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The Efficacy of Essential Oil of *Syzygymium aromaticum* and Fixed Oil of *Cocos nucifera* on Some Selected Bacteria and Fungi

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Abstract

The recent failure of antibiotics in curbing the menace of multidrug resistance microorganisms has necessitated the search for alternative therapies from plant materials. Therefore, in this research work, the antibacterial and antifungal activity of the essential oil of *Syzygium aromaticum* (clove) and fixed oil of *cocos nucifera* (coconut) were tested against selected bacteria and dermatophytes. The susceptibility of the isolates to conventional antibiotics was compared with that of the oils. The Minimum Inhibitory Concentration (MIC) of the oils was also determined. The isolates obtained were identified as *Proteus mirabilis, Staphylococcus aureus, Escherichia coli* and *Moraxella catarrhalis* and the dermatophytes were *Epidermophyton floccosum* and *Microsporum sp.* The highest zone of inhibition by clove oil was recorded in *Staphylococcus aureus* with a zone of inhibition of 30 mm followed by *Proteus mirabilis, Moraxella catarrhalis* and *Escherichia coli* with 19 mm, 17 mm and 14 mm respectively while coconut oil could not inhibit all the bacterial isolates. Furthermore, clove oil had higher activity than *coconut* oil on the dermatophytes as it inhibited both isolates while coconut oil could not inhibit *Epidermophyton floccosum.* The MIC of clove oil on the dermatophytes was 3.125 mg/ ml. This result reveals the antimicrobial potential of the essential oil of clove and thus requires further enquiries into its pharmacokinetics, toxicity and dose regiment.

Keywords: Clove oil; Coconut Oil; Antimicrobial potential; Minimum inhibition concentration

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Introduction

Many plants are being used for their antimicrobial traits, which is due to compounds synthesized in their secondary metabolism. These compounds contain active substances which are generally classified as essential oils. Essential oils and some other extracts of plants have evoked interest as sources of natural products; hence, plants are screened for their potential use as alternative remedies for the treatment of many infectious diseases [1]. Essential oils of plants are of great use in folk medicine, food flavouring, fragrance, and pharmaceutical industries [2]. They are aromatic volatile oily hydrophobic liquid concentrates that are extracted from plant material, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits, roots and whole plant. They are highly complex mixtures of 20 to 60 volatile compounds, albeit some may contain more than 100 different components [3-4]. They contain varieties of volatile molecules such as Terpenes, Terpenoids (primary constituent responsible for aroma and flavour) and phenol [5-6] Essential oils have been traditionally used for treatment of infectious diseases all over the world for centuries [7]. According to World Health Organization (WHO), plants that contains substances (such as essential oils) that can be used for therapeutic purposes or which are precursor of chemo-pharmaceutical semi-synthetic new drug is referred to as a medicinal plant [8]. Medicinal plants, such as cloves and coconuts, have the ability to inhibit the growth of a wide range of pathogenic microorganisms due to presence of essential oils. [9-11]. Clove (*Syzygium aromaticum*) is one of the most valuable spices that have been used from centuries as food preservative and for many medicinal purposes. The oil of

cloves has been used in a variety of health conditions including indigestion, generalized stress, parasitic infestations, cough, toothaches, headache, nausea, and blood impurities. In fact, the expert pane German Commission approved the use of its essential oil as a topical antiseptic and anesthetic. The cloves are anti-mutagenic, anti-inflammatory, antioxidant, anti-ulcer genic, antithrombotic and antiparasitic. Cloves' flower bud has many medicinal proprieties like antiviral, antimicrobial, antifungal general stimulating, hypertensive aphrodisiac, light stomachic, carminative, anesthetic useful in cataract [12-14]. The essential oil extracted from the dried flower buds of clove are used for acne, warts, scars and parasites [15]. In Tropical Asia, it has been given to treat such diverse infections as malaria, cholera and tuberculosis [13].

