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Research Article

Pharmacodynamics and Pharmacokinetics of Zingiber Officinale / Syzygium Aromaticum Oils Extracts on Albino Rats /Surgical Wound Isolates from Four Selected General Hospitals

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Abstract

Surgical wound infections are a serious problem that can delay healing and increase the risk of complications and death. Antibiotics are often not enough to treat these infections, because of the growing resistance of bacteria, the adverse effects of drugs, and the high cost of treatment. Therefore, there is a need to explore alternative therapies, such as natural plant extracts, that can have antimicrobial properties and enhance wound healing. Previous studies have shown that some plants, such as ginger (Zingiber officinale) and clove (Syzygium aromaticum), have antibacterial, anti-inflammatory, antioxidant, and wound-healing effects. However, the optimal methods of extracting and applying these plant oils, and their efficacy and safety in animal and human models, are not well established. This study aimed to investigate the effects of oil extracts of ginger and clove on wound healing in albino rats and their antibacterial activity against surgical wound isolates. The oil extracts were prepared using two different solvents (N-hexane and olive oil) and concentrations (100%, 50%, 25%and 12.5%), and applied topically to the wounds of albino rats. The wound healing process was evaluated by measuring the serum and tissue levels of inflammatory cytokines, oxidative stress markers, and tissue repair enzymes. The antibacterial activity of the oil extracts was tested against ten bacterial strains isolated from surgical wounds of patients in four hospitals in Ondo State, Nigeria, using the agar well diffusion method. The results showed that the oil extracts improved the wound healing process in rats by reducing inflammation, oxidative stress, and tissue damage, and increasing tissue regeneration and repair. The oil extracts also showed antibacterial activity against the wound pathogens. The N-hexane solvent extraction was the most effective method for obtaining ginger oil with high antibacterial activity, while the other methods and oils were ineffective against bacterial pathogens. The study concluded that oil extracts of ginger and clove have potential benefits for the treatment of wounds and wound infections in albino rats. The study suggested that further research is needed to optimize the extraction and application methods and to evaluate the clinical efficacy and safety of these plant oils in human trials.

Keywords: Pharmacodynamics and Pharmacokinetics.

1. Introduction

The quest for effective natural wound healing solutions has reignited interest in traditional herbal remedies. Notably, Zingiber officinale (ginger) and Syzygium aromaticum (Clove) have extensive historical use in traditional and folk medicine. Extracted from the rhizomes and buds of these plants, ginger and clove oils contain various bioactive compounds, including terpenes, phenols, and flavonoids. These oils have been reported to possess antioxidant, anti-inflammatory, antimicrobial, analgesic, immunomodulatory, and wound healing properties [1-4]. Nevertheless, the combined pharmacodynamics and pharmacokinetics of these oils remain poorly understood. Pharmacodynamics studies the effects of drugs on the body, while pharmacokinetics examines drug absorption, distribution, metabolism, and excretion. These facets are crucial for evaluating drug efficacy, safety, and optimal dosage. This project aims to investigate the pharmacodynamics and pharmacokinetics of ginger and clove oils in albino rats and surgical wound isolates from four selected general hospitals in Ondo State, Nigeria. It is hypothesized that these oils have synergistic effects on wound healing and infection prevention, and that their pharmacokinetic profiles are influenced by factors such as route of administration, dose, and formulation. The outcomes of this project will provide novel insights into the pharmacological effects and mechanisms of ginger and clove oils, potentially expanding their applications in wound management. Additionally, this endeavor will contribute to the scientific validation and rational use of these traditional medicinal plants.

2. Materials and Methods

Plant Collection and Identification

Fresh rhizomes of Zingiber officinale Roscoe (Zingiberaceae) and Syzygium aromaticum L. (Myrtaceae) were purchased in November, 2023 from the local market of Akungba-Akoko, Ondo State, Nigeria, which shares boundary with neighboring states; on East- Edo and Delta, on West-Ogun and Osun, on the south- bright Atlantic Ocean, on the north-Ekiti and Kogi State. Ondo State is located on the longitude and latitude of approximately 5.0833° E and 7.1667° N respectively. The fresh rhizomes were washed thoroughly under running tap water to remove adhered soil and foreign matter. The fresh rhizomes were cut into small pieces and identified by a botanist in the herbarium of the Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Voucher specimens were deposited in the herbarium for future reference.

Extraction Methods

Two different solvents were used to extract the oils from the plant materials: n-hexane and olive oil. N-hexane (99% purity, Sigma-Aldrich) were used to extract the oil from fresh ginger rhizomes, while olive oil (extra virgin, Bertolli) were used to extract the oils from fresh ginger, fresh clove, and a mixture of fresh ginger and clove rhizomes.

Ginger Extracted with N-Hexane

A 600 g of the fresh ginger rhizomes was soaked in 900 ml of n-hexane in a glass jar. The jar was sealed and kept in a dark place for ten days. After ten days, the soaked sample was filtered using a sterile muslin cloth. The filtrate, which contained the n-hexane and the oil, was transferred to a round-bottom flask and connected to a rotary evaporator. The solvent was evaporated at 40°C and 100 mmHg for 2 h, leaving the oil in the flask. The oil was collected and stored in a dark glass bottle at 4°C until further analysis. The yield of the oil was calculated as the ratio of the weight of the oil to the initial weight of the fresh ginger rhizomes, expressed as a percentage. The yield of the oil obtained from the fresh ginger was 2% [5].

Ginger Soaked in Olive Oil

An 80 g of the fresh ginger rhizomes was soaked in 200 ml of olive oil in a glass jar. The jar was swirled for 30 min and then left aseptically on the laboratory work bench for 7 days. After 7 days, the soaked sample was filtered using a sterile muslin cloth. The filtrate, which contained the olive oil and the ginger oil, was collected and stored in a dark glass bottle at room temperature until further analysis.

Ginger and Clove Soaked in Olive Oil

An 80 g of the fresh ginger rhizomes and an 80 g of the fresh clove rhizomes were soaked in 400 ml of olive oil in a glass jar. The jar was swirled for 30 min and then left aseptically on the laboratory work bench for 7 days. After 7 days, the soaked sample was filtered using a sterile muslin cloth. The filtrate, which contained the olive oil and the oils, was collected and stored in a dark glass bottle at 4°C until further analysis.

Clove Soaked in Olive Oil

An 80 g of the fresh clove rhizomes was soaked in 200 ml of olive oil in a glass jar. The jar was swirled for 30 min and then left aseptically on the laboratory work bench for 7 days. After 7 days, the soaked sample was filtered using a sterile muslin cloth. The filtrate, which contained the olive oil and the oil, was collected and stored in a dark glass bottle at room temperature until further analysis.

Preparation of Extract Concentration

Previously prepared and stored extracts were reconstituted using DMSO, distilled water. 50mg/ml of the extract was prepared by dissolving 5ml of the concentrated extract in 5ml of DMSO and 15mls of water to form 20% concentration in 1:3 ratios. Simultaneously ampicillin was used as a positive control at a concentration 25mg/ml respectively. The dilution medium used for the positive control was sterile distilled water.

Collection of Surgical Wound Isolates

In this present study, 10 different surgical wound isolates were collected aseptically in sterile universal bottles from the Microbiology section of the University's Health Centre at Adekunle Ajasin University, Akungba-Akoko in Ondo State. The samples were then transported to the laboratory within 15 minutes for microbial analysis.

The isolates were maintained on nutrient agar slant and stored at 40C in a refrigerator. They were sub-cultured on nutrient agar at regular intervals to avoid contamination and loss of viability.

Preparation of Glass Wares and Equipment

Petri dishes, conical flasks, test tubes, McCartney bottles, glass slides, beaker and measuring cylinder, were thoroughly washed, air dried and sterilized in the hot air oven at 1700C for hours prior to use. Inoculating loop was sterilized by holding in the Bunsen flame until redness and allowed to cool before using it for inoculation. The work bench was cleansed with alcohol swab, before starting any bench work.

