IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 17, Issue 4 Ser. I (Jul. –Aug. 2022), PP 39-43
www.Iosrjournals.Org

Antibacterial Activity of Honey and Palm Kernel Oil on *Staphylococcus Aureus*.

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Abstract
Honey and Palm kernel oil exhibit antibacterial activities against a wide range of bacteria in different milieu. This study aims to compare the antibacterial activity of Honey, Palm kernel oil, and the synergistic effect of both honey and palm kernel oil against Staphylococcus aureus. The inhibitory effect of both honey and palm kernel on bacterial growth was evident at concentrations 100%, 75%, 50%, and 25% (v/v). Susceptibility testing with standard antibiotics, gentamicin, methicillin, and ampicillin showed zone of inhibition of 0, 15, and 18mm respectively. honey, Palm kernel oil, and their mixture showed better antibacterial activity against the test organism when compared with the standard antibiotics. The Minimum Inhibitory Concentration (MIC) of honey was observed to be at a concentration of 50% while that of palm kernel oil at a concentration of 25%. MIC showed that Palm kernel oil is more potent against the test organism even at low concentrations than honey.

Keywords: *Honey, Palm Kernel Oil, Staphylococcus aureus, Antibacterial.*

Date of Submission: 17-07-2022 Date of Acceptance: 02-08-2022

I. Introduction

Infectious diseases are the leading causes of death in tropical countries accounting for approximately one-half of all deaths [1]. Many infectious diseases are known to be treated with herbal remedies derived from plant materials throughout history. Today plant materials continue to play a major role in primary healthcare as therapeutic remedies in many developing countries [2][3][4] The use of synthetic antibiotics in the treatment of infectious diseases has played a role in the existence of drug-resistant varieties and the spread of resistance between bacterial species [5] The importance of antimicrobial agents in reducing the global burden of infectious diseases, however, call for the urgent need of alternative antimicrobial strategies because the effectiveness of synthetic antibiotics has been declining. This situation, therefore, has led to re-evaluation and adoption of the therapeutic use of ancient remedies including essential oils such as coconut oil, palm kernel oil, black cumin oil, green tea, etc. extracts of different plants and biologically active compounds isolated from natural species such as honey, *Garcinia Kola* (bitter kola), etc. used in herbal medicine [6] benefits derived from using medicine obtained from plants are relatively safer than synthetic alternatives by offering profound therapeutic benefits and give more affordable treatment[7]

The antibacterial effect of honey has been in existence dating centuries back [8] but had limited use in medicine due to lack of scientific support [9]. It has been rediscovered and it is gaining acceptance as an antibacterial treatment for topical infections relating to burns and skin ulcer [10]. It is also employed in wound therapy [11] and gastritis caused by enteropathogenic bacteria. It is well known that honey inhibits a broad spectrum of bacterial species as studies have been conducted on some commercial honey in Nigeria and internationally, honey (e.g. Manuka) for instance has demonstrated effectiveness against several human pathogens including *Escherichia coli, Enterobacter aerogenes, Salmonella typhimurium* and *Staphylococcus aureus,* [12]. Two well-characterized antibacterial factors identified in honey are hydrogen peroxide and high osmolarity[13]. Other investigators have identified flavonoids in honey, particularly caffeic acid and ferulic acid as the most likely contributors. Also, the phytochemical component and in-vitro antibacterial activity of honey in the induction of increased lymphocytes and phagocytes' activities attributes to the antimicrobial activities of honey [14].

