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EFFECT OF GINGER (ZINGIBER OFFICINALE) EXTRACT ON CARIOGENIC ORGANISMS WITH PARTICULAR EMPHASIS ON STREPTOCOCCUS MUTANS AND LACTOBACILLUS ACIDOPHILUS

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ABSTRACT

This study attempted to examine the anti-microbial properties of ginger *(Zingiber officinale)* rhizome on cariogenic organisms with emphasis on *Streptococcus mutants* and *Lactobacillus acidophilus,* the known causative agents for dental caries (tooth decay). The ginger crude extract used for the study was obtained by both cold and hot extraction methods. The cold extraction was obtained by using distilled water in the rotary evaporating set at 40°C, while the hot extract was obtained by using petroleum spirit as the extracting solvent. The cold extract was found to exhibit antimicrobial effect on the test organism, in which the average zones of inhibition for *Streptococcus mutants* through cup-plate method and disk

Introduction:

The oral cavity harbors a large diverse microbial population which often comes into contact with it from air, water, food and other environmental segments. Although majority of these microorganisms are rapidly destroyed and eliminated by the host defense mechanisms or as a result of the hostile oral environment such as change in temperature, change in pH and increase in salivary flow with its corresponding viscosity, some species despite the

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diffusion technique was found to be satisfactory. In like manner, the average zones of inhibition for the two methods on *Lactobacillus acidophilus* was observed. From the result, the minimum inhibitory concentration of the crude extract shows that at 3ml in each 10^3 microbial load, it had antibacterial effect, which was bacteriostatic. However, the results of the petroleum spirit extract did not show any effect on the test organism. This could be attributed to the fact that the extract was immiscible and hence not effective against the test organisms. Chemical analysis of the plant ginger *Zingiber officinale* showed that it contains carbohydrates, alkaloids, flavonoids, sterols, tannins, glycosides, saponins, and resins. Additionally, it contains two principal components-the aromatic and pungent components. Thu this study shows that the extract of crude ginger have antibacterial activities at the range of 3.0 x 10^3ug/ml to 5.0 x 10^3ug/ml.

Keyword: Anti-microbial, *Zingiber officinale,* cariogenic *Streptococcus mutans* and *Lactobacili acidophilus.*

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above prevailing unfavorable factors still survive and consequently colonize one or more regions of the oral cavity and form eco niches. This latter group has poor oral hygiene and weak defense mechanisms of the host to their advantage (Izang, 2009).

Mouths as an organ plays a vital role in human anatomy. It plays a role of establishing a communication route between the internal environment of the body and the external. It's basically made up of tissues and organs which are in complex forms. Some of the tissues and organs are mucous membranes, lymphatic and blood vessels, salivary glands, gingival walls, muscles, nerves, tongue, palates, jaws and teeth. These organs collectively play a vital role in man's anatomy (Izang, 2009). Because of the complexity of the mouth's anatomy, warm temperature, relatively neutral pH, moist environment and its consistent contact with a varieties of food, mouth is prone to infections due to microbial attacks which may be directly by action or indirectly by their metabolites (Lavelle, 2012).

With respect to oral infection, several defects have been identified. These includes, Malocclusion, trench mouth, gingivitis. dental erosion or fluorosis (stain) and periodontal disease. The microbial flora in the mouth and the primary causative agents are fungi, viruses and bacteria (Geddes, 2003).

Although most of the oral infections could be treated by administration of antibiotics. Dental caries (tooth decay) is exceedingly the most widespread and resistant infection; and so, it is of medical importance both to individuals, government at all levels and non-governmental organizations (Naveena and Menderithan, 2010). Dental caries occurs globally and cuts across wide range of ages. Children between the ages of 4-6 years old have one or more tooth decay, while adults between the ages of 35-44 years increasingly loss their teeth. The percentage estimate placed the two categories of people 49% and 19% respectively. In Nigeria, a survey carried out on the prevalence of dental caries among individuals between the ages of 7-9 years in Ekpoma, Edo State has a mean percentage of 39.06, (WHO, 2015).

Loesche, (2011) has reported a 10- fold increase in dental problem in most Nigerian urban children.