Isolation, Identification and Characterization of Surgical Wound Organisms

Preparation of Wound Samples for Culture

9ml of sterile physiological saline was aseptically dispensed into 10 sterile test tubes and the mouth was corked with cotton wool wrapped with aluminum foil, each test tube was then labelled as 10-1 _ 10-5 respectively. Wound swabs were dispensed into 9ml of sterile physiological saline. 1ml of the stock culture was serially transferred to other 9ml of sterile physiological saline in other test tubes in an aliquot manner up to the tenth dilution. Pour plate method of inoculation was used for the enumeration of bacteria; 0.5ml of the five-fold dilution of 10-3 and 10-5 wound samples (inoculum) was put into sterile petri dishes.

Identification of Isolates

Microscopic and Microscopic Examination of Isolates from Wound Sample

Cultural and microscopic examinations were performed to

determine the identity of the isolated wound microorganisms. The cultural characteristics of the isolates were examined; the creamy pigmentation, round and slightly elevated shape, irregular and thread-like some of which are swarmy, with no distinct colony and cellular morphology characteristics. Furthermore, conventional identification of the isolates was carried out using various biochemical tests such as, indole, motility, gram staining, fermentation of sugars (Sucrose, Lactose, and Dextrose), Urease, Hydrogen sulphide, gelatin liquefaction and nitrate reduction test.

Preparation of Oil Extract for Antimicrobial Susceptibility Test

Antimicrobial Assay of the Oil Extract

The antimicrobial activity of the oil extract was evaluated by the agar well diffusion method against ten bacterial strains: Klebsiella oxytoca (2), Staphylococcus aureus, Escherichia coli (2), Acinetobacter baumanii, Bergeyella zoohelcum, Coagulase –ve Staphylococcus aureus, Pseudomonas aeruginosa, and Chryseobacterium meningosepticum. The bacterial strains were obtained from the Microbiology section of the University's Health Centre at Adekunle Ajasin University, Akungba-Akoko in Ondo State, Nigeria. The bacterial cultures were maintained on nutrient agar plates at 4°C and subcultured before each experiment [6].

Inoculation and Incorporation of Test Organisms and Oil Extract

Antimicrobial susceptibility test was carried out using Mueller-Hinton agar and the organisms were seeded on the Mueller-Hinton agar using sterile swab-sticks. Sterile swab-sticks were used to pick inoculum from standardized inoculum suspension to sterile petri-dishes containing molten Mueller-Hinton agar. Agar well diffusion method was used to dispense antimicrobials (oil extracted). A sterile cork borer of 1mm diameter was used to bore wells on the agar medium that has been seeded with test organisms. Using syringe, the graded concentrations (100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of the extracted oil were gently dispensed into each designated well. Positive controls (Ampicillin) were also incorporated into one of the designated wells. After 24 hours the results were taken by observing, measuring and recording the zone of inhibitions formed around the agar wells [7].

Determination of Minimum Inhibitory Concentration

Dilutions of 100, 50, 25 and 12.5 were prepared from the stock solutions of the extracts. Two drops of each extract and control were put in each hole under aseptic condition, kept at room temperature for 1hour to allow the agent to diffuse into the agar medium and incubated accordingly. Chloramphenicol was used as control. The plates were incubated at 37oC for 24hours. The zones of inhibition were then measured [8].

Measurement of Death Rate of the Isolates Using Ultra Violet Spectrophotometer

This procedure used by Osuntokun was used during this test [9]. Growth dynamic test was done to determine the rate of growth of the isolates as well as their killing time in due time. A loopful colony was picked from the stocked culture slant and inoculated into 9ml nutrient broth which was incubated for 24 hours at 370C. A loopful of each organism was picked from the broth culture into another 9ml nutrient broth in three sets which are labelled as set A, B, C and D respectively. The growth rate was measured using ultraviolet (UV-vis) spectrophotometer; the spectrophotometer was set at 620λ wavelength, warmed up for 15 minutes and calibrated; during the determination of the growth, sterilized nutrient broth was used to calibrate the spectrophotometer for set A. during the determination of the killing rate sterilized nutrient broth, in which the oil to be assay has been incorporated into was used. The first reading was taken at zero hour, eight hour and it continues after every twelve hours for six times [6].

Animal Care

Thirty (30) male albino rats with weight of 0.18–0.2 kg were used during the study. The rats were purchased from the Department of Biochemistry, Obafemi Awolowo University, Ile Ife, Osun state. Animal care and handling was carried out as described by Hemmati et al., while the rats were fed with commercial pellet diet and water ad libitum [10].

Experimental Design

The animals were housed groups (n=2) in aluminum cages throughout the research. All the animals were kept in a holding room illuminated with 12 h light/dark cycles at room temperature of 23 ± 2 °C with relative humidity of 45% to 55%.

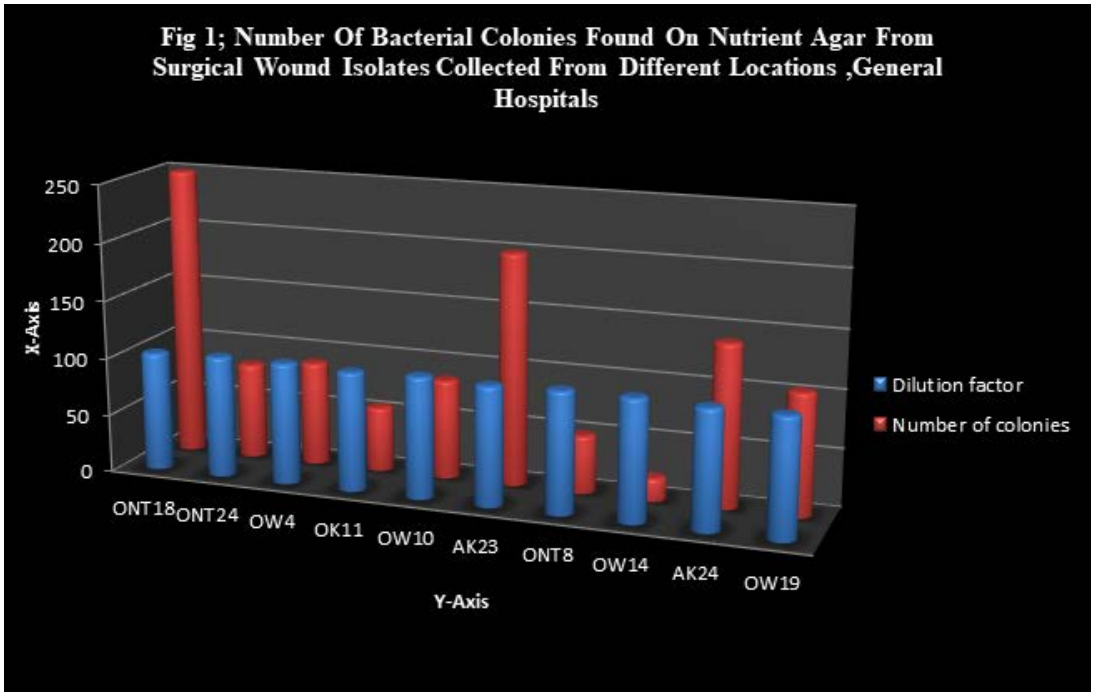
Toxicological Assay of Zingiber officinale (ginger) and Syzygium aromaticum (clove) Oil on *Experimental Animal*

The hair on the limb region was shaved 24 h before the test. The rats were generally anesthetized with intraperitoneal injection of ketamine: xylazine (0.4 mL: 0.1 mL, 40 mg/kg: 10 mg/kg). Ethanol (70%) was used as an antiseptic for the shaved region before introducing the wound. A circular excision wound was made using a biopsy punch (4 mm in diameter) as mentioned by Shailajan *et al.* [11]. The wounding day was counted as day zero. The wounds were treated topically twice daily with the oil samples respectively until the 7th day of the experiment. Wound size was observed, measured and photographed. On the final day the rats were sacrificed, blood was taken and small portion of the wound area was extracted, rinsed in saline buffer which was kept in freezer for further analysis [12].