Palm trees have a plethora of therapeutic uses in traditional medicine practice. Every part of the plant can be used medicinally. The leaves of *E. guineensis* are squeezed and the juice that is obtained is placed on wounds to enhance healing [15]. The sap of this plant is also used as a laxative and the partially fermented palm

wine is administered to nursing mothers to improve lactation. Fruit husk ash is used for the preparation of a soap used for skin infections. A root decoction is used in Nigeria for headaches. The pulverized roots are added to drinks for gonorrhea, menorrhagia and as a cure for bronchitis [16]. The fruit mesocarp oil and palm kernel oil are administered as a poison antidote and used externally with several other herbs as lotions for skin diseases. Palm kernel oil is applied to convulsant children to regulate their body temperature. Recent studies also reported various pharmacological activities of this plant extract namely standardization of the extract's antimicrobial activity, antioxidant activity and infected wound healing activity [17][18][19]. The toxicity of *E. guineensis* leaf methanol extract against brine shrimp (*A. salina*) and mice has also been tested. The results of both tests confirmed that *E. guineensis* is nontoxic and recommended as a safe natural product for commercial utilization [21]. Palm oil is extracted from the fresh mesocarp of the fruit, which contains 45-55% oil, but varies from light yellow to orange-red in color, and melts at 25^{O}C. The palm kernel oil is obtained from the kernels enclosed in the endocarp. Palm oil is obtained from the kernels enclosed in the endocarp. Palm oil contains saturated palmitic acid, oleic, and linoleic acid, giving it a higher unsaturated acid content than palm kernel or coconut oils.

Palm kernel oil has also been reported to be anodyne, antidotal, aphrodisiac and diuretic. It is a folk remedy for headaches, pains, rheumatism, cardiovascular diseases, arterial thrombosis, and atherosclerosis [22]**.** Palm kernel oil is also used as a liniment for indolent tumors [23]

The present study is designed to access the antimicrobial efficacy of the synergy of honey and Palm kernel oil and their antimicrobial effect separately on *Staphylococcus aureus* from a clinical sample.

II. Materials And Methods

2.1. Equipment used for laboratory experiment

They include; Autoclave**,** incubator**,** Bunsen burner**,** Refrigerator

2.2. Materials used for the laboratory work

Pasteur pipette, measuring cylinder, Inoculation wire loops, Sterile Petri dishes (disposable), Millimeter ruler, Aluminum foil, Cotton wool, Masking tape/marking pen, Distilled water, Raw honey, Palm kernel oil, 95% ethanol, Mueller Hinton agar, Test tubes, Sterile spreader etc.

2.3. Sample collection

Palm kernel oil was extracted traditionally from fresh seed (Ojukwu) obtained from *Elaeis guineensi.* The sample was obtained from Afor Awo – Omamma Oru East Local Government Area Imo State. Honey was obtained from "Everyday Supermarket" at World Bank Owerri Imo State. The honey was undiluted and directly from the bee.

2.3.1. Culture collection

Staphylococcus aureus was obtained from the Microbiological Laboratory, Federal Medical Centre (FMC) Umuahia. The identity and confirmation of these test microorganisms were carried out in the collection centre. The isolate was sub-cultured on a nutrient agar slant and stored at 4^{o}C until required for the study.

2.4. Antibacterial assay

The effect of honey and palm kernel oil on *Staphylococcus aureus* was assayed by Agar well diffusion method (Stroke, 1975)

2.4.1. Sample preparation

Honey dilutions were prepared immediately before testing by diluting honey to the required concentrations 100%, 75%, 50%, and 25%, v/v. The Palm kernel oil was also prepared into the required concentrations 100%, 75%, 50%, 25%, v/v, using two-fold serial dilutions which is a stepwise dilution of a substance by reducing the concentration by a factor of two. The mixture of honey and palm kernel oil was made effective by making an emulsion using polysorbent (Tween 80). To get an oil emulsion, 1ml of oil was added in 4ml 25% Tween 80 solution. (25% Tween 80 solution is got by adding 1ml of Tween 80 to 3ml of warm distilled water). The following concentrations were obtained after the mixture was obtained. Honey (25%) kernel oil (75%), honey (50%) kernel oil (50%), and honey (75%) kernel oil (25%) (Sahm and Washington 1990).