Despite un parallel advanced in dentistry and series of research breakthrough, it is on record that dental caries and other periodontal diseases remain a serious dental health problem. Nine out of ten people are affected by it in the cause of their lives and has been a factor responsible for not only the loss of teeth or oral infection, it is linked as a triggering factor of sub-acute bacterial endocarditis (SBE), an infection of the inner lining of the heart, especially in rheumatic patients. It is also a factor of bacteraemia, a situation resulting from an individual having high concentration of bacterial cells in the blood, (Geddes, 2003).

Sadly, despite the persistent warning and campaigns by World Health Organizations on this subject, some human predisposal habits such as current high trends in modernization, high rate of smoking, high intake of sugared confectionaries, poor oral hygiene, instability on government health policies, depressed economic stigma on individuals and the emergent of resistant mutants by the causative agents on formulated

antibiotics will always make scientific discoveries and advancement a mockery.

Dental caries has resisted attempts of antibiotic therapy partly due to the persistence contact with carbohydrate substrate by the causative agents, its ability to synthesized both intracellular and extra cellular glycan and the emergence of mutants, (Loesche, 2011)

It is on this basis that this study is aimed at finding out the effect of ginger extract on cariogenic organisms with particular emphasis on *Streptococcus mutants* and *Lactobacillus acidophilus.*

MATERIALS AND METHODS

Collection and Preparation of Ginger Rhizome

The root stock of ginger rhizome was bought from Wunti market in Bauchi state. This was followed by screening and removal of the physically infected parts. The dried ginger was then pulverized in a mortar into semi-fine powder ready for extraction, (Daniel, 2009).

Collection and Preparation of Test Organisms

The parent culture was collected at medical microbiology laboratory, University of Jos. And was sub-cultured in an appropriately selective media.

Extraction Procedure for Ginger Extracts

Distilled water was used for the cold extraction. 60g of the pulverized sample was weighted on a whatman filter paper. The content of the paper was there after transferred into a sterile beaker and 500ml of distilled water was added. The content was stirred periodically with a glass rod and allowed to soak overnight at room temperature. The sample was carefully filtered and the filtrate was concentrated in a rota evaporator, set at 40°C, (Zero, 2004)

Phytochemical Analysis Of Ginger Rhizome Extract AOAC, (2003)

Test for Carbohydrates

A few drops of Molisch reagent was added to 2ml of each extract in a test tube, this was followed by slowly addition of 1ml H_2SO_4 down the inclined

tube so that the acid formed a layer beneath the solution without mixing with it. A water extract turned light brown on addition of molisch reagent, while the petroleum spirit extract tube reddish brown. Also a ring was observed at the interface of the two extracts on addition of conc. H_2SO_4.

Fallings' Test: Standard Test for Free Reducing Sugar

This was done by adding 5mls of mixture of equal volumes of felling solution A and B to 2m1 of each extract in a test tube, and heated till boiled for a minute or two. A brick red precipitate of cupper was observed in both tubes and this was taken for the presence of free reducing sugar.

Test for Alkaloids

For the test of the presence of alkaloids, the extracts sample were acidified by adding 1ml of 1% HCL to 3m1 extract in a test tubes, allowed to dilute for few seconds. After the dilution, 1ml each was pipetted into separate test tubes and few drops of Mayer's reagent was added. The tube with water extract shows a creamy-white precipitate while a tube of petroleum spirit shows dark reddish brown which gradually turn to creamy-white was observed. This was taken as an evidence for the presence of alkaloids.

Test for Tannins

Two drops of 5% $Fecl_3$ was added to 1ml of each extract. The tubes were allowed to stand for a minute and a dirty-greenish precipitate was observed in both water and petroleum spirit extract. This indicates the presence of tannins.

Test for Flavonoid (Shendo test)

Magnesium powder (0.1g) was diluted with few drops of concentrated HCL acid. This was added to 3m1 each of the extract. A red colouration was observed in the two test tubes. This indicates the presence of flavonoids.

Test for Sterols (Liebermann Burchard Reaction) 1ml of each extract was pipetted into separate test tubes and 1ml of concentrated H_2SO_4 was carefully introduced. A reddish coloration was observed and this was taken

for the presence of gingeral shogoal and zingerone, which collectively forms the sterol components of the ginger.

Test for Glycosides

In two separate test tubes, 1ml of each extract was dispensed. This was followed by adding 10ml of 50% H_2SO_4 and the mixture was heated in boiling water for 10-15 minutes. After cooling, 5m1 of Fehling's solution was added to each tube and the mixture re-boiled. A brick-reddish colouration was observed in each tube. This was taken as the evidence for the presence of glycosides.