3. Results

Figure 1: Shows the number of bacterial colonies found on nutrient agar from surgical wound isolates collected from different locations (General hospitals) in four towns namely: Owo, Akure, Ondo and Okitipupa, Southwest, Nigeria. The results showed that the swabs varied widely across the sample codes, ranging from 20 to 250. The highest colony counts were observed in ONT18 and AK23, which had a dilution factor of 10-3, while the lowest colony count was observed in OW14, which had a dilution factor of 10-5. ONT18 had the highest colony count (250) but also the lowest dilution factor 10-3, which means that the sample was not diluted much before being cultured. This suggests that ONT18 had

a very high bacterial load in the wound sample, which could indicate a serious infection. On the other hand, OW14 had the lowest colony count (20) but also the highest dilution factor 10-5, which means that the sample was diluted more before being cultured. This suggests that OW14 had a very low bacterial load in the wound sample, which could indicate a mild infection. The sample codes with higher colony counts and lower dilution factors (such as ONT18, AK23, and AK24) had higher bacterial loads and potentially higher infection risks than the sample codes with lower colony counts and higher dilution factors (such as OW14, OK11, and ONT8).



Key: ONT: Isolate from Ondo State Teaching Hospital, Ondo. **OW**: Isolate from Federal Medical Centre, Owo. **OK**: Isolate from General Hospital, Okitipupa. **AK**: Isolate from State Hospital, Akure.

Table 1: Shows that the isolates varied in colony color, elevation, opacity, margin, shape, Gram's stain reaction, and bacterial shape. The most common colony color was whitish, followed by yellow and creamy. The most common elevation was convex, followed by flat. The most common opacity was opaque, followed by translucent. The most common margin was entire, followed by irregular. The most common colony shape was circular, followed by irregular. The most common Gram's stain reaction was positive, followed by negative. The most common bacterial shape was rod, followed by cocci. Based on the results, the isolates can be classified into different groups of bacteria. The isolates that were Gram-positive cocci (AK24, ONT24, and ONT8). The isolates that were Gram-negative cocci (OW4 and OW14). The isolates that were Gram-positive rods (OK11 and AK23). The isolates that were Gram-negative rods (OW10, ONT18, and OW19).

Table 1: Morphology and Colonial Characteristics of Surgical Wound Isolate on Agar from Four General Hospital.

Isolate code	Colony colour	Elevation	Opacity	Margin	Colony shape	Grams stain reaction	shape
AK24	Whitish	Convex	Transcluscent	Entire	Circular	Positive	Cocci
ONT24	Golden Yellow	Convex	Opaque	Entire	Circular	Positive	Cocci
OW4	Greyish White	Convex	Opaque	Entire	Circular	Negative	Cocci
OK11	Creamy	Flat	Opaque	Entire	Circular	Positive	Rod
OW10	Creamy	Convex	Opaque	Entire	Circular	Negative	Rod
AK23	Greyish White	Convex	Transcluscent	Entire	Circular	Positive	Rod
ONT8	White	Convex	Opaque	Entire	Circular	Positive	Cocci
OW14	Yellow	Convex	Opaque	Entire	Circular	Negative	Cocci
ONT18	Whitish	Convex	Transcluscent	Entire	Circular	Negative	Rod
OW19	Yellow	Flat	Opaque	Irregular	Irregular	Negative	Rod

Key: The isolates from Ondo State Teaching Hospital (**ONT**), Federal Medical Centre, Owo (**OW**), General Hospital, Okitipupa (**OK**), and State Hospital, Akure (**AK**).

Table 2: Shows the biochemical test and sugar fermentation result of the surgical wound isolate. In this table, AK24, OW4, AK23, ONT8, OW14 and OW19 were positive for indole while ONT24, OK11, OW10 and ONT18 were negative. All isolates were positive for catalase, citrate and urease test and negative to methyl red test respectively. It was observed in this table that OW4, OW10, ONT18 and OW19 were Vorges-Proskauer positive while the remaining isolates were negative. ONT24, OW14 and ONT18 were negative for motility test while others were positive. It was observed that OW4, ONT8 and OW19 were oxidase negative while otherswere positive. Also, it was observed that all isolates were positive for mannitol fermentation test except for ONT8 and OW14which are negative. Furthermore, all isolates were positive for Glucose, Dextrose, Fructose and Sucrosefermentation test and negative for lactose fermentation respectively. It was observed that all isolates were positive for hydrogen sulphide test except AK24 and OW19 which are negative. The table shows that all isolates were negative for coagulase test except ONT24, OW10 and OW19 which are positive.

Table 2: Biochemical, Motility Test, Gas Production and Sugar Fermentation of the Surgical Wound Isolate from Four General Hospital.

	Biochemical tests										G. P						
Isolat ecode	Mo-tility	In-dole	Meth-yl red	Cata-lase	Ci-trate	Oxi-dase	Ure-ase	Vog-es	pros-kaeur	Coag-ulase	H_2S	Man-nitol	Glu-cose	Lac-tose	Dex-trose	Fruc-tose	Su-crose
AK24	+	+	_	+	+	+	+	_		_	_	+	+	_	+	+	+
ONT24	_	_	_	+	+	+	+	_		+	+	+	+	_	+	+	+
OW4	+	+	-	+	+	_	+	+		_	+	+	+	_	+	+	+
OK11	+	_	_	+	+	+	+	_		_	+	+	+	_	+	+	+
OW10	+	_	_	+	+	+	+	+		+	+	+	+	_	+	+	+
AK23	+	+	_	+	+	+	+	_		_	+	+	+	_	+	+	+
ONT8	+	+	_	+	+	_	+	_		_	+	_	+	_	+	+	+
OW14	_	+	_	+	+	+	+	_		_	+	_	+	_	+	+	+
ONT18	_	_	_	+	+	+	+	+		_	+	+	+	_	+	+	+
OW19	+	+	_	+	+	_	+	+		+	_	+	+	_	+	+	+

Key: (+) = **Positive**, (-) = **Negative**, the isolates from Ondo State Teaching Hospital (**ONT**), Federal Medical Centre, Owo (**OW**), General Hospital, Okitipupa (**OK**), and State Hospital, Akure (**AK**).

Table 3: Using Bergey's Manual, Klebsiella oxytoca, Staphylococcus aureus, Escherichia coli, Acinetobacter baumanii, Bergeyella zoohelcum, Coagulase –ve Staphylococcus aureus, Pseudomonas aeruginosa, and Chryseobacterium meningosepticumwere identified.

Table 3: List of Surgical Wound Isolate from four General Hospital Characterize Using Bergey's Manual of Determinative Bacteriology

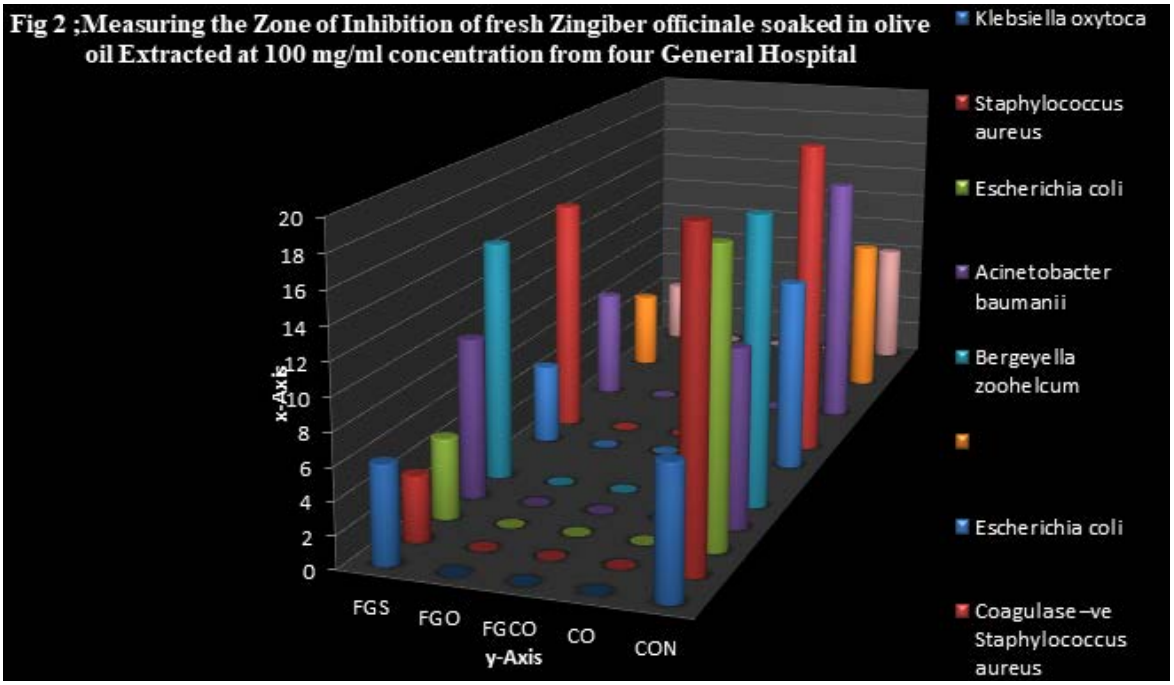
Isolate code	Probable isolates
AK24	*Klebsiella oxytoca*
ONT24	*Staphylococcus aureus*
OW4	*Escherichia coli*
OK11	*Acinetobacter baumanii*
OW10	*Bergeyella zoohelcum*
AK23	*Escherichia coli*
ONT8	*Coagulase –ve Staphylococcus aureus*
OW14	*Pseudomonas aeruginosa*
ONT18	*Klebsiella oxytoca*
OW19	*Chryseobacterium meningosepticum*