2.4.2. Determination of antibacterial activity

Principle: The antimicrobials present in the honey and kernel oil are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Procedure: Antimicrobial effect of the essential oils was determined by agar well diffusion method [27] using the test organism; *Staphylococcus aureus*. Mueller Hinton agar was prepared according to manufacturer's instruction, autoclaved and dispensed into sterile Petri dishes, and allowed to set before use. The test organism in the broth was inoculated in the Mueller Hinton agar using a cotton swab and spread evenly on the surface of the media. The media was allowed to stand for 15 minutes. Three wells of about 6mm diameter were aseptically cut on the already prepared Mueller Hinton agar plate containing the organism using a sterile cork borer. Aliquot 2.0ml of the honey, kernel oil, and mixture of both oils of different concentrations were introduced into each well respectively. The plates were then incubated at 37°C for 24 hours. The resultant zones of inhibition were measured using a ruler calibrated in millimeter.

2.4.3. Determination of minimum inhibitory concentration (mic)

The Minimum Inhibitory Concentration (MIC) of both honey (*Triacanthos*) and Palm kernel oil (*Elaeis guineensis)* was determined from the results of the antimicrobial test. This is shown in table 4.3. The table shows the Minimum Inhibitory Concentration (MIC) of the test organisms which is the lowest concentration of the honey and Palm kernel oil that can inhibit the growth of the organism as determined from the zone of inhibition.

3.1. Results

The present study shows the evaluation of the antibacterial properties and synergistic effect of honey and palm kernel oil on *Staphylococcus aureus.* The antibacterial activity in this study was expressed as a measure of the diameter of the inhibition of growth in millimeters.

Table 3.1 shows the antibacterial activity of different concentrations of honey and palm kernel oil with zone of inhibition ranging from 0 to 15mm and 0 to 23mm respectively.

Table 3.2 shows the antimicrobial activity of the synergy of honey and palm kernel oil with zone of inhibition ranging from 15 to 25mm at concentration of 75%honey:25%palm kernel oil, 50%honey:50%palm kernel oil, and 25%honey:75%palm kernel oil.

Table 3.3 shows the MIC of the samples on the test organism from the zone of inhibition observed. The MIC for honey is at 50% concentration and that of Palm kernel oil is at 25% concentration.

Table 3.4 shows the susceptibility testing of standard antibiotics (gentamicin, methicilin and ampicilin) with zone of inhibition of 0, 15 and 18mm respectively.

Table 3.1: Antimicrobial activity of honey and Palm kernel oil against *Staphylococcus aureus*

Concentration	Zones of Inhibition (mm)	
	Honey	Palm kernel oil
12.5%	0	0
25%	0	8
50%	13	15
100%	18	23

Table 3.2: Antimicrobial activity of the synergy of honey and palm kernel oil against *Staphylococcus aureus*.

Concentration Honey : Palm kernel oil	Zones of Inhibition (mm) on Growth of *Staphylococcus aureus*
25 : 75%	15
50 : 50%	25
75 : 25%	18

Table 3.3: Minimal Inhibitory Concentration (MIC) of honey and Palm kernel oil

Test Organism		Minimum Inhibitory Concentration (MIC) In percent (%)		
		Honey	Palm kernel oil	Honey+Palm kernel oil
Staphylococcus aureus	50	25	25	

Table 3.4: Antimicrobial testing of standard antibiotics.

Test Organism		Zones of Inhibition (mm)		
		Gentamicin	Methicilin	Ampicilin
Staphylococcus aureus	0	15	18	

4.1. Discussion

The investigation done on honey and palm kernel oil revealed that samples possess antimicrobial activities against *Staphylococcus aureus*. Presence of an inhibiting factor in honey which is hydrogen peroxide and flavonoids have been observed to be responsible for its antimicrobial activity. The data obtained showed that the inhibitory effect of the sample on the investigated test organism was concentration dependent. This observation is in agreement with the findings [24][25] that the efficacy of honey and most plant extract was concentration dependent. Honey at 50% and 100% concentration inhibited the test organism while honey at 25% and 12.5% concentration showed no inhibition of the test organism (Table 4.1).

