated at 37^0C for 4 days. Broth tubes that appear turbid (not clear)

Table 1. Total Bacterial Count.

	Shea butter	Coconut oil	Palm Kernel oil
Total Heterotrophic bacteria	9.5×10^{-4} - 2.1×10^{-6}	7.5×10^{-4} -1.6×10^{-6}	6.0×10^{-4} - 1.2×10^{-6}
Total Coliform	9.0×10^{-2} - 9.9×10^{-3}	NG	NG
Total Salmonella Shigella	1.2×10^{-2} - 1.4×10^{-4}	NG	NG

NG = No growth

Table 2. Morphology and biochemical characteristics of Bacteria isolate.

Sample code	Gram Morp	Spore	Cat	Oxi	Ind	MR	VP	H_2S	Gas	Slant	Butt	Glu	Mot	Probable Organism
BEO1	+ rod	+	+	-	-	-	-	-	-	B	A	A	+	*Bacillus* sp
BEO2	- rod	-	+	+	-	-	+	-	-	B	B	-	+	*Pseudomonas* sp
BEO3	- rod	-	+	-	+	+	-	-	+	A	A	A/G	+	Ecoli
BEO4	- rod	-	+	-	+	+	-	+	+	B	A	A/G		*Proteus* sp
BEO5	- rod	-	+	-	-	-	+	-	+	A	A	A	+	*Seriatia* sp
BEO6	+ cocci	-	+	-	-	+	-	-	-	A	A	A	+	*Micrococcus* sp
BEO7	+ rod	-	+	-	-	-	+	-	+	A	A	A/G	-	*Klebsiella* sp
BEO8	+ cocci	-	+	-	-	-	+	-	-	A	A	-	-	*Staphylococcus* sp
BEO9	- rod			+	+	-	+	-	+	A	A	A	-	*Flavobacteria* sp
BEO10	- rod			-	+	-	+	-	-	A	A	A	+	S. marcescens
BEO11	- rod			-	+	-	-	+	+	A	A	A	+	*Salmonella* sp

Note
Cat = catalase; Oxi = Oxidase; Ind = Indole; MR = Methyl red; VP = Voges proskauer; Glu = Glucose; Mot = motility

are indicative of fungi growth while tubes that remain clear indicate no growth. The minimum inhibitory concentration of the antifungal is the tubes having the lowest concentration that does not show growth.

DISCUSSIONS

Shea butter, coconut oil and palm kernel oil are edible oil that are fast becoming house-hold items, this is because of their nutritive, cosmetic, pharmaceutical and medical values. The study evaluated the microbial state and antifungal screening of edible oil sold in Port-Harcourt Markets.

The total heterotrophic bacteria count (THBC) had Shea butter oil samples greater than coconut oil samples greater than palm kernel oil samples as shown in Table 1. The value of THB in the various samples were above the permissible load of not more than two in a dilution of 10^{-4} ml (Yusuf et al., 2017; Okechala et al., 2011) as stated by the National Agency for Food and Drug Administration Control (NAFDAC).

The genera of organism and their frequency of occurrence are; *Bacillus* sp 27.27 %, *Pseudomonas* sp 18.18 %, *E.coli* 6.06 % , *Proteus*, 3.03 % , *Serratia* 12.12 %, *Micrococcus* sp 6.06 %, *Klebsiella* sp 3.03 %, *Staphylococcus* sp 21.21 %, *Flavobacteria* sp, 2.08 %, *S. marcescens* 6.06 %, *Salmonella* sp 6.06 % . The genera of organisms are similar to those isolated by other researchers as revealed in Table 2. Okechalu et al., 2011 worked on Palm oil and isolated *Bacillus* sp, *Proteus* sp, *Micrococcus* sp, *Staphylococcus aureus*. Ohimain et al., (2013) worked with palm oil mill effluents, they isolated *Micrococcus* sp, *Bacillus* sp, *Pseudomonas* sp. and *Staphylococcus* sp . Esiegbuya et al., (2015) worked with Shea butter and isolated Escherichia coli, *Micrococcus* sp, *Enterobacte*r sp and *Pseudomonas* sp. Ezediokpu et al., (2015) isolated *Staphylococcus* sp, *Alcaligene* sp, *Enterococcus* sp, *Salmonella* sp, *Achromobacter* sp, *Micrococcus* sp and Klebsiella

Table 3. Cultural and Morphological Characteristic of fungal Isolate.

Sample code	Cultural characteristics	Microscopic appearance using lactophenol blue	Probable Organisms
FEO1	Blackish brown sporing mycelia with scalloped margin, smooth and entire with light cracked revered	Septate hyphae with long conidiosphere that support spherical vesicles which gives rise to large metulae	*Aspergillus* sp
FEO2	Blue-greenish colonies with a white halo a radiating margin, the reverse is white-golden	Hyphae are hyaline and septate and produce branch-like conidiosperes, tiny finger-like projection	*Penicillin* sp
FEO3	Fluffy whitish mycelia which becomes dark with age	Non-septate sporandiosphere vary in light. presence of rhizoids	*Rhizopus* sp
FEO4	White cotton with pnk center and alight periphery, reverse is light.	Hyphae are small and septate, and give rise to philides that produce large multicelled micro conidia	*Fusarium* sp
FEO5	White or creamy, 1 mm, raised, round, opaque.	Round shaped single and clustered cells appearing purple with simple staining using crystal violet.	*Candida* sp
FEO6	Light yellow-orange velvet surface with ligt reverse	Single round sporandium	*Mucor* sp
FEO7	Powdery yellow-brown pigmentation.	Conidia are sub-spherical, chlamydospores present single or in short chain	*Paecilomyces* sp
FE08	Gray surface with dark pigmented reverse.	Long chain of budded conidia that have dark septate scar.	*Cladosporium* sp
FEO9	White, 1 mm, raised, opaque, circular, shiny and light reverse	Oval shaped purle cells as stained with crystal violet	*Saccharomyces* sp

sp, they worked with Palm kernel oil. Madhusudhan et al., (2015) isolated *Pseudomonas aeruginnosa*, *Klebsiella pneumonia*, *shigella* sp while working with vegetable oil and locally produce oil.