Key: The isolates from: Ondo State Teaching Hospital (**ONT**), Federal Medical Centre, Owo (**OW**), General Hospital, Okitipupa (**OK**), and State Hospital, Akure (**AK**).

Figure 2: Shows antimicrobial screening of fresh Zingiber officinale soaked in olive oil at 100mg/ml using Ampicillin as positive control. The results in this table indicate that fresh Zingiber officinale soaked in n-Hexane exhibited the highest zone of inhibition against Bergeyella zoohelcum (OW10) and Coagulase –ve Staphylococcus aureus (ONT8), each with a value of 15mm, while the lowest zone of inhibition was observed against Staphylococcus aureus (ONT24) and Chryseobacterium meningosepticum (OW19)with a value of 4mm. Fresh Zingiber officinale soaked in olive oil showed

no zone of inhibition against all the isolates, Fresh Zingiber officinale and clove soaked in olive oil showed no zone of inhibition against all the isolates, Clove soaked in olive oil showed no zone of inhibition against all the isolates. Ampicillin showed clear zone of inhibition against all the isolates with the highest zone of inhibition against Staphylococcus aureus (ONT24)and Coagulase –ve Staphylococcus aureus (ONT8)with a value of 20mm respectively while the lowest zone of inhibition against Klebsiella oxytoca (AK24) and Chryseobacterium meningosepticum (OW19) with a value of 8mm each.

Figure 3: Shows antimicrobial screening of fresh. Zingiber officinale soaked in olive oil at 50mg/ml using Ampicillin as positive control. The results in this table indicate that fresh Zingiber officinale soaked in n-Hexane exhibited zone of inhibition against Acinetobacter baumanii (OK11) with a value of 7mm, 6mm zone of inhibition against Klebsiella oxytoca (ONT18), 5mm zone of inhibition against Chryseobacterium meningosepticum (OW19) and 4mm zone of inhibition against Pseudomonas aeruginosa (OW14) while the others showed no zone of inhibition. Fresh Zingiber officinale soaked in olive oil showed no zone of inhibition against all the isolates, Fresh Zingiber officinale and clove soaked in olive oil showed no zone of inhibition against all the isolates, Clove soaked in olive oil showed no zone of inhibition against all the isolates.



