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Evaluation of antimicrobial activity of ginger extracts (oil, juice and starch) on selected microorganisms

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Abstract

Ginger (*Zingiber Officinale*) has been traditionally used for its medicinal properties. It possesses a noticeable antimicrobial activity against most microorganisms. This study evaluated the antimicrobial properties of ginger oil, juice and starch, in order to establish their antimicrobial potentials. Different parts of the ginger rhizome were extracted (oil, juice and starch), and cultures of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans and Aspergillus niger* were used to evaluate the antimicrobial capacity of the ginger imploring the Agar-Well Diffusion Assay method. The physicochemical properties, proximate composition and characterization of ginger were also determined using standard methods. The ginger extracts showed a noticeable antimicrobial activity which was confirmed by checking the susceptibility of different strains of bacteria and fungi by measuring the zone of inhibition. The result of zone of inhibition measured were as follows; at 100 concentration *Staphylococcus aureus* had 19.6mm, *Bacillus subtilis* 9.3mm, *Salmonella typhi* 4.3mm, *Escherichia coli* 21.3mm while P*seudomonas aeruginosa* and C*andida albicans* had 4.6mm respectively. The ginger starch/powder did not show any effect on the test organisms. It was only the ginger oil that showed significant effects against the test organisms except *Aspergillus niger*. The proximate composition of the ginger was found to be moisture content 47.80 ± 0.44, fat/lipids 19.30± 0.82, crude protein 3.71 ± 0, Ash 6.27 ± 0.15, crude fibre 7.22 ± 0.029 and carbohydrate 15.83 ± 1.01. The ginger oil was found to have the following properties: P^{H} 5.3± 0.26, Specific gravity 0.593 ± 0.11, Refractive index 0.239± 0.08, Acid value mgKOH/g 1.81 ± 1.35, saponification value mgKOH/g 30.77 ± 5.1 and iodine value mg/g 3.156 ± 0.89. In this study, it was established that the oil extract exhibited a very high antimicrobial potential due to its manifestation on six out of the seven isolates used, with *E. coli* being highly inhibited. This implies that ginger oil is effective in the treatment of infections caused by the six test organisms.

Keywords: Ginger; Antimicrobials; Oil; Juice; Starch; Microorganisms

1. Introduction

Plants are valuable antimicrobial agents known for the treatment of various microbial and non-microbial diseases, which has its origin from time immemorial. Medicinal plants have been of great importance to the healthcare needs in Nigeria. They are the pillar and backbone of traditional medicine. Lack of Healthcare facilities and the high cost of orthodox treatment in local communities is a major challenge (Zhu and Mekalanos, 2003; Okochi, 2003).

Zingiber officinale is commonly grown in all Tropical regions around the World. Ginger oleoresin is a dark amber to dark brown viscous liquid, with an aromatic, warm, spicy, sweet smell, and a pungent/warm taste. The constituents responsible for the pungent taste are non-volatile phenols, gingerols, shogaols and zingerone. Ginger is botanically known as *Zingiber officinale*. The rhizome (underground stem) is a spice that can be used fresh, dried and powdered or as a juice or oil. The ginger spice have unique aroma and flavor which are derived from compounds known as phytochemicals or

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secondary metabolites (Avato *et al*., 2002, Melvin *et al.,* 2009). Antimicrobial agents with selective toxicity are especially, useful as a chemotherapeutic agent in treating infectious diseases and may be a function of specific receptor requirement for drug attachment or it may depend on the inhibition of biochem ical events essential to the pathogen but not to host (Omoya and Akharaiyi, 2010). Many scientists have reported antimicrobial properties of several plants. The antimicrobial anti-tumor, anti- inflammatory and anti-necrotic (Omoya and Akharaiyi 2012) activities have been reported from the use of plants extracts. The flesh of the ginger rhizome can be yellow, white or gray in colour depending on the variety. It is covered with a brownish skin that may either be thick or thin depending upon whether the plant was harvested when or young. This plant ginger is native to Southeastern Asia, but it is being used and consumed by so many people mature across the globe. The plant is used as spice in various dishes, ranging from Soy sauce, baked food, pureed sweet potatoes, sautéed vegetables and salad dressing because of its aroma and taste. Apart from the aromatic property of ginger, it has long been priced for its culinary and above all medicinal properties (GMF, 2015). Ginger is a medicinal plant that has been widely used all over the world to treat various types of stomach problems including motion sickness, morning sickness, colic, gas, diarrhea, nausea caused by cancer treatment, nausea and vomiting after surgery, as well as loss of appetite (Auta, *et al* 2011). Others include pain relief from arthritis or muscle soreness, menstrual pain, upper respiratory tract infections, cramps, rheumatism, sprains, sore throats, constipation, hypertension, fever and other infectious disease (Ali, 2008). The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have become a glaring predicament. As a result, there is an urgent need to find alternative drugs in disease treatment especially those of plant origin which are easily available with considerably less side effects (Khulbe and Sati, 2009). The use of plants and their extracts for treatment of infectious diseases has long been adopted in many parts of the world. The plant derived medicines may be used in many different forms including: powder, liquid, oil or mixture which could be raw or boiled. They contain large amount of beta-caryophyllene, which is being investigated as an anti-inflammatory agent. It also contains significant proportions of secondary metabolites (phytochemicals) are secondary metabolites which have different health benefits and with respect to plants, they possess color, aroma and flavor and chemical compounds produced by plants generally to helped them thrive or thwart competitors, predators, or pathogen. Antibiotics are also known to be one of the most vital tools used in fighting bacterial infections and they have greatly improved the quality of health since their introduction in the fight against infectious disease (Boakye *et al*., 2016). Medicinal plants range from those used in the production of mainstream pharmaceutical products to plants used in herbal medicine preparations in various cultures of the world.