The susceptibility of some test organisms to honey could be due to the emergence of resistant strain. Besides, several factors such as botanical and entomological origin may influence the antibacterial activity of honey. Reports by [26] show that honey from different phytogeographic regions varied in their ability to inhibit the growth of bacteria, suggesting that botanical origin plays an important role in influencing antimicrobial activity. Various researchers have shown that honey exerts antimicrobial activities against various microorganisms [27]

Honey has been successfully used in the treatment of surgical wounds, burn wounds and ulcers. It has also been shown as a good medium to store skin grafts [28] Honey also inhibits bacterial growth due to its concentration, low pH, hydrogen peroxide, proteineceous compounds, or other unidentified antimicrobial activity. Shrinking and disruption of bacteria may be due to the presence of antibacterial substance such as inhibine. Besides its antimicrobial properties, honey can clear infection in a number of ways *in vivo,* like boosting the immune system, anti-inflammatory and antioxidant activities and via stimulation of cell growth.

Palm kernel oil alone showed antimicrobial effectiveness on *Staphylococcus aureus* in this study. This was evident on the zones of inhibition recorded, even at 25% concentration unlike that of honey alone (table 4.1). Palm kernel oil show antimicrobial effect due to its lauric acid content. Lauric acid is the antimicrobial agent in palm kernel oil. The method of extraction seems to affect the level of lauric acid present in the sample. The bacteria, *Staphylococcus aureus* which can cause boils and infections of wounds were inhibited minimally. Research has shown that the use of this type of inhibitory agents does not lead to the development of resistant organisms. Palm kernel oil can be used as an ointment for the body to minimize infections by microorganisms and this justify its usage amongst the populace in some parts of Nigeria.

The mixture of honey and Palm kernel oil produced greater zone of inhibition for the test bacteria than honey and Palm kernel oil used separately as shown in Table 4.2. The synergistic effect of honey and Palm kernel oil mixture on the test bacteria showed in descending order of concentration as 50%honey:50%Palm kernel oil > 25%honey:75%Palm kernel oil > 75%honey:25%Palm kernel oil.

The susceptibility testing with standard antibiotics, gentamicin, methicilin and ampicilin show zone of inhibition of 0, 15 and 18mm respectively. From the observation, honey, Palm kernel oil and their mixture showed better antimicrobial activity against the test organism when compared with the standard antibiotics as shown in Table 4.4. The result implies that *Staphylococcus aureus* showed significant difference in the susceptibility to the synergistic mixture and separate use of honey and Palm kernel oil.

The Minimum Inhibitory Concentration (MIC) was also determined from the result of zone of inhibition observed. The MIC of honey was observed to be at concentration of 50% while that of palm kernel oil at concentration of 25%. MIC showed that Palm kernel oil is more potent against the test organism even at low concentration than honey as shown in Table 4.3.

4.2. Conclusion

In conclusion, the results obtained are visible evidence that the synergistic effect and separate use of honey and Palm kernel oil portrays inhibitory effects on *Staphylococcus aureus*. This inhibitory effect can be attributed to the presence of hydrogen peroxide in honey and lauric acid in palm kernel oil. This research has brought about the valuable therapeutic knowledge of common essential oils. The efficacy of honey and Palm kernel oil against *Staphylococcus aureus* justify and explain the scientific basis for their use in traditional medicine in prevention and treatment of bacterial infections such as gastrointestinal tract infection, wound, ulcer etc. caused by this pathogenic bacterium which have developed resistance to antibiotics.

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Peace Onyinyechi Madu, et. al. "Antibacterial Activity of Honey and Palm Kernel Oil on Staphylococcus Aureus." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 17(4), (2022): pp. 39-43.