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Antibacterial Activity of Lauric Acid on Some Selected Clinical Isolates

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Abstract

This was an in vitro study to scrutinize the intensity of lauric acid on inhibiting some clinical isolates from patients with urinary tract infection, respiratory tract infection and digestive tract infection. Isolates used for this study were gotten from General Hospital Maitama, Abuja. Media were prepared according to manufacturer's instructions and all biochemical tests of the presumptive organisms were conducted according to standard methods. Organisms isolated were *Streptococcus pneumonia* and *Mycobacterium tuberculosis* from respiratory tract, *Escherichia coli* and *Staphylococcus aureus* from Urinary tract and *Salmonella spp*. from intestinal tract accordingly. Lauric acid was esterified from coconut oil through freezing method. Bauer-Kirby disc diffusion assay was adopted for the inhibitory experiment. Zones of inhibition were measured in diametre. Lauric acid demonstrated the highest zones of inhibition on *Staphylococcus aureus* 15 mm ± 1.414 mm and *Streptococcus pneumoniae* 15 mm ± 0.000 mm at the highest dilution factor of 1:10 followed by Mycobacterium tuberculosis spp. having a diametre of 14 mm ± 1.414 mm and the lowest inhibition on *Escherichia coli* and *Salmonella* with a diametre 8 mm ± 0.000 mm at the same dilution factor while the lowest inhibition was 1 mm ± 1.414 mm and 1 mm ± 0.000 mm on *Staphylococcus aureus* and *Streptococcus pneumonia* at the lowest dilution concentration 1:100000. The acid generally proved effective against Gram positive bacteria used in this study even at the lowest concentration while other Gram-negative organisms showed resistance at the lowest concentration. This study recommends the use of this acid in combating some of the microbial strains that are resistance to antibiotics. Further study of this acid on other pathogenic organisms including the non-cellular strains (viruses) should be considered.

Keywords: Investigation; Lauric acid; Clinical isolates

Introduction

Lauric acid or systematically dodecanoic acid is saturated fatty acids with a 12 carbon atom chain thus falling into the medium chain fatty acids. This acid is formed in many vegetables, fats particularly in coconut oil and palm kernel oil. Lauric acid a twelve (12) carbon chain acids, is one of the medium chain fatty acids gotten from some plants oil particularly coconut oil and others related oil such as palm kernel oil which has been known as one of the most active ingredient and is more predominant in the total saturated fat present. Lauric acid has been known as one of the most active ingredient and composed over 52% of the total 92% saturated fats present in the coconut oil and is claimed to play a significant role in the healing miracle that is revealed in coconut oil. Virgin coconut oil, a potent nondrug or natural yeast fighter, contains three medium chain fatty acids, i.e. lauric acid (50% to 53%), caprylic acid, and capric acid, all of which have antibacterial and antifungal effect against lipid coated bacteria and fungi such as *Candida spp*. Medium-chain free fatty acids have been found to have a broad spectrum of microbicidal activity though the mechanisms by which the lipids kill bacteria is not known, but electron microscope studies indicate that they disrupt cell membranes. On the other hand, free fatty acids (FFA) of various chain lengths (C8-C18) have antibacterial activity against a range of Gram-positive bacteria, but not against a number of Gram-negative bacteria. Lauric acid is a minor sebum component (1% to 2% of total sebum FFA) Bach and Babayan, but it is the most active antimicrobial FFA [1-6].

Variations in composition plant and genetic disparity among bacteria and fungi of the same or different species have been found to be responsible for the few inconsistencies in the antibacterial and antifungal properties of plant extract. The esterification of coconut oil which yielded a carbon chain has proved beyond reasonable doubt that, lauric acid 12-carbon chain fatty acid is more biological active and has the highest antiviral activities than coconut oil which is the parent substance. This resulted from the medium chain triglycerides (MCTs) present in coconut oil which anti-bacterial influence because it has the ability to disintegrate bacterial cell walls;

MCTs are also presenting the ability to treat severe bacterial infections that are antibiotic resistant. This acid is used for the treatment of viral infections, bacterial infections, fungal infection and protozoal infections and it is traditionally used for the production of soap and cosmetics as such, it is neutralize with sodium hydroxide (NaOH) and give sodium laurate by saponification. Some previous studies demonstrated that lauric acid (sodium laurate) has antimicrobial efficacy against both E. faecalis biofilms and multispecies biofilms. Several claims have been made on the use of lauric acid and its parent substance coconut oil on its health benefits and medicinal effect [7-10]. This study therefore aimed at investigating the invitro microbicidal effect of lauric acid on some clinical isolates.