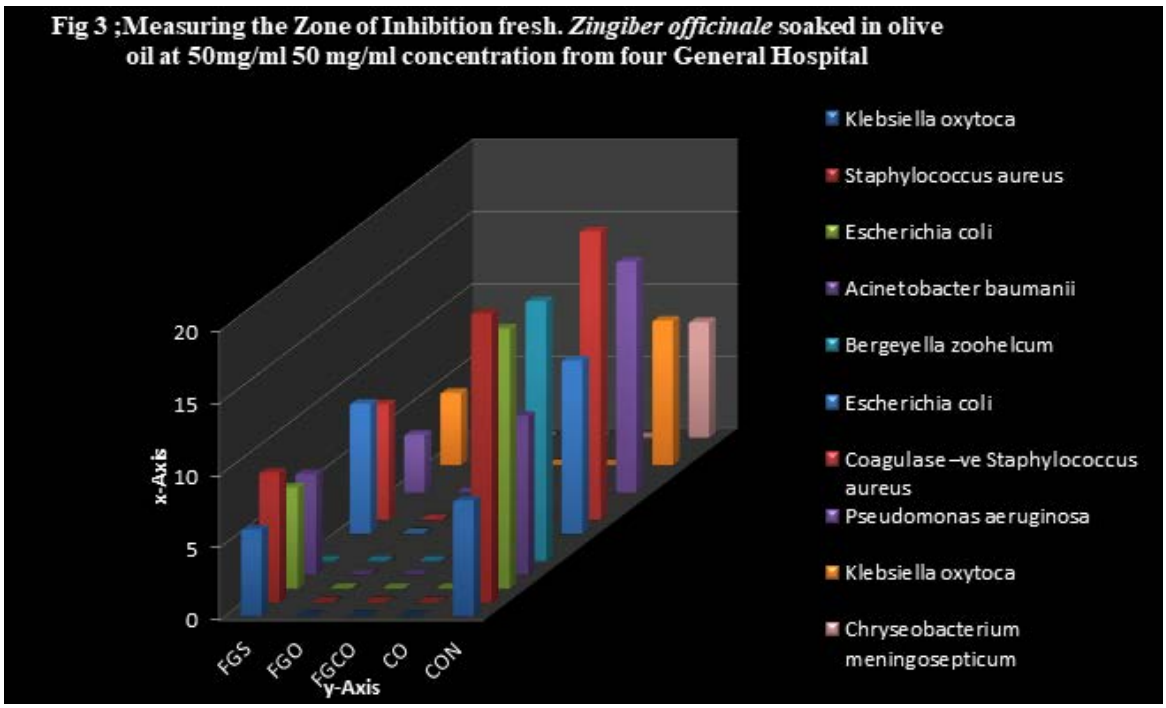


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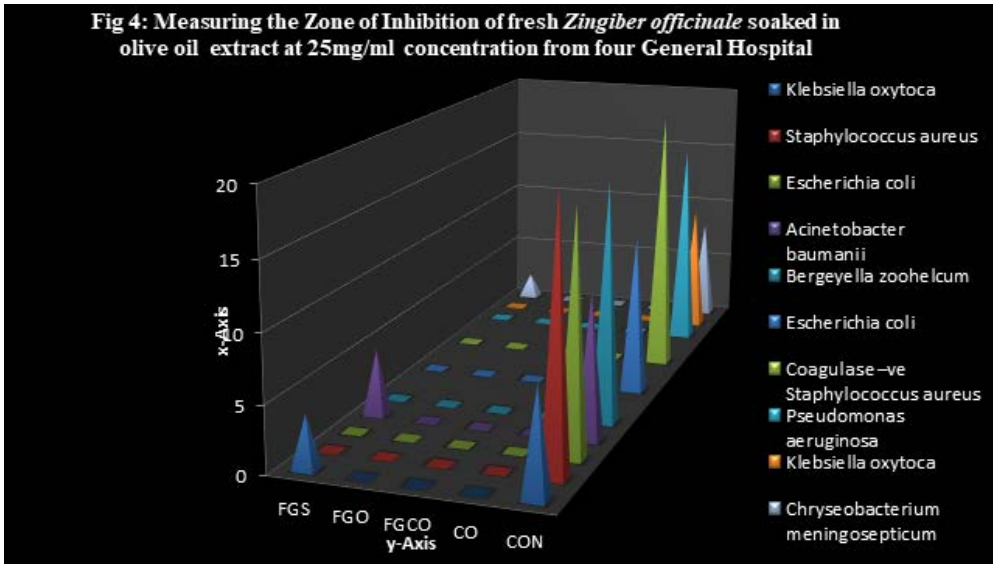
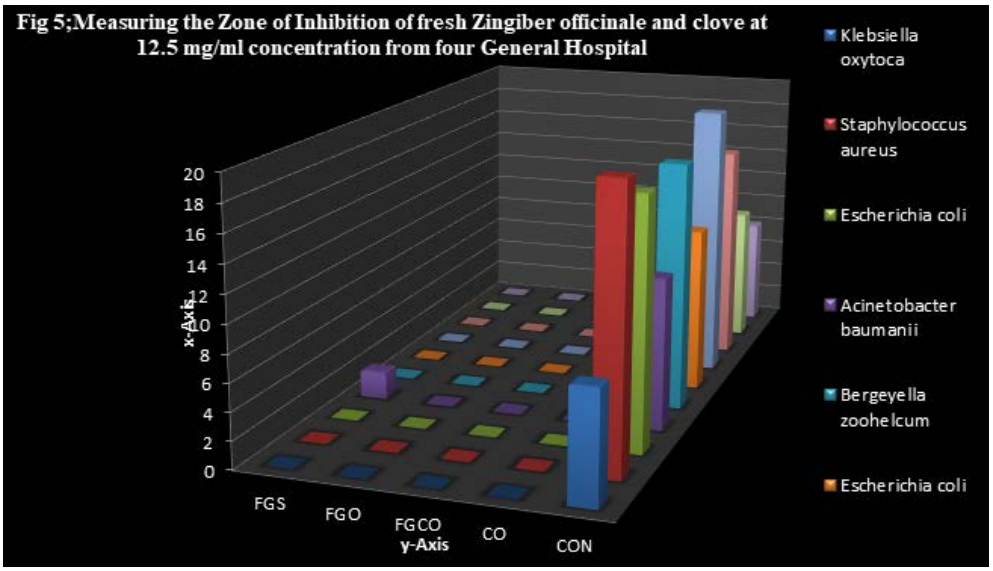
Figure 4: Shows antimicrobial screening of fresh Zingiber officinale soaked in olive oil at 25mg/ml using Ampicillin as positive control. The results in this table indicate that fresh Zingiber officinale soaked in n-Hexane exhibited zone of inhibition against Acinetobacter baumanii (OK11) with a value of 5mm, and against Klebsiella oxytoca (ONT18) with a value of 4mm as well as against Chryseobacterium meningosepticum (OW19) with a value of 2mm while others showed no zone of inhibition. Fresh Zingiber officinale soaked in olive oil showed no zone of inhibition against all the isolates, Fresh Zingiber officinale and clove soaked in olive oil showed no zone of inhibition against all the isolates, Clove soaked in olive oil showed no zone of inhibition against all the isolates.

Figure 5: Shows antimicrobial screening of fresh Zingiber officinale and clove soaked in olive oil 12.5mg/ml using Ampicillin as positive control. The results in this table indicate that fresh Zingiber officinale soaked in n-Hexane only showed zone of inhibition against Acinetobacter baumanii (OK11) and while other no zone of inhibition was observed in the remaining isolates. Fresh Zingiber officinale soaked in olive oil showed no zone of inhibition against all the isolates, Fresh Zingiber officinale and clove soaked in olive oil showed no zone of inhibition against all the isolates, Clove soaked in olive oil showed no zone of inhibition against all the isolates.

Figure 6: Shows the MIC and MBC of fresh Zingiber officinale oil against the surgical wound isolates examined. The MIC of fresh Zingiber officinale oil against the isolate as observed in the table ranges between 12.5mg/ml to 25mg/ml while the MBC ranges between 25mg/ml to 100mg/ml. Fresh Zingiber officinale oil has MIC of 12.5mg/ml effect on Acinetobacter baumanii, Bergeyella zoohelcum, Coagulase –ve Staphylococcus aureus, Pseudomonas aeruginosa and Chryseobacterium meningosepticum. And MBC of 25mg/ml effect on Escherichia coli but no effect was observed on Klebsiella oxytoca and Staphylococcus aureus. Zingiber officinale oil MIC against the isolate as observed ranges between 25mg/ml to 100mg/ml while the MBC has 100mg/ml effect on Coagulase negative staphylococcus, Escherichia coli and 50mg/ml effect on Acinetobacter baumanii while it has no effect on Klebsiella oxytoca and Staphylococcus aureus.





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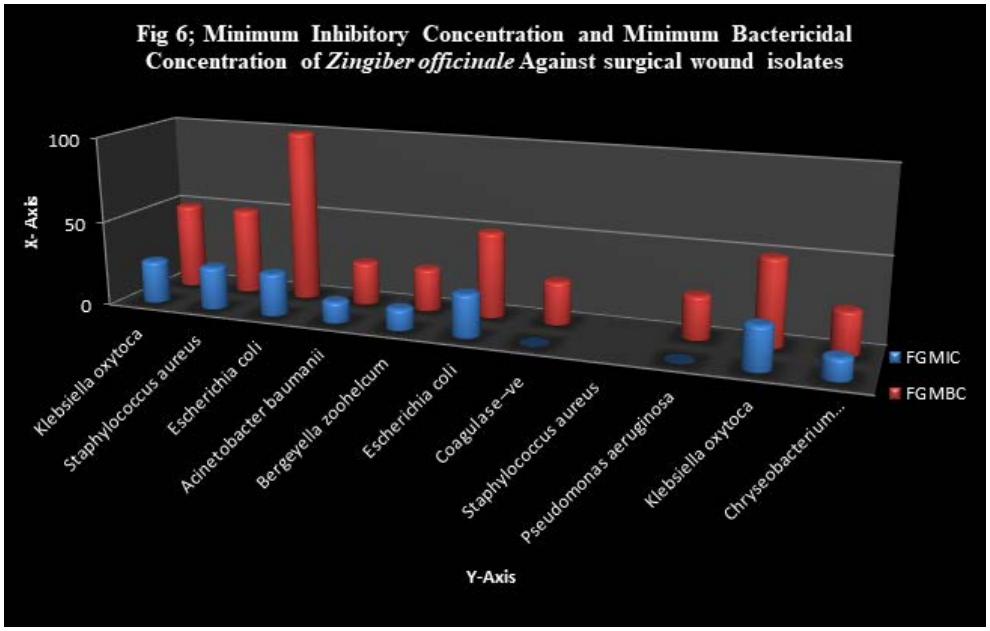

Key : FGMIC= Fresh Zingiber officinale oil minimum inhibitory concentration.

FGMBC = Fresh Zingiber officinale oil minimum bactericidal concentration.

Table 4: Shows the growth rate and killing kinetics of 50mg of Zingiber officinale oil onsurgical wound isolates using a UV spectrophotometer with 620λ. In this table, it was observed that at 0 hours, Staphylococcus aureus had the highest growth rate of 0.039λ while Escherichia coli had the lowest growth rate of 0.036λ. At 7 hours, all the isolates have the growth rate of 0λ.

Table 4.1: Shows the growth rate and killing kinetics of 25mg of Zingiber officinale oil on surgical wound isolates using a UV spectrophotometer with 620λ. In this table, it was observed that at 0 hours, Staphylococcus aureus had the highest growth rate of 0.212λ while Klebsiella oxytoca had the lowest growth rate of 0.208λ. At 7 hours, all the isolates have the growth rate of 0λ.

Table 4.2: Shows the growth rate and killing kinetics of 12.5mg of Zingiber officinale oil on surgical wound isolates using a UV spectrophotometer with 620λ. In this table, it was observed that at 0 hours, Klebsiella oxytoca had the highest growth rate of 0.198λ while Chryseobacterium meningosepticum had the lowest growth rate of 0.086λ. At 7 hours, all the isolates have the growth rate of 0λ.

Table 4: Killing Time of Bacteria Isolate with 50mg of Zingiber Officinale Oil Using Ultraviolet Spectrophotometer with Wavelength 620λ.