E coli are indicator organisms isolated from the samples, these organisms are index of possible water contamination by human pathogens. It is important to note that some species of *E coli* causes infantile diarrhea, which is the greatest singular killer of babies (Talaro and Talaro, 1994). These edible oils (Shea butter, coconut oil and palm kernel oil) are used as traditional remedies for cough, especially in children. *Klebsiella* sp, *Proteus sp* and *Seratia* sp are opportunistic pathogens isolated from the samples which causes secondary infection in individuals whose immune system have been compromised (Talaro and Talaro, 1993).

Total Coliforms count and total Salmonella Shigella count occurred only in Shea butter sample, there were none in coconut oil and palm kernel oil samples, this results were similar to those obtained by Yusuf et al., (2017) who worked with extracted palm kernel and coconut oil although Madhusudhan et al., (2015) isolated Coliforms from some of the vegetable oil

Table 4. Minimum Inhibitory Concentration Assay for Isolated Fungi.

Fungi isolate	NYSTATIN				GRISEFLUVIN				KETOCONAZOLE			
	100 %	50 %	25 %	12.25 %	100 %	50 %	25 %	12.25 %	100 %	50 %	25 %	12.25 %
***Aspergillus* Sp**				√			√		√			
***Penicillin* Sp**				√	√							√
***Rhizopus* Sp**				√				√				√
***Fusarium* Sp**			√			√						√
***Candida* Sp**				√				√				√
***Cladosporium* Sp**			√			√						√
***Saccharomyces* Sp**				√				√				√

samples. Ezediokpu et al., (2015) research with refined palm kernel oil and obtained Coliforms from all samples used. *Salmonella* and *Staphylococcus* are primary pathogenic organisms isolated from the samples, *Salmonella* causes salmonellosis while *Staphylococcus* produces toxin which causes staphylococcus food poisoning (Okechalu et al., 2011; Talaro and Talaro, 1993).

Eight (8) genera of fungi were isolated and identified tentatively, these are *Aspergillus* sp, *Penicillium* sp, *Rhizopus* sp, *Fusarium* sp, *Candida* sp, *Mucor* sp, *Paeciloryces* sp, *Cladosporium* sp (Table 3). These are similar to those isolated by other researches (Okechalu et al., 2011; Esiegbuga et al., 2015, Ezediokpe et al., 2015; Madhusudhan et al., 2015). *Aspergillus* sp, *Mucor* sp and *Penicillium* sp are scatter in the form of spores, making them omnipresent in both air and dust (Apiniisi, 2005 Makun et al., 2009). The samples were almost always sold exposed in the market, particularly Shea butter, making it easy for the microorganisms to contaminate it. *Aspergillus* sp causes Aspergillosis and *Aspergillus flavus* has been implicated for the production of alflatoxin which is capable of inducing toxic syndrome especially cancer.

The antifungal agent used were Nystatin, Griseofulvin and Ketoconazole (Table 4). The minimum inhibitory concentration (MIC) of Nystatin for *penicillium* sp was 12.25 mg, *Aspergillus* sp 12.25 mg, *Candida* sp 12.25 mg, *Fusarium* sp 25 mg, *Rhizopus* sp 12.25 mg, *Cladosporium* sp 25 mg, *Saccharomyces* sp12.25 mg. Nystatin had 71% inhibition at 12.25 mg, 29 % at 25 mg and 0 % at both 50 mg and 100 mg (Table 4). Griseofulvin MIC for penicillium sp was 100 mg, *Aspergillus* sp 25 mg, *Candida* sp 25 mg, *Fusarium* sp 50 mg, *Rhizopus* sp 12.25 mg, *Chadosporium* sp 50 mg, *Saccharamyces* sp 25 mg; having 42.8 % inhibition at 12.25 mg, 14.3 % at 25 mg, 28.6 % 50 mg and 14.3% at 100 mg. Ketoconazole MIC for penicillium sp was 12.25 mg, *Aspergillus* sp 100 mg, *Candida* sp 12.25 mg, *Fusarium* sp 25 mg, *Rhizopus* sp 12.25 mg, *Chadosporium* sp 12.25 mg, *Saccaramyces* sp12.25 mg; having 85.7 % inhibition at 12.25 mg, 14.3 % at 100 mg and 0 % at both 25 mg and 50 mg.

CONCLUSION

There are the presences of microorganisms in most of the edible oil, which are at a level above the permissible standard. The organisms are pathogenic which is of health importance. It is possible the edible oil got contaminated during manufacturing and handling process, therefore, important stringent aseptic procedure should be maintained. The MIC result indicated that antifungal Ketoconazole and Nystatin could be employed for the treatment of possible Mycoses. It is crucial that further research be carried in this area.

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