Preparation of the Media

Preparation of Nutrient Broth for *Lactobacilli Acidophilus*

In a sterile conical flask, 3.5g agar was weighted and dispensed and 50m1 of sterile distilled water was added. The content of the flask was shaken to dissolve and The flask was then corked with a sterile cotton wool and aluminium foil paper and sterilization by autoclaving at 121°C for 15 minutes. After sterilization period, the broth was allowed to cool down to 45°C. before inoculation.

Preparation of Nutrient Broth for *Streptococcus mutants*

In a sterile conical flask, 1.8g of blood agar base powder was weighted on an electronic balance and 50ml of sterile distilled water added to it. The content of the flask was plugged with a sterile cotton wool, shake to dissolve and sterilize in an autoclave at 121°C for 15 minutes. After sterilization the agar was allowed to cool down to 45°C. before inoculation.

Seeding and Incubation of Test Organisms

Lactobacilli acidophilus

l0ml of the sterile Demans Rogosa broth was pipetted in to two tubes. Seeding of the organism by using a sterile wire loop to pick a colony from the stock culture. This was seeded in tube A, while tube B was left unseeded; and serves as the control. The tubes were immediately corked and allowed to stand at room temperature for 15 minutes, after which they

were incubated at 36°C for 18-24 hours. The appearance of opacity in tube A suggested the presence and viability of the organism. Biochemical tests were further carried out to confirm the presence of the cell. This was achieved by dropping 1ml of bromocresol green indicator. The appearance of reddish-colour from the initially yellow colour confirmed the presence of the organism. On gram reaction, the cells appear deep-blue on microscopy. Ahmed *et al*., 2005)

Streptococcus mutants

For this organism, 2m1 of sterile blood base agar was pipetted into sterile Petri-plates, and 1ml of sterile blood was added in the plates. Both plates were allowed to gel and labelled A and B respectively. In plate A, a loop of the test organism was aseptically streaked, while plate B was left un-streak. This served as the control. Both plates were immediately transferred into an anaerobic gar and a burning candle was introduced into the gar .and was then left for 15minutes at room temperature. The jar with the plates was then incubated at 36°C for 18-48 hours (Usman, 2005). After the incubated period, plate 'A' on physical examination revealed the presence of whitish patches. This indicates partial haemolysis of the red blood cells (RBCs). Further test was done through biochemical analysis by introducing a drop of alkaline phosphate in a broth culture tube. The changing in colour of the tube from light yellow to deep purple confirmed the presence and viability of the organism (Bulman *et al.,* (2006).

Preparation of Nutrient Agar

Nutrient agar for the seeking of the test organisms was prepared by dissolving 0.96g of the agar base in 40ml of sterile distilled water in a 50m1 conical flask. The flask was corked with sterile cotton wool and it was sterilized in an autoclave at 121°C for 15 minutes.

Culture and Sensitivity Tests

Two methods were employed in these tests:

Cup-Plate Method

Sterile nutrient agar was dispensed into 16 pre-labelled petri plates and was allowed to gel at room temperature. A colony *Streptococcus mutants* was then aseptically seeded into 4 plates. The same was done in the remaining 4 plates in which 0.lml of *Lactobacilli* broth colony was injected with the aid of sterile needle and syringe. Using a Durham tube, holes were bone in the gelled plates, and this was followed by filling 4 of the plates with the extract. All practical were done in duplicates the plates were left at room temperature to ensure proper diffusion of the exacts. The plates in the gas jar were then incubated at 36°C for 18-24 hours. After the incubation period, the zones of inhibitions on each plate was observed and measured. The values were recorded to the nearest millimetres. (Oyewale, and uroji, 2014).

Disk Diffusion Method

A whatsman No. 1 filler paper was carefully perforated into 200 disks, and dispensed into 2 separate vials with each having 100 disk. The vials were than screwed up and sterilized in an oven at 160°C for 60 minutes.