However, Coconuts (*Cocos nucifera*), likewise, contain essential oils that are of therapeutic purposes. One of the primary natural products from dry coconut fruit is the coconut oil, which has been used from time immemorial as functional food and in pharmaceuticals. It is referred to as "miracle oil". Coconut oil is one of the few thyroid-activating substances that support the body's use of thyroid hormones, thus increasing metabolism by preventing obesity, overweight problem, boost energy and fights fatigue. In the tropics, coconut oil is widely used in skin care to; moisturize the skin, relieve dryness, flaking and prevent stretch marks. It is used for wounds, bruises, burns, rashes, eczema, and dermatitis [16]. It supports the natural chemical balance of the skin and provides protection from the damaging effects of ultraviolet radiation from the sun. Coconut oil, due to its contents of caprylic acid, which is fungicidal, is used in the treatment of fungal skin infections such as athlete's foot, thrush, ringworm and candidiasis [17-18]. The increasing spread of multi-drug resistant strains of bacterial and dermatophytes, and the reduced number of drugs available makes it necessary to discover new classes of antibacterial, antifungals and compounds that inhibit these resistant mechanisms [19]. The predominant bacteria with multiple resistant to existing antibiotics are *Staphylococcus aureus, Escherichia coli, Moraxella catarrhalis*, *Proteus mirabilis* all of which are associated with urinary tract infection. *Epidermophyton floccosum* and *Microsporum sp*. are dermatophytes that are displaying resistance to antifungal agents [20]. The recent failure of antibiotics in curbing the menace of multidrug resistance has necessitated the search for alternative therapies from plant materials. Therefore, this research aims to determine the efficacy of the essential oils of *Syzygium aromaticum* and fixed oil of *cocos nucifera* on some selected bacteria and fungi

Materials and Methods

Collection of samples

Plant materials: The buds of clove (*Syzygium aromaticum*) were purchased from Lafenwa Market, Ogun State, Nigeria while the coconut (*Cocos nucifera*) was purchased from a local retailer in Badagry, Lagos State, Nigeria.

Collection and maintenance of clinical isolates: The bacterial isolates (*Proteus mirabilis, Staphylococcus aureus, Moraxella catarrhalis* and *Escherichia coli)* were obtained from the Department of Microbiology of the state hospital, Sokenu road, Ijaye, Abeokuta, Ogun State, Nigeria while the fungi isolates (*Epidermophyton floccosum* and *Microsporum sp*.) were obtained from Federal Medical Centre, Idi-Aba, Abeokuta, Ogun State. The stock cultures obtained were maintained in a sterile nutrient agar slant and Potato Dextrose Agar slant respectively. The slants were aseptically refrigerated to preserve for further use.

Characterization of isolates: The identity of test organisms were characterized using morphological and biochemical characteristics which included size, shape, colour, consistency, edges, elevation, opacity, staining (Gram, Spore and Capsule staining), indole, motility, catalase, urease, citrate, methyl-red, Voges proskauer, hydrogen sulphide and sugar fermentation test. The results were interpreted according to Bergy's Manual of Determinative Bacteriology.

Extraction of plant material

Extraction of clove oil: The clove extraction was carried out using the method described by Ayoola GA [21]. The extraction of clove oil was done through hydro- distillation. 250 g of dried clove was weighed and poured into a round bottom flask and water was added to approximately three-quarter full. The Clevenger apparatus was then connected to the flask. The trap arm was filled with water to allow the oil to condense on the water layer, heat was applied from the heating mantle, and as the water in the flask boiled, steam carrying the volatile oil rose through the neck of the flask condensing on the surface of the condenser onto the water on the graduated trap arm. The oil sank because it is denser than water. Distillation was continued until there was no more difference in successive readings of the oil volume. The oil was drained off and dried over anhydrous sodium sulphate (BDH). The oil was then pipetted using a Pasteur pipette and kept in an amber bottle for further use.