Herbal medicine is one of the oldest forms of medical treatment known to man and could be considered one of the forerunners of the modern pharmaceutical trade hence some modern drugs have been synthesized from plants derivatives. Plants of medical values are all over the world, thus, different cultures use different plants in disease treatment. The use of medicinal plants for the treatment and control of diseases around the world has gained popularity and acceptability in different ethnic groups because of their effectiveness and accessibility by the common man that cannot afford the cost of some modern medicines over the years, plants extract and plant derived medicines have made immense contributions to the overall health and well-being of man (Anyanwu and Nwosu, 2014). The anti-microbial ability of plant extracts and oils has established a platform for the processing and transformation of these plant products into pharmaceuticals preservatives and natural medicine. As a result of this, there is a growing interest in plants usage for medicinal purposes due to the presence of several antibacterial compounds present in them. Khulbe and Sati (2009) stated that Plant-derived medications have gained much interest as chemotherapeutic medication alternatives for treating diseases. The most important classes of these bioactive constituents of ginger plants are Alkaloids, flavonoids, Tannins, saponins, Terpernoids phenolic compounds, steroids, glycosides and carotenoids.

In recent years, considerate attention has been directed towards identifying natural antioxidants, namely the *Cymbopogon citrates* (lemon grass)*, Zingiber officinale* (ginger) and eucalyptus plants. They are used for human consumption regarding health promotion and disease prevention. Many ailments have been discovered, but the challenge is the treatment without adverse effects. There are also resistances to drugs used in treating these cases like cancer, skin infections, rashes, inflammations, cough and diabetes mellitus.

According to World Health Organization, micro-organisms like *Escherichia coli* and *Staphylococcus aureus* are developing resistance to several drugs which is posing severe threat like inflammation and other infections to the general public health. The importance of plants in medicine remains even of greater relevance with the current global shift to obtain drugs from plants sources as a result of which attention has been given to the medicinal value of herbal remedies for safety, efficiency and economy (Akindahunsi and Salawu, 2011). However, the need for a non-synthetic approach to fight these microorganisms, hence the research on extraction, characterization and antimicrobial analysis of aqueous extracts of ginger (zingiber officinale) oils, juice and starch. The aim of this work was to evaluate and compare the antimicrobial activity of ginger extracts (oil, juice and starch) on selected microorganisms (Bacteria, fungi and yeast).

2. Materials and methods

2.1. Culture Media

All media components and chemicals used in the studies were of analytical grade. Culture media used were Sensitivity Test agar and Sabouraud Dextrose agar for the antimicrobial evaluation. Selective media including Manitol salt agar, Centrimide agar, Mac Conkey agar and Salmonella Shigella agar were used for isolation of specific organisms. Culture media were prepared according to the instructions of the manufacturers.

2.2. Reagents

The reagents used include; Tetraoxosulphate (vi) acids (H_2SO_4), Hydrochloric acids (HCL), Sodium hydroxides (NaOH) Potassium tetraoxo Manganate($KMnO_4$), Manganese (iv) Oxide, trioxonitric (v) acids (HNO_3), Phenolphthalein Indicator, Methyl Orange Indicator, Buffer Solutions, Potassium Iodides, $Sncl_2$, Dimethyl sulfoxide, Tween 80 Ammonium Pyroline Dithiocarbamate, Ammonium Pyrrolidine Dithiocarbarmate, Methyl Isobutyl Ketone, Hydrogen Peroxide, phosphate buffer, magnessium sulphate solution, calcium chloride solution, ferric chloride solution.