Isolates	0hour	1hour	2hour	3hour	4hour	5hour	6hour	7hour	8hour	control
Klebsiella oxytoca	0.038	0.014	0.106	0.086	0.046	0.13	0.002	0.000	0.000	0.000
Staphylococcus aureus	0.040	0.182	0.092	0.048	0.019	0.14	0.002	0.000	0.000	0.000
Escherichia coli	0.036	0.188	0.098	0.082	0.041	0.14	0.002	0.000	0.000	0.000
Acinetobacter baumanii	0.038	0.189	0.101	0.079	0.043	0.12	0.003	0.000	0.000	0.000
Bergeyolla zotheleum	0.038	0.088	0.106	0.083	0.047	0.15	0.002	0.000	0.000	0.000
Escherichia coli	0.039	0.014	0.106	0.086	0.046	0.13	0.002	0.000	0.000	0.000
Coagulase–ve Staphylococcus aureu	0.038	0.014	0.092	0.048	0.019	0.14	0.002	0.000	0.000	0.000
Pseudomonas aeruginosa	0.038	0.014	0.098	0.082	0.041	0.14	0.002	0.000	0.000	0.000
Klebsiella oxytoca	0.038	0.088	0.101	0.079	0.043	0.12	0.003	0.000	0.000	0.000
Chrysobacterium meningosepticum	0.038	0.014	0.106	0.083	0.047	0.15	0.002	0.000	0.000	0.000

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Table 4.1: Killing Time of Bacteria Isolate with 25mg of Fresh *Zingiber Officinale* Oil Using Ultraviolet Spectrophotometer with Wavelength 620λ.

Isolates	0hour	1hour	2hour	3hour	4hour	5hour	6hour	7hour	8hour	control
Klebsiella oxytoca	0.211	0.096	0.088	0.080	0.044	0.014	0.003	0.000	0.000	0.000
Staphylococcus aureus	0.210	0.094	0.094	0.043	0.016	0.012	0.003	0.000	0.000	0.000
Escherichia coli	0.211	0.092	0.092	0.082	0.047	0.012	0.003	0.000	0.000	0.000
Acinetobacter baumanii	0.208	0.094	0.088	0.089	0.043	0.014	0.002	0.000	0.000	0.000
Bergeyolla zotheleum	0.210	0.096	0.079	0.043	0.014	0.012	0.003	0.000	0.000	0.000
Escherichia coli	0.211	0.096	0.094	0.080	0.014	0.012	0.001	0.000	0.000	0.000
Coagulase–ve Staphylococcus aureu	0.210	0.094	0.088	0.043	0.016	0.012	0.003	0.000	0.000	0.000
Pseudomonas aeruginosa	0.211	0.092	0.092	0.082	0.047	0.014	0.002	0.000	0.000	0.000
Klebsiella oxytoca	0.208	0.094	0.088	0.043	0.043	0.014	0.003	0.000	0.000	0.000
Chrysobacterium meningosepticum	0.210	0.092	0.079	0.089	0.014	0.012	0.003	0.000	0.000	0.000

Table 4.2: Killing Time of Bacteria Isolate with 12.5mg of Fresh *Zingiber Officinale* Oil Using Ultraviolet Spectrophotometer with Wavelength 620λ.

Isolates	0hour	1hour	2hour	3hour	4hour	5hour	6hour	7hour	8hour	control
Klebsiella oxytoca	0.196	0.088	0.080	0.047	0.014	0.010	0.001	0.000	0.000	0.000
Staphylococcus aureus	0.182	0.092	0.041	0.082	0.043	0.012	0.002	0.000	0.000	0.000
Escherichia coli	0.188	0.079	0.047	0.015	0.047	0.014	0.001	0.000	0.000	0.000
Acinetobacter baumanii	0.189	0.083	0.043	0.12	0.025	0.012	0.002	0.000	0.000	0.000
Bergeyolla zotheleum	0.088	0.048	0.019	0.014	0.038	0.010	0.002	0.000	0.000	0.000
Escherichia coli	0.182	0.088	0.080	0.047	0.014	0.010	0.001	0.000	0.000	0.000
Coagulase–ve Staphylococcus aureu	0.196	0.092	0.041	0.082	0.043	0.012	0.002	0.000	0.000	0.000
Pseudomonas aeruginosa	0.188	0.079	0.047	0.015	0.047	0.014	0.001	0.000	0.000	0.000
Klebsiella oxytoca	0.189	0.083	0.043	0.12	0.025	0.012	0.002	0.000	0.000	0.000
Chrysobacterium meningosepticum	0.088	0.048	0.019	0.014	0.038	0.010	0.002	0.000	0.000	0.000

Figure 7: The table shows the serum bioassay parameters of albino rats treated with oil extractsof Zingiber officinale (ginger) and Syzygium aromaticum (clove). The parameters are;
Alanine amino transferase ALT (U/L), Alkaline phosphastase ALP (U/L), Aspartate aminotransferase AST (U/L), Total protein TP (mg/dL), Glutathione GSH (ug/dL), Alkaline Albumin ALB (mg/dL), Globulin GLB (mg/dL).

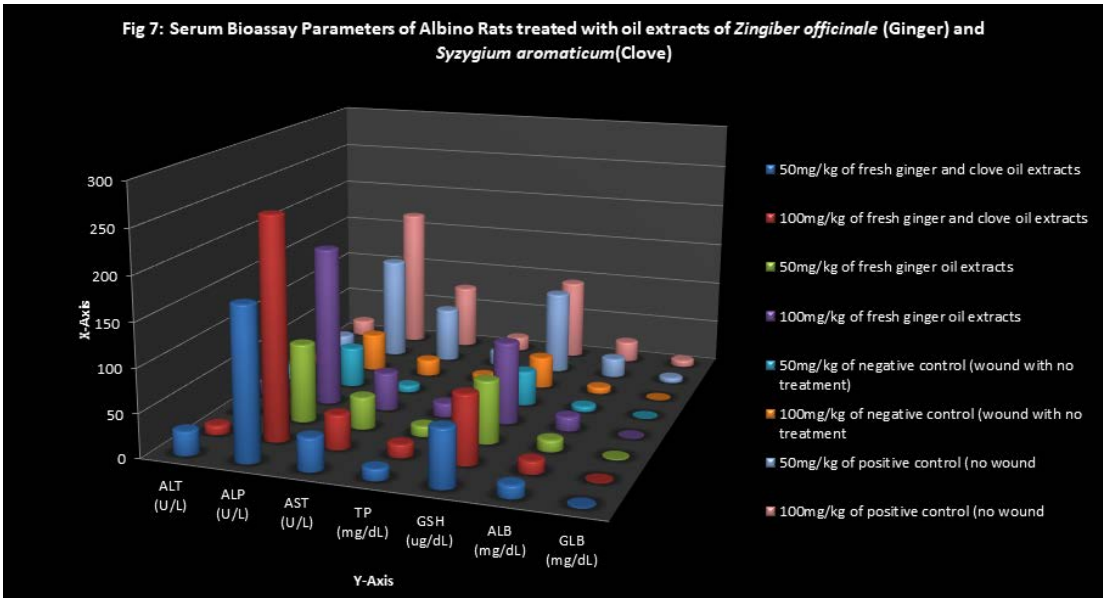
The table compares the values of these parameters among eight groups of rats, each treated with different doses of ginger and clove oil extracts, as well as with positive or negative controls. The positive control groups (G and H) have no wound, while the negative control groups (E and F) have wound with no treatment. The other groups (A, B, C, and D) have wound and are treated with various combinations and doses of ginger and clove oil extracts.