Materials and Methods

Abuja is the capital city of Nigeria. It is located in the centre of Nigeria, within the Federal capital territory (FCT) which was built mainly in the 1980s and officially became Nigeria's capital on 12 December 1991, replacing, Lagos, though the latter remains the country's most populous city (Murray, 2011). Abuja's geography is defined by Aso Rock, a 400 metre (1,300 ft) monolith left by water erosion.

Clinical isolates

Recent isolates of bacterial organisms were isolated from clinical specimens of patients with urinary tract infection, respiratory tract infection and digestive tract infection attending health care facilities at the General Hospital Maitama.

Preparation of coconut oil extract

Fresh coconut (Cocos nucifera) was obtained from Lafia modern market Lafia, Nigeria. The fresh coconut meat was grated and pressed using a sterilized sieve to produce coconut milk, which was further allowed to ferment for 48 hours under anaerobic condition. After the fermentation, three layers were formed: the water layer, lipid layer and the protein coat layer. Protein coat and the water layer were separated from the oil (lipid layer). The oil was then heated slightly to remove remaining moisture. After which the oil was filtered by passage through a 25 m-pore size filter (Millipore, St. Quentin, France) to give an aqueous extract of coconut oil. This was collected in a sterile vial and stored at 4°C until use.

Esterification of lauric acid from coconut oil

Coconut oil freezes at 25.1°C while lauric acids freezes at 47°C and if coconut is freeze under controlled temperature of 25.1°C, it will freeze leaving lauric acids unfreeze. Extra virgin coconut oil was poured into a temperature glass container, manufacturer filter to remove impurities, digital freezer was set at 25.1°C to freeze coconut oil. This was done because coconut oil freezes at 25.1°C while lauric acid freezes at 47°C. The frozen coconut oil was removed from the glass container and the unfreeze part which is lauric acid was further poured into an air tide container for the experiment.

Test organisms suspension

Suspension of each of the test organisms was made by collecting a loopful of colony from each plate and was incubated overnight at 37°C in Nutrient broth. The overnight broth culture of organisms was diluted in nutrient broth to an inoculum load of approximately 1×10^6 cfu/ml. It was standardized according to National Committee for Clinical Laboratory Standards (NCCLS, 2002) by gradually adding normal saline to compare its turbidity to McFarland turbidity standard of 0.5 which is approximately 1.0 × 106 cfu/ml.

Sterile swab sticks were dipped into each of the bacterial solution and were used to inoculate the solidified Nutrient agar plates ensuring that the plates were completely covered for uniform growth as described by Aboh et al. [11-14].

Sterility test

Extracted lauric acid was cultured on prepared media plates and incubated overnight at 37°C. This was done to ensure that the extract is completely sterile. All media prepared were picked at random and incubated overnight at 37°C for the same purpose.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was carried out in each of the plate using agar disc diffusion method as described by Bauer-Kirby. This involves a heavy inoculation of an agar plate with the test organisms. A disc of filter paper (Whatman filter paper) was impregnated with a known volume and appropriate concentration of lauric acid and was placed on a plate of susceptibility testing agar uniformly inoculated with the test organism and equally spaced on the inoculated plate. The antimicrobial agent diffused from the disc into the medium and the growth of the test organism was inhibited at a distance from the disc that is related (among other factors) to the susceptibility of the organisms. Strains susceptible to the antimicrobial were inhibited at a distance from the disc whereas resistant strains have smaller zones of inhibition or grow up to edge of the disc. Following incubation, the agar plate was examined for zones of inhibition (areas of no growth) surrounding the discs. Zone of inhibition indicates antimicrobial activity against the organisms. Absence of zone of inhibition indicates that the acid was ineffective against the test organisms or the organisms are resistant to the acid [15-21].