2.3. Test Microorganisms

The isolates used are *Escherichia coli*, Bacillus *subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans and Aspergillus niger.*

2.4. Ginger Sample Processed Prior to Analysis

Two (2) kg of the ginger rhizome were weighed, peeled and crushed with electric blender. After grinding, 2kg of the crushed sample was transferred into a round bottomed flask containing 500ml of water. The extraction or production of ginger oil was done using steam distillation method via Clevenger apparatus. (Kumar and Tripathi, 2011)

- Extraction of Ginger Essential Oil, Juice and Starch
- Extraction of ginger Essential oil
- Fresh ground ginger was used to obtain essential oils at low temperature steam distillation using the Clevenger.

Procedure: The Clevenger apparatus consists essentially of three parts (a) a round – bottomed flask in which was placed the material containing the volatile oil and 500mls of water. (b) A separator in which the oil was automatically separated from distillate in a graduated tube, thereby permitting a direct reading of the quantity of oil and a convenient condenser. A heating mantle was used to achieve this purpose. Up to 500g of ginger was placed directly in the flask. Water was added until the flask was half full. The contents were boiled for approximately two hours, until all of the volatile oils have been driven off. The steam carrying the volatile oil condenses and falls into the graduated tube of the separator. The water was separated from the oil by gravity and automatically flows back into the distillation flask

Essential oil being less dense than water was collected through a burette attached to the Clevenger via a tap. The essential oils were analyzed to obtain their percentage yield, acid value, saponification value, iodine value, ester value, refractive index, specific gravity and pH

2.5. Production/Extraction of Ginger Juice

The juice was prepared by getting 100g of the ginger rhizome peeled and washed thoroughly. This was blended into a mesh added 50ml of clean water. After blending the mixture, was allowed to stay for 30mins before decanting the juice into a clean sterile container and then stored in the refrigerator.

2.6. Production/extraction of Ginger Starch

In preparing the ginger starch, 2 kg of the rhizome was weighed, peeled and washed. This was ground in a clean sterile grinding machine. After grinding, the mixture was sieved using a clean sterile muslin cloth. After sieving, the fiber which was a rough mesh was discarded. The sieved portion was allowed to settle for 30mins and decanted. The water and the starch were collected in a clean container. The starch was allowed to dry and the dried powder poured into a clean container.

2.7. Antibacterial assay

The antibacterial assay for each of the crude extract was carried out using the agar well diffusion method as described by (Aida *et al*., 2001). The antimicrobial activity of ginger extracts (i.e crude, juice and starch extracts) were tested against five standard clinical bacteria isolates namely: *Staphylococcus aureus, Salmonella typhi, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* and two fungi isolates namely *Aspergillus niger* and *Candida albicans.* A 0.5 McFarland standard bacterial and fungal suspensions of each of the test isolates was prepared and these formed the bacteria and fungi stock solutions used in the agar well diffusion assays as outlined below.

2.8. Agar Well Diffusion Assay

The media i.e. Sensitivity Test Agar (Oxoid, USA) was prepared and treated according to the manufacturer's specification, where 40 g of the media was dissolved in 1L of sterile distilled water and sterilized at 121°C for 15 mins. The media was allowed to cool to 50°C and later dispensed into 90 mm sterile agar plates and left to set. The sterile Sensitivity Test Agar plates were inoculated with the test culture by surface spreading method using sterile cotton swab which was dipped into each of the inoculums and excess was removed by rotating the swab several times against the inside wall of the tube above the level of the fluid. This was done to obtain uniformity of the inoculums. A sterile cork borer was used to make six wells (6 mm in diameter) on each of the STA and SDA plates. Aliquots of 60 ul of each extract dilutions, reconstituted in dimethyl sulfoxide (DMSO) at concentrations of 100, 50, 25, 12.5, 6.25, and 3,13 mg/ml for each of the three (3) extracts were applied in each of the wells in the culture plates previously seeded with the test organisms. Ciprofloxacin (5μg), Nystatin (5μg) and DMSO served as the positive and negative controls respectively. The cultures were incubated at 37°C for 18-24 hr and 25-27 °C for 48 hr respectively to allow the growth of microorganism. The antimicrobial potential for each extract was determined by measuring the zone of inhibition across each well. If the test materials have antimicrobial activity, it will inhibit the growth of the microorganisms, giving a clear, distinct zone called "Zone of Inhibition". The antimicrobial activities of the extracts were determined in terms of millimeter by measuring the diameter of zone of inhibition. The greater the zone of inhibition the greater the activity of the extracts against the microorganism. For each extract, three replicates test were conducted against each organism. Each extract was tested against all the bacterial and fungal isolates.