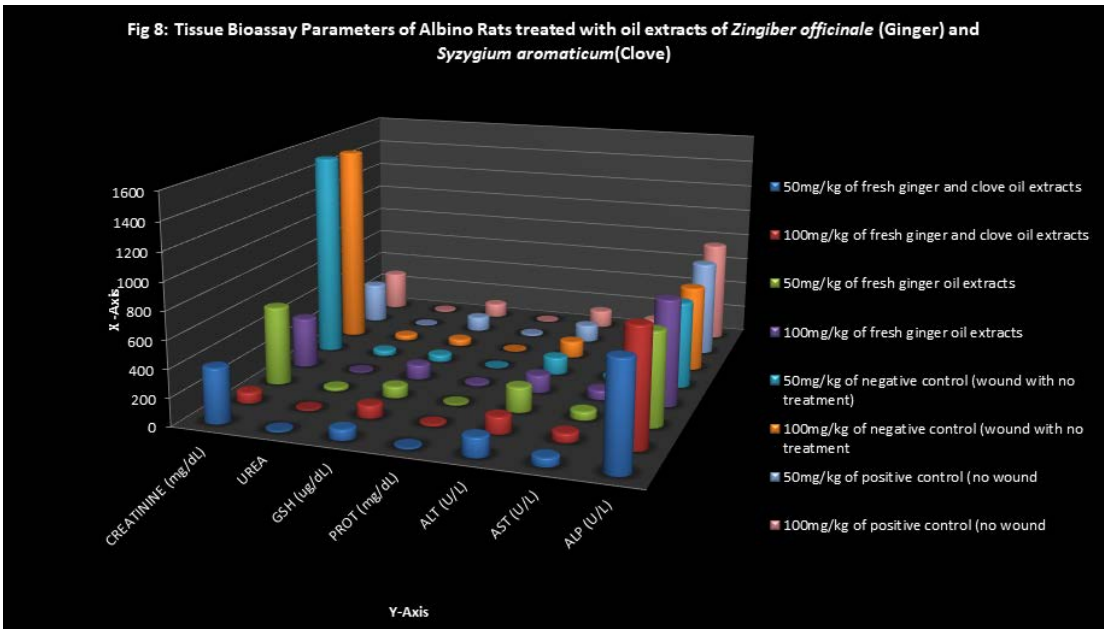
• The groups treated with ginger and clove oil extracts (A, B, C, and D) have lower ALT, ALP, and AST levels than the negative control groups (E and F), which may suggest that the extracts have a protective effect on the tissues from the wound-induced damage or inflammation.
• The groups treated with ginger and clove oil extracts (A, B, C, and D) have higher TP, GSH, ALB, and GLB levels than the negative control groups (E and F), which may indicate that the extracts improve immune function of the rats.
• The groups treated with higher doses (100mg) of ginger and clove oil extracts (B and D) have lower ALT, ALP, and AST levels, and higher TP, GSH, ALB, and GLB levels than the groups treated with lower doses (50mg) of the extracts (A and C), which may imply that the extracts have a dose-dependent effect on the serum bioassay parameters.
• The groups treated with ginger and clove oil extracts (A, B, C, and D) have similar or slightly higher ALT, ALP, and AST levels, and similar or slightly lower TP, GSH, ALB, and GLB levels than the positive control groups (G and H), which may suggest that the extracts do not cause any significant adverse effects on the serum bioassay parameters compared to the normal rats.

Figure 8: Shows the effects of different doses and combinations of ginger and clove oil extracts on some biochemical and hematological parameters of albino rats. The parameters are: Creatinine (mg/dL), Urea, Alanine amino transferase ALT (U/L), alkaline phosphastase ALP (U/L), Aspartate amino-transferase AST (U/L), Protein PROT (mg/dL), Glutathione GSH (ug/dL).

The Figure Shows that:

- The rats treated with ginger and clove oil extracts (groups A and B) had lower creatinine and urea levels than the negative control groups (groups E and F), indicating better wound healing.
- The rats treated with ginger and clove oil extracts (groups A and B) also had higher GSH levels than the negative control groups (groups E and F), indicating better wound healing.
- The rats treated with ginger and clove oil extracts (groups A and B) had higher total protein levels than the negative control groups (groups E and F), indicating better wound healing.
- The rats treated with ginger and clove oil extracts (groups A and B) had lower ALT and AST levels than the negative control groups (groups E and F), indicating lession flammation.
- The rats treated with ginger and clove oil extracts (groups A and B) had similar ALP levels to the negative control groups (groups E and F), indicating less toxicity.
- The rats treated with ginger oil extracts only (groups C and D) had similar or slightly worse results than the rats treated with ginger and clove oil extracts (groups A and B), indicating that clove oil may have some synergistic or additive effects with ginger oil.
- The rats in the positive control groups (groups G and H) had the best results in all parameters, indicating that they were healthy and unaffected by any treatment or wound.





Keys: ALT: Alanine amino transferase, ALP: Alkaline phosphastase, AST: Aspartate aminotransferase, TP: Total protein, GSH: Glutathione, ALB: Alkaline Albumin, GLB: Globulin GSH: Glutathione, PROT: Protein, ALT: Alanine aminotransferase AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

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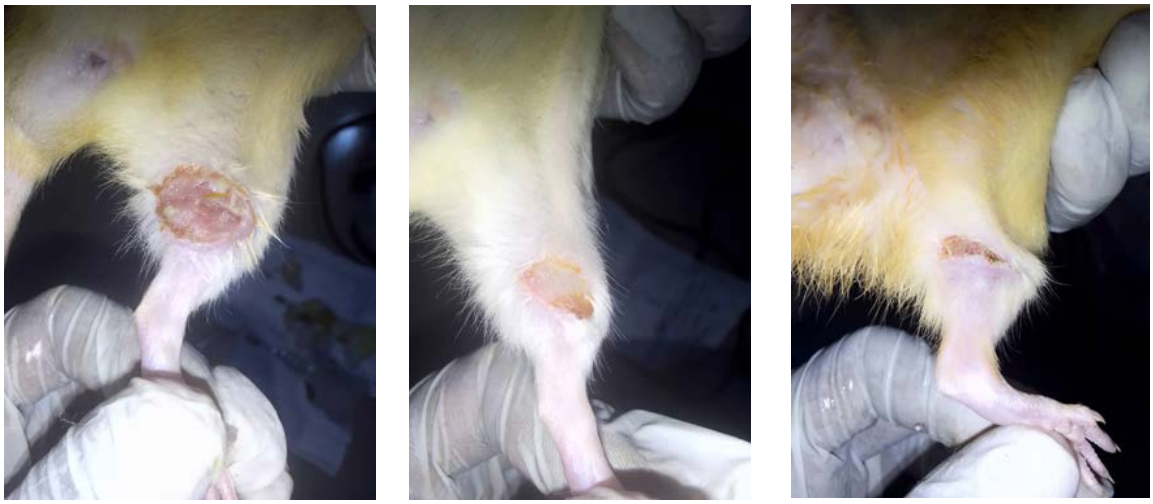

Plate1: Macroscopic observations of the healing processes of the excision wounds inflicted on Albino rats.

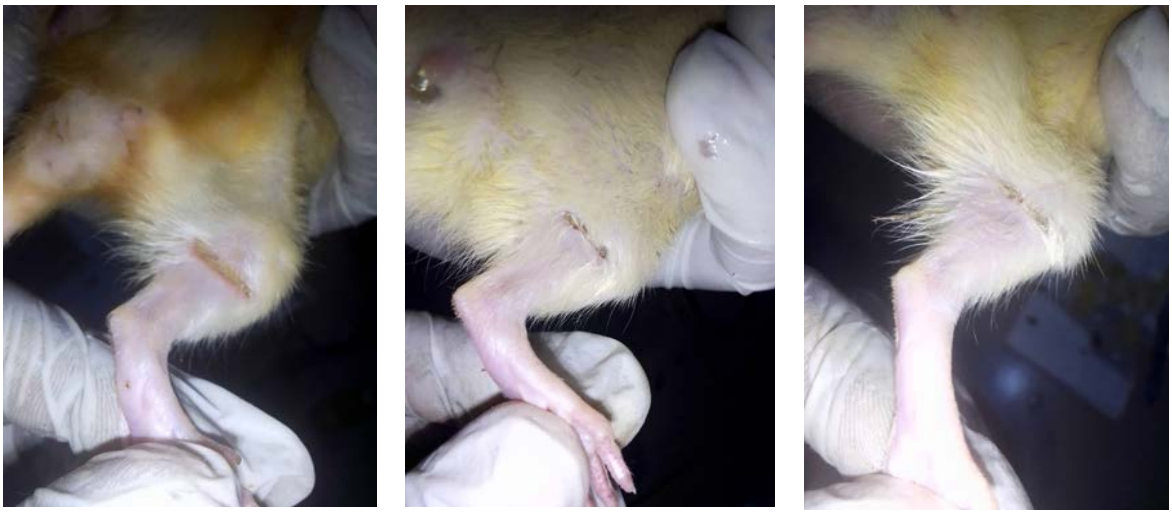

Plate 2: Macroscopic observations of the healing processes of the excision wounds inflicted on Albino rats.