Result of the morphological identification, biochemical reaction, carbohydrate utilization and haemolytic reaction of the test organisms is shown in **Table 1** below.

Table 1 Morphological characteristics and biochemical identification of the clinical isolates.

Biochemical Examination	*S. ureus*	*S. pneumonia*	*M. Tuberculosis*	*E. coli*	*Salmonella species*
Morphology	Cocci	Cocci	Rod	Rod	rod
Arrangement	Irregular in Cluster	Pairs in Chain		Single	single
Pigmentation	Yellow		Golden	Non	Pale colour
Catalase	+	-	-	+	
Oxidase	-	-	-	-	-
Coagulase	+	-	-	-	
Motility	-	-	-	+	+
Indole	-	-	-	+	-
Nitrate	+	-	+		-
Methyl Red	+	+	-	+	-
V. P	+	+	-	-	-
Urease	+	-	-	-	+
H_2S	-	-	-	-	+
Endospore	-	-	-	-	-
Glucose	+	+	-	+	-
Sucrose	+	+	-	+	-
Mannose	+	+	-	-	+
Mannitol	+	-	-	+	+
Gas	-	-	-	+	-
Haemolysis	+	+	-	-	-
Acid fast stain	-	-	+	-	-

Keys: + = Positive; **-** = Negative; **VP** = Voges Proskauer

The result of the agar disc diffusion antimicrobial assay of lauric acid on some clinical isolates is shown in **Table 2** below. The clinical isolates used for the sensitivity assay were: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Salmonella species* and *Mycobacterium tuberculosis* showing varying susceptibility pattern.

Table 2 Diametre zones of inhibition of lauric acid on the clinical isolates.

Dilution per mL	**Bacterial organisms used in the antimicrobial experiment**				
	S. aureus	*E. coli*	*S. pneumoniae*	*Salmonella spp.*	*M. tuberculosis*
1:10	15 ± 1.414	8.0000	15 ± 1.414	8 ± 0.000	8 ± 1.414
1:100	11 ± 0.000	6 ± 1.414	10 ± 1.414	6 ± 1.414	7 ± 0.000
1:1000	10 ± 1.414	2 ± 0.000	10 ± 0.0000	2 ± 0.000	3 ± 1.000
1:10000	6 ± 0.000	R	6 ± 0.000	R	
1:100000	1 ± 0.1.414	R	1 ± 0.000	R	R

Keys: R=means resistance

Figure 1 shows pictorial representation of the antimicrobial sensitivity of the selected clinical isolates at the highest dilution (1:10) concentration with the highest zones of inhibition observed on *Staphylococcus aureus* and *Streptococcus pneumonia* and the lowest on *Escherichia coli* and *Salmonella* spp.

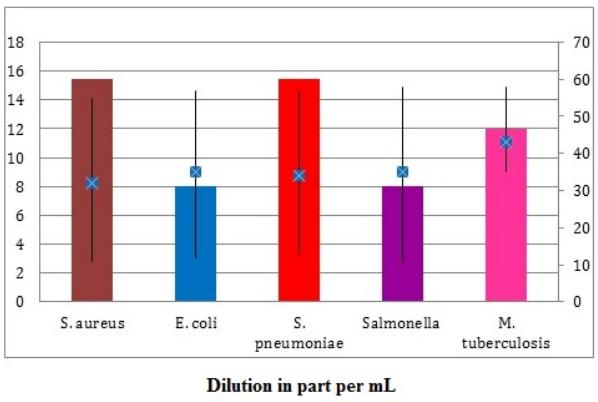

Figure 1 Antimicrobial sensitivity assay of lauric acid on isolates.

Figure 2 showing the highest dilution concentration (1:00000) with the highest zones of inhibition on *Staphylococcus aureus* and *Streptococcus pneumoniae* while the lowest zone of inhibition was observed on *Mycobacterium tuberculosis*. *Escherichia coli* and *Salmonella* spp. however showed no zones of inhibition at the lowest dilution concentration.