2.9. Determination of Minimum Inhibitory Concentration (MIC)

Determination of Minimum Inhibitory Concentration (MIC) of ginger (oil, Juice and starch) extracts: The MIC for the crude extract was determined by agar-well diffusion method. A two-fold serial dilution of the test extracts was prepared by first reconstituting it in DMSO (5 g dissolved in 5 mL of DMSO= 1000 mg/mL). It was then diluted in sterile DMSO to achieve a decreasing concentration range of volume (3 mL) by 2-fold serial dilution. Then, each dilution was added (1 mL) aseptically into sterile Petri dishes.

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in Petri dishes. To 1 mL of varying concentrations of the extracts (100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0. 78, 0.39 and 0.19 mg/mL) in sterile Petri dishes, Nutrient agar (9 mL) was added and then a loop ful of each of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced by streaking. The culture plates were then incubated at 37°C for 24 h and 28°C for 48 h for bacteria and fungi plates, respectively. After incubation, the plates were then examined for microbial growth by observing for visible growth. The lowest concentration of extract following the concentration showing no visible growth was considered as the MIC. The procedures were conducted under strict aseptic conditions.

2.10. Determination of Minimal Bactericidal Concentration (MBC)

The MBC is an extension of the MIC procedure and it was determined after completing the MIC first, the agar plates showing no growth in the MIC tests were used to determine the MBC.

Here, antibiotic disc were cut from each agar plate (MIC) and transferred into corresponding containers of fresh nutrient medium. The medium were incubated for 48 hrs at 37^{0}C. after incubation, microbial growth was ascertained by checking for the presence of growth in the recovery medium. The minimal concentration of the antimicrobial agent that produces total cell death was taken as the MBC.

2.11. Analytical Tool

The statistical tool for the analysis is ANOVA (Analysis of Variance). ANOVA (Analysis of Variance) is a statistical tool used to compare the means of three or more groups. It determines if there are significant differences between group means by examining the variance within and between groups. ANOVA is particularly useful when you have more than two groups and want to avoid making multiple t-tests, which can increase the risk of errors. Analysis of

Variance (ANOVA) is a statistical formula used to compare variances across the means (or average) of different groups. A range of scenarios use it to determine if there is any difference between the means of different group.

3. Results

Among the seven isolates used, the five bacteria and the yeast (*Candida albicans*) were sensitive to only the ginger oil of all the extracts. Table 1 showed that ginger oil extract was effective against all the isolates except *Aspergillus niger*. *Salmonella typhi* and *Pseudomonas aeruginosa* was effective only on 100th concentration with IZD of 4.3 and 4.6 respectively. The ginger oil was more effective on *Staphylococcus aureus* up till the 6th concentration. The 100th value which is the first was 19.3 while the 3.13 value which is the sixth was 0.7. This showed that the value decreases with the decrease in concentration. The evaluation is as shown in Table 1.

Table 1 Antimicrobial properties of different concentrations of ginger oil (mg/ml/ Inhibition Zone diameter (IZD) (mm)

Test Organisms	**CONCENTRATIONS**					
	100	**50**	**25**	**12.5**	**6.25**	**3.13**
Staphlococcus aureus	19.30	18.60	8.30	6.00	5.30	0. 70
Bacillus subtilis	9.30	6.60	4.00	3.00	1.60	0.00
Salmonella typhi	4.30	0.00	0.00	0.00	0.00	0.00
Escherichia coli	21.30	14.60	6.60	3.60	0.00	0.00
Pseudomonas aeruginosa	4.60	0.00	0.00	0.00	0.00	0.00
Candida albicans	4.60	4.00	3.30	0.00	0.00	0.00
Aspergillus niger	0.00	0.00	0.00	0.00	0.00	0.00

In Table 2, among the five (5) bacteria isolates used, *Staphylococcus aureus* was affected the more. Its minimum inhibition concentration (MIC) was up to the seventh (7th) concentration (1.56 mg/ml), while the *Escherichia coli* and *Bacillus subtilis* were affected at the fifth (5th) concentration (6.25mg/ml), finally *Pseudomonas aeruginosa* was with the least effect, having a minimum inhibitory concentration of 12.5mg/ml (4th concentration)