4. Discussion

Surgical wound infections are a common and serious complication of surgery, affecting the healing process and increasing the risk of morbidity and mortality [13]. The use of antibiotics to treat these infections is often limited by the emergence of resistant bacteria, the side effects of the drugs, and the cost of the treatment [14]. Therefore, alternative and complementary therapies, such as the use of natural plant extracts, are gaining attention as potential sources of antimicrobial agents. Ginger (Zingiber officinale) and clove (Syzygium aromaticum) are two widely used spices and medicinal plants, with a long history of traditional use for various ailments. Both plants have been reported to possess antimicrobial properties, mainly due to their essential oils, which contain various bioactive compounds, such as gingerol, zingerone, and shogaol in ginger, and eugenol, acetyleugenol, and caryophyllene in clove [15].

These compounds can act on different targets in bacterial cells, such as the cell membrane, the cytoplasm, the proteins, and the nucleic acids, and interfere with their functions and viability. However, the extraction and preservation of these oils can affect their quality and quantity, as well as their antimicrobial activity. Different solvents, such as non-polar (e.g., N-hexane) or polar (e.g., olive oil), can have different efficiencies and selectivities in dissolving the oil components [16]. Moreover, the concentration, duration, and temperature of the extraction can also influence the yield and the composition of the oil [17]. Additionally, the storage and exposure of the oil to light, heat, and oxygen can cause oxidation and degradation of the oil components, reducing their stability and activity [16].

This study investigated the effects of oil extracts of Zingiber officinale (ginger) and Syzygium aromaticum (clove) on wound healing Albino rats and antibacterial activity. The oil extracts were obtained using different solvents and concentrations and applied topically to the wounds of rats. The serum and tissue bioassay parameters of the rats were measured to evaluate the wound-healing process. The oil extracts were also tested against ten bacterial strains isolated from surgical wounds of patients in four general hospitals in Ondo State, Nigeria. The bacterial strains were Klebsiella oxytoca (2), Staphylococcus aureus, Escherichia coli (2), Acinetobacter baumanii, Bergeyella zoohelcum, Coagulase –ve Staphylococcus aureus, Pseudomonas aeruginosa, and Chryseobacterium meningosepticum. The study found that the oil extracts improved the wound-healing process in rats

by modulating the biochemical markers of inflammation, oxidative stress, and tissue repair [18]. The study also found that the oil extracts inhibited the growth and survival of the wound bacteria and had synergistic effects [18]. Two methods of extraction were used in the extraction process: N-hexane solvent extraction and olive oil maceration. The oils were tested at four concentrations: 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. The agar well diffusion method, the minimum inhibitory concentration (MIC) method, and the minimum bactericidal concentration (MBC) method as well as killing kinetics were used to measure the antimicrobial activity. The results showed that only the ginger oil extracted with N-hexane solvent exhibited significant antibacterial activity against the tested wound isolates, while the other oils were ineffective. The N-hexane ginger oil inhibited all the bacterial strains with zones of inhibition ranging from 4.0 mm to 15.0 mm at 100 mg/ml concentration.

The MIC and MBC of the N-hexane ginger oil were 12.5 mg/ml and 25 mg/ml, respectively, for most of the bacterial strains, except for Klebsiella oxytoca, Staphylococcus aureus and Escherichia coli, which had MICof 25 mg/ml and MBC of 50 mg/ml, respectively. The N-hexane ginger oil also showed some activity at lower concentrations (50 mg/ml and 25 mg/ml), but not at 12.5 mg/ml. The ginger rhizome soaked in olive oil, the ginger and clove rhizomes soaked in olive oil, and the clove rhizome soaked in olive oil showed no antimicrobial activity against any of the bacterial strains at any concentration. Only the ginger rhizome soaked in N-hexane solvent exhibited antimicrobial activity, while the remaining oils were ineffective. This could be due to several factors, such as the type and concentration of the solvent, the duration and temperature of the extraction, the quality and quantity of the plant material, and the composition and volatility of the oil. N-hexane is a non-polar solvent that can dissolve the lipophilic components of ginger oil, such as gingerol, zingerone, and shogaol, which are responsible for its antimicrobial properties [19].

Olive oil, on the other hand, is a polar solvent that may not extract these components efficiently or may dilute them to a level below the minimum inhibitory concentration. Moreover, olive oil may have some antimicrobial activity of its own, which could interfere with the detection of ginger oil activity [20]. Adding clove to the ginger may also affect the extraction and the antimicrobial activity, as clove contains eugenol, which is a potent antimicrobial agent, but may also react with the ginger components and alter their structure and function [21]. Therefore, the extraction method, the solvent, and the plant material are important factors that influence the antimicrobial activity of ginger oil.

The pharmacodynamic and pharmacokinetic mechanisms of the oil extracts of ginger and clove on wound healing activity were explored in this study. The oil extracts modulated the biochemical markers of inflammation and oxidative stress, such as ALT, AST, ALP, GSH, TP, ALB, and GLB, which are involved in the protection and repair of the wounded tissues. These effects are consistent with previous studies that reported the anti-inflammatory and antioxidant activities of ginger and clove extracts in various animal models of wound healing [15, 22]. The phytochemical constituents of the oil extracts, such as gingerols, shogaols, zingerone, eugenol, and flavonoids, could be responsible for these effects, as they have been shown to affect the expression and activity of various enzymes, cytokines, and transcription factors involved in the inflammatory and oxidative pathways [23, 24].

The oil extracts also improved renal and liver functions by reducing the levels of creatinine, urea, ALT, AST, and ALP, which are indicators of renal and hepatic injury and dysfunction [25]. These effects indicate that the oil extracts enhanced the clearance and detoxification of the oil extracts and their metabolites, and prevented the accumulation of toxic substances in the blood and tissues [16, 17]. These effects are in agreement with previous studies that demonstrated the nephroprotective and hepatoprotective effects of ginger and clove extracts in various animal models of renal and liver injury [26, 27]. The oil extracts could modulate the activity and expression of various enzymes and transporters involved in the renal and hepatic excretion and biotransformation of the oil extracts and their metabolites, such as cytochrome P450, uridine diphosphate glucuronosyltransferase, multidrug resistance protein, and organic anion transporter [16, 27]. The oil extracts exhibited antibacterial activity against the surgical wound isolates, which included gram-positive and gram-negative bacteria. The oil extracts inhibited the growth and survival of the wound pathogens by disrupting their cell wall and membrane integrity and function. The oil extracts also exhibited synergistic effects by enhancing penetration and efficacy against the bacteria [15, 23]. These effects are consistent with previous studies that reported the antibacterial activity of ginger and clove extracts against various bacterial strains [27]. The phytochemical constituents of the oil extracts, such as gingerols, shogaols, zingerone, eugenol, and flavonoids, could be responsible for these effects, as they have been shown to affect the structure and function of the bacterial cell wall and membrane [28].

5. Conclusion

This study concludes that oil extracts of ginger and clove have pharmacodynamic and pharmacokinetic effects that improve the wound healing process in Albino rats by modulating the serum and tissue bioassay parameters related to inflammation, oxidative stress, and protein synthesis. The oil extracts also have antibacterial activity against the surgical wound isolates from four selected general hospitals in Ondo state, Nigeria. The oil extracts of ginger and clove could be used as potential natural remedies for the treatment of wounds and other inflammatory and oxidative stress-related conditions in humans. The oil extracts of ginger and clove also have synergistic effects, which could be useful for the treatment and prevention of wound infections. The oil extracts of ginger and clove are rich sources of phytochemicals, such as gingerols, shogaols, zingerone, eugenol, and flavonoids, which have various pharmacological properties and mechanisms of action. The oil extracts of ginger and clove are safe and effective alternatives to synthetic drugs, which may have adverse effects or resistance problems.

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