Extraction of coconut oil: Coconut oil extraction was carried out using the method described by Oyi AR, et al. [22]. The fresh endosperm or coconut meat was separated manually from the shells and then washed to remove dirt. The product was cut into pieces using a knife. It was weighed and milled using blender. The resulting mass was mixed with lukewarm water and chaff was filtered out using a cotton cloth. (The ratio of milled meat to water was 1:1). The residue (marc) was firmly pressed and process was repeated to ensure thorough extraction of the milky liquid oil. The liquid was heated to boiling. At this point, the source of heat was removed to avoid boiling over and wastage of milk. Heating effected the floating of oil as the top layer, which was skimmed off and gradually evaporated to dryness over gentle heat in a shallow aluminium pan, and was constantly stirred to avoid burning and discoloration. The oil filtered off and the deposit or "brownie" was firmly pressed to release remaining oil. The oil was thereafter decanted. Finally, the product was gently heated to dry all traces of moisture and filtered through a cotton cloth to obtain the oil for further use.

Antimicrobial studies (sensitivity test of extracts)

Sensitivity test of extracted oils on Bacterial Isolates:

Two approaches were used for the evaluation of antimicrobial activity of both plant extract and commercial antibiotics.

Agar well diffusion method: Agar well diffusion method as described by Hugo and Russel was employed. An overnight culture of *Escherichia coli*, *Proteus mirabilis* and *Staphylococcus aureus and streptococcus* was standardized to contain approximately 1.5×10^8 cfu/ ml and this was inoculated into 20 ml of already prepared Muller-hinton agar. The culture medium was allowed to set. Thereafter, a sterile cork borer N. 4 (8.0 mm diameter) was used to punch wells in the seeded nutrient agar. The agar plugs was removed with a flamed and cooled wire loop. Different concentrations of the various clove oil and coconut oil was poured into separate wells in different plates and properly labelled and allowed to diffuse. The plates were then incubated at 37°C for 24 hours and the zone of inhibition was measured using a calibrated ruler.

Disk diffusion method: Disk diffusion method was employed to determine the effect of standard antibiotics against the test microorganism. Standard antibiotic disc with eight different antibiotics was used against *Escherichia coli, Proteus mirabilis and Staphylococcus aureus and Streptococcus*. The Muller-Hinton agar plates was seeded with the test organisms and filter paper strips of standard antibiotics Augmentin (Amoxycilin/Clavulanic acid) (AMC) 30 µg, Ciprofloxacin (CIP) 5 µg, Gentamycin (CN) 120 µg, Ampicillin (AMP) 10 µg, Cefoxitin (FOX) 30 µg, Chloramphenicol (C) 30 µg, Nahidixol/Nalidixic acid (NA) 30 µg, Sulphomethoazole or Trimethoprim (SXT) 25 µg were laid aseptically on the plate using a pair of sterile forceps. The plates were then incubated at 37°C for 24 hours. After incubation, zone of growth inhibition was then measured and recorded.

Sensitivity test of extracted oils on dermatophyte (Fungi)

Two-fold dilution was conducted for the two extracts (clove essential oil and coconut fixed oil) using absolute methanol and tween 80 respectively. 18 ml of Potato Dextrose Agar was prepared and sterilized after which the diluted extract solution was added and was shaken for homogeneity. The solution was poured into two petri-dishes simultaneously and was allowed to set. The test organisms *Epidermophyton floccosum* and *Microsporum sp*. were then introduced on the plates using the inoculating pin. The plates were incubated at 27°C for 72hours after which the result was observed and recorded.

Determination of minimum inhibitory concentration

Dilution susceptibility tests for bacteria isolate: This was done by two-fold dilution of the extracts; clove oil and coconut oil where the former was diluted with absolute methanol and the latter with tween80, using a concentration of dissolved solution 100 mg/ ml, 50 mg/ ml, 25 mg/ ml, 12.5 mg/ ml, 6.25 mg/ ml, 3.125 mg/ ml, 1.563 mg/ ml, 0.782 mg/ ml, 0.391 mg/ ml, and 0.195 mg/ ml respectively. 18 ml of nutrient agar was prepared and sterilized into ten McCartney bottles, after which the bottles were labelled according to the dissolved solutions. The solutions were then poured into the McCartney bottles containing 18 ml nutrient agar, it was allowed to cool before pouring it into ten petri-dishes respectively. The culture medium was allowed to set, after which the agar was seeded by carefully streaking the test organisms that has been standardized to contain approximately 1.5×10^8 cfu/ml and then placed in the incubator at 37°C for 72hrs, after which the plates were checked and the result was recorded. The MIC was taken as the least concentration of the test sample that inhibited the growth of the organism.