Table 2 Effect of Ginger oil Concentrations on Bacteria Isolates (Minimum Inhibition Concentration (MIC) mg/ml)

Concentration (mg/ml)	***Staphylococcus aureus***	***Bacillus subtilis***	***Salmonella typhi***	***Escherichia coli***	***Pseudomonas aeruginosa***
100	-	-		-	-
50	-	-		-	-
25	-	-	-	-	-
12.5	-	-	-	-	_
6.25	-	-	+	-	+
3.13	-	+	+	+	+
1.56	-	+	+	+	+
0.78	+	+	+	+	+
0.39	+	+	+	+	+
0.19	+	+	+	+	+

Key: + means no inhibition, - means inhibition

In Table 3 the ginger oil has effect on *Candida albicans* from the 1st (100) to the 3rd (25) concentration, but has no effect at all on Aspergillus *niger*

Table 3 Effect of Ginger oil Concentrations on Fungi Isolates (Minimum Inhibition Concentration (MIC) mg/ml)

Concentration (mg/ml)	*Candida albicans*	*Aspergillus niger*
100	+	-
50	+	-
25	+	-
12.5	-	-

All the test organisms were not affected by the whole ginger mesh used.

For the minimum bactericidal concentration (MBC), each of the bacterial isolate were affected (killed) at different concentrations. The isolates *S. aureus* and *B. subtilis* had the same MBC of 6.3, while *E. coli, P. aeruginosa* and *S. typhi* had the same MBC of 25.

That is to say that the extract (ginger oil) was able to inhibit all the bacterial isolates at higher concentrations.

Table 4 Result of MBC

Concentration in mg/ml/Inhibition	Zone diameter (IZD) (mm				
	P. aeruginosa	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*
100	-	-	-	-	-
50	-	-	-	-	-
25	-	-	-	-	-
12.5	+	-	-	+	+
6.3	+	-	-	+	+
3.1	+	+	+	+	+
1.6	+	+	+	+	+
0.8	+	+	+	+	+
0.4	+	+	+	+	+
0.2	+	+	+	+	+

Key – means no growth, + means growth

4. Discussions

The antimicrobial activity of spices is due to specific phytochemical or essential oils (Akintobi *et al*, 2013). The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, type of microorganism, composition of the environment (Gull *et al*, 2012). The result of this study showed that only ginger oil extract had an inhibitory activity against the test organisms. Among the seven organisms used for this study, five (5) bacterial isolates and one (1) fungus (*Candida albicans*) responded to the effect of the ginger oil extract while the remaining fungal isolate (*Aspergillus niger*) was not affected. In the result of this study from Table 1, *E. coli* showed the highest susceptibility towards the plant oil extract. This is not in agreement with the work of Riaz *et al* (2015), who reported *S. aureus* as the most susceptible. It could be as a result of the method of extraction or the solvent used. From Table 1, the result would have corresponded with that of Raiz *et al.,* (2015) but for the error in getting the 3rd plate in the triplicate. This brought down the value of the zone of inhibition for *S. aureus.* The oil extract had no effect on *Aspergillus niger*, this is against the report of Teles *et al* (2019) where the extract has effect on *Aspergillus niger* using Disk diffusion method. Table 1 also showed that *Staphylococcus aureus* recorded 19.3,18.6,8.3 which completely agreed with the work of Rahman *et al* (2020) who stated that in the case of Ethanolic Extract, sensitivity was seen against Staphylococcus aureus using disc diffusion method. Zones of inhibition were 8, 13 and 19 mm at 25, 50 and 100 μg/10 μl respectively.

From this study it is the ginger oil that showed very significant result of antimicrobial effect on all the test organisms, except *Aspergillus niger.*

From the analysis on the Antimicrobial properties of different concentrations of ginger oil, the f-ratio for the grand mean for the test of dependent variable effect i.e. effect of antimicrobial properties due to level of concentration of ginger oil is 886.498 which are significant at 0.000 level. This suggests that the different levels of concentration of ginger oil is a good model and hence was able to indicate valid variation in the dependent variation, i.e. the differences in the effect on antimicrobial properties.

5. Conclusion

This study has established that the oil extract exhibited a very high antimicrobial potential due to its manifestation on six out of the seven isolates used, with *E. coli* being highly inhibited. This implies that ginger oil is effective in the treatment of infections caused by the six test organisms. This effect could be as a result of the bioactive compounds present the ginger oil such as gingerol and shogoal. It was also discovered that the juice and starch had no effect on the test organisms.

Compliance with ethical standards

Disclosure of conflict of interest

There was no conflict of interest in any part of this work.

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