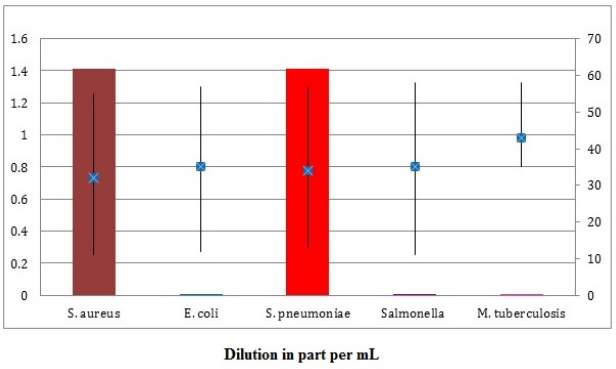

Figure 2 Lowest Dilution Concentration (1:10) of lauric acid on isolates.

This research work investigates the efficacy of lauric acid against some clinical isolates from patients with respiratory tract infection, digestive tract infection and urinary tract infection. Organisms isolated were *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella species* and *Mycobacterium tuberculosis*. Lauric acid in this study showed considerable inhibitory effect on *Staphylococcus aureus* with decrease in effects corresponding to the concentration of the acid. The acid demonstrated highest zones of inhibition on *Staphylococcus aureus* with the following diametre 15 mm ± 1.414 mm and *Streptococcus pneumoniae* with the following diametre 15 mm ± 0.000 mm at the highest dilution concentration of 1:10, followed by *Mycobacterium tuberculosis* 12 mm ± 0.000 mm, and the lowest inhibitory effect was observed on *Escherichia coli* and *Salmonella spp*. having a diametre of 8 mm ± 0.000 mm at the same dilution concentration. In general, the acid was more effective against *Staphylococcus aureus* and *Streptococcus pneumonia* even at the lowest concentration in this study, at 1:100000 dilutions with little zones of inhibition as compare to 1:10 dilution followed by *M. tuberculosis* which showed resistance at the 1:10000 dilution concentration whereas *E. coli* and *Salmonella* species proved resistant at the lowest concentration. Statistically, the results obtained showed significant difference in Analysis of Variance (ANOVA) at 5% level of significance (p=0.05). Similarly, Ja-Hyung and Young-Wook, reported that Synthetic sodium laurate (lauric acid) fatty acid exhibit significantly high antimicrobial activity by inhibiting microbial survival and biofilm growth against *Streptococcus mutans*. Arguably, Padgett *et al*. reported that high level of lauric acid addition (8%) significantly lower the film water permeability. This result conforms to the popular assertion that says the higher the concentration, the higher the antimicrobial effect of agent against organisms. *Escherichia coli* and *Salmonella spp* which are Gram negative bacteria showed resistance to the acid tested at a lower concentration compare to other Gram-positive bacteria such as *S. aureus* and *S. pneumoniae*. This finding conforms to the findings of Mamman *et al*. that says Gram negative bacteria exhibit much resistance compare to Gram positive bacteria. The acid was effective from dilutions of 1:10 to 1:100000 except for *E. coli* and *Salmonella* species which were effective at 1:10, 1:100 and 1:1000 only. The highest zone of inhibition was observed at 1:10 dilution, having 15 mm ± 1.414 mm and the lowest was observed at 1:00000 with 2 ± 0.000. Lauric acid exhibited appreciably high antimicrobial activity in some clinical isolates than others and the zones of inhibition varied based on their dilution concentration declining as the dilution concentration decreases [22-25] (**Figures 1 and 2**).

Conclusion and Recommendation

This study establishes the fact that lauric acid has antibacterial effect on Gram positive bacteria more compare to the Gram-negative bacteria. This however recommends that lauric acid beneficially be used in treating some of the microbial infection caused by some Gram-positive bacteria. More studies should be done to ascertain the mechanisms of actions of this acid on the bacterial cell including the non-cellular (viruses) strains.

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