Dilution susceptibility tests of dermatophyte: Dilution susceptibility tests can be used to determine minimum inhibitory concentration (MIC) which was done by two-fold dilution of the extracts; clove oil and coconut oil where the former was diluted with absolute methanol and the latter with tween80, using a concentration of dissolved solution 100 mg/ ml, 50 mg/ ml, 25 mg/ ml, 12.5 mg/ ml, 6.25 mg/ ml, 3.125 mg/ ml, 1.563 mg/ ml, 0.782 mg/ ml, 0.391 mg/ ml, and 0.195 mg/ ml respectively. 18 ml of Potato Dextrose Agar was prepared and sterilized into ten McCartney bottles, after which the bottles were labelled according to the dissolved solutions. The solutions were then poured into the McCartney bottles containing 18 ml Potato Dextrose Agar; it was allowed to cool before pouring it into ten petri-dishes respectively. The culture media were allowed to set, after which the test organisms *Epidermophyton floccosum* and *Microsporum sp.* were inoculated respectively on the media using the inoculating pin. The culture media was then incubated at 27°C for 72hours after which the result was observed and recorded.

Minimum bactericidal concentration: The samples from the concentration before the MIC assay for bacteria isolate that showed no growth was sub-cultured in a freshly prepared nutrient agar and was incubated for 48hrs. The lowest concentration that showed no growth indicates the minimum bactericidal concentration.

Minimum fungicidal concentration: The samples from the concentration before the MIC assay that showed no growth was sub-cultured on a freshly prepared Potato Dextrose Agar and incubated for 48hrs.

Results

Antibacterial activity of clove and coconut

The antibacterial activity of the essential oil of *Syzygium aromaticum* (clove) and *Cocos nucifera* (coconut) oil were tested on different bacterial species and the result are presented in **Table 1**. Using the agar well diffusion method the essential oil of clove and coconut oil were tested for their antibacterial activity on *Proteus mirabilis*, *Staphylococcus aureus*, *Moraxella catarrhalis* and *Eschercia coli*. After incubation for 24 hours, the zones of inhibition for the essential oil of clove was 19 mm for *Proteus mirabilis*, 30 mm for *Staphylococcus aureus*, 14 mm for *Moraxella catarhalis* and 17 mm for *Escherichia coli* while coconut oil was observed to have no effect on the all the bacterial species.

MIC of clove and coconut oil

The Minimum Inhibitory Concentration (MIC) of the essential oil of clove on all bacteria species had a value of 0.391 mg/ ml while there was no minimum inhibitory concentration for coconut oil because the entire double fold dilution of 100 mg/ ml final active concentration had no effect on the four test bacterial species. On the other hand, out of the five concentrations of the double fold dilution used, 0.39 mg/ ml was the MIC for *Epidermophyton floccosum* and *Microsporum species* (**Table 2). Table 3** shows the MIC of coconut oil against the test organism where the entire double fold dilution of 100 mg/ ml final active concentration did not inhibit the growth of *Epidermophyton floccosum* but was effective against *Microsporum species* up till 0.39 mg/ ml making it the MIC.

Antifungal activity of clove and coconut oil

Table 4 shows the antifungal effect of clove oil and coconut oil on the test fungi. 100 mg/ ml concentration of the clove oil was effective against the test fungi while 100 mg/m concentration of coconut oil was only effective against *Microsporum species* and not against *Epidermophyton floccosum*.

Discussion

In this study, the antimicrobial activity of essential oil of clove and coconut oil was evaluated against selected bacterial (*Staphlylococcus aureus, Moraxella catarrhalis, Pseudomonas aeruginosa and Citrobacter species*) and fungal species (*Epidermophyton floccosum and Microsporum species*). Clove oil was observed to inhibit microbial growth of the entire four test bacterial with inhibition zone ranging from 12 to 30 m. It was also effective against *E. floccosum and M. species* which was in accordance with the study conducted by Eugenia P, et al. [23] who evaluated the antifungal activity of essential of clove against Candida strains, *Aspergillus species*, *Microsporum gypseum* and *Epidermophyton floccosum*, and reported that essential oil of clove showed wide spectrum activity and inhibited all the fungal species. Likewise, the strong antifungal activity of clove conforms to the report of Rana IS, et al. [24]. Clove was observed to exhibit stronger activity against *Staphyloccocus aureus* and *Citrobacter species* with zone of inhibition of 30 mm and 21 mm respectively. However, on evaluation of the effectiveness of the extract during MIC, using double dilution with concentrations from 100 mg/ ml to 0.19 mg/ ml for the four test bacteria. A minimum inhibitory concentration of 0.39 mg/ ml for *S. aureus* and *Moraxella catterhalis* and 0.78 mg/ ml for *Pseudomonas aeruginosa* and *Citrobacter species.* The stronger antibacterial activity of clove oil is in accordance with the report of Sabahat and Perween. Also, during the course of this study, it was observed that coconut oil was not effective against any of the bacteria species (*Staphyloccocus aureus, Moraxela cattarhalis, Pseudomonas aeruginosa* and *Citrobacter species*). This is in contrast to [25] which stated that coconut oil showed antibacterial activity. This can could be due to lack of surface active emulsifying agents, strain of the organism used, geographical location of the coconut used, species of the coconut and the growth conditions of the plant. On the other hand, coconut oil exhibited great antifungal activity against *Microsporum speices* which is in accordance with the report of Oyi AR, et al. [22]. Also, the strong antifungal activity of virgin coconut oil against fungi conforms to the report of Ogbolu DO, et al. [25] who reported that coconut oil has high antifungal activity against *Candida species*.

Table 1 Antibacterial effect of extracted oils on the bacterial test.

Test organisms	Zone of Inhibition (mm) (Clove oil)	Zone of inhibition (mm) (Coconut oil)
Proteus mirabilis	19	No inhibition
Staphylococcus aureus	30	No inhibition
Moraxella catarrhalis	14	No inhibition
Escherichia coli	17	No inhibition

Table 2 Minimum inhibitory concentration of essential oil *S. aromaticum* on the bacterial test.

Test Organisms	Concentration (mg/ml) 100 50 25 12.5 6.25 3.125 1.563 0.782 0.391 0.195 0.0975
Proteus mirabilis	- - - - - - - - - + +
Staphylococcus aureus	- - - - - - - - - + +
Escherichia coli	- - - - - - - - - + +
Moraxella catarrhalis	- - - - - - - - - + +
Epidermophyton floccosum	- - - - - - - - - + +
Microsporum sp.	- - - - - - - - - + +

-= No growth
+ = Growth

Table 3 Minimum inhibitory concentration of fixed oil of *Cocos nucifera* on the dermatophytes.

Isolate	Concentration (mg/ml) 100	50	25	13	6.25	3.1	1.563	0.78	0.391	0.2
Epidermophton floccosum	+	+	+	+	+	+	+	+	+	+
Microsporum sp.	-	-	-	-	-	-	-	+	+	+

- = No growth
+ = Growth

Table 4 Antifungal effect of extracted oils on dermatophytes.

Test organisms	Concentration (mg/ml) *S. aromaticum* oil (100mg/ml)	*C. nucifera* oil (100mg/ml)
Epidermophton floccosum	-	+
Microsporum sp.	-	-

- = No growth
+ = Growth

Conclusion

This research has revealed that clove oil is effective against bacterial and fungal species and even shows cidal effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa* even at low concentrations as well as against *Microsporum species*. Thus, essential oil of clove could be a promising source of bactericidal and fungicidal agents while coconut oil could be a good source of fungicidal agent that could be applied *in vivo*.

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