For this method, a nutrient agar was used as the growth medium for the microbes. The agar medium was prepared by dissolving 38g of the nutrient agar powder in 1000ml of distilled water, heated to dissolved and sterilized in an autoclave at 121°C for 15 minutes. It was cooled and poured into 8 pre-labelled plates to solidify. Standard stains of *Streptococcus mutants* and *Lactobacilli acidophilus* previously sub-cultured in a nutrient broth which had stayed overnight were injected. The 0.lml microbial load plates were smeared on the surface of the plates using a sterile glass rod spreader. The sterilized disk incorporated with the extract were carefully planted on the agar, with each organism having 3 plates. The remaining two plates were used for the control experiment. All the plates were incubated in an anaerobic jar and a lighted candle placed in the jar with the plates were incubated at 37°C'for 24 hours after standing at room temperature 15 minutes to ensure proper diffusion of the antimicrobial agents. The zone of inhibitions on each plate was measured using a

transparent plastic ruler. All measurements were observed and recorded to the nearest millimetres. Oyewale and Uroji, (2014).

Determination of Minimum Inhibitory Concentration (MIC)

For the determination of the (MIC) of the ginger rhizome extract, the tube dilution method as described by Oyewole and Uroji, (2014) was adopted. From the powdered ginger sample, 60g was soaked in 500m1distilled water overnight. . The filtrate was concentrated in a rotary evaporator set at 40°C. The concentrate was dried into a powder from in an oven set at 37°C. From this, dried concentrated separate weights were made and dispensed in pre-labelled test tube with each tube having 1, 2, 3, 4, and 5g in that order. In each of the tubes, 3m1 of sterile distilled water was dispense and shake to dissolve. This was followed by pipetting 5m1 of nutrient broth into each tube and then in each tube, a wire loop of test organism A *(Streptococcus mutant)* was seeded. The same procedure was performed for organism B, *Lactobacilli acidophilus.* The tubes were corked and incubated at 37°C for 24 hours in an incubator after standing for 15 minutes at room, temperature in an anaerobic jar. By this method, the lowest concentration tube in which no visible growth of the test organism was observed by level in the tube was recorded as the MIC. Further analysis was done by spectrophotometric method. (Oyewale, and uroji, 2014).

Determination of Minimum Bactericidal Concentration (MBC).

For the determination of the MBC of the ginger rhizome extract, streak-plate method was adopted as described by (Usman, 2011; Oyewale, and uroji 2014). A nutrient agar was prepared and poured into a petri plate and allowed to solidify. From the tube had no growth, a wire loop of the sample was streaked on the surface of the agar, the plate was incubated at 37°C for 24 hours and observed. All plates were streaked in duplicates. The plate that showed no growth after the incubation period was recorded as the MBC. (Oyewale, and uroji, 2014).

Statistical analysis

ANOVA was used to test for significance difference in all the data obtained from zones of inhibition. All statistical analysis were carried out using the SPSS 17.0 window based program. Significance difference and non-significance was defined when $p<0.05$ and $p>0.05$ respectively.

RESULTS: (Conversion factor: 1cm = 10mm)

table 1: Phytochemical analysis of the rhizome extract of *zingiber officinale*

Plant Extract	Carbohydrate	Reducing sugar	Alkaloids	Tannins	Flavonoids	Sterols	Glycosides
P S	+	+	+	+	+	+	+
Water	+	+	+	+	+	+	+

Key: P S. = Petroleum Spirit + Present.

Table 2: Average Zones of Inhibition (cm) of Bacterial Growth of the Rhizome extract of *Zingiber officinale*: Cup-plate method

Test org.	Petroleum spirit					Water				
	Concentration (ml)									
	$1x0^3$	$2x10^3$	$3x10^3$	$4x10^3$	$5x10^3$	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$	$5x10^3$
S. mutant	0	0	0	0	0	0.49	0.50	0.51	0.62	0.73
L.cidophilus	0	0	0	0	0	0.69	0.73	0.79	0.83	0.82

Table 3: Zones of Inhibition (mm) of Bacterial Growth of the Rhizome extract of *Zingiber officinale*: cup-plate method

Test org.	Petroleum Spirit					Water				
	Concentration (ml)									
	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$	$5x10^3$	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$	$5x10^3$
S. mutants	0	0	0	0	0	0.049	5.00	5.10	6.20	7.30
L. acidophilus	0	0	0	0	0	6.90	7.30	7.70	8.30	8.20

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Table 4: Average Zones of Inhibition (cm) of Bacterial Growth of the Rhizome extract of *Zingiber officinale*: Didk Diffusion Technique

Test org.	Petroleum Spirit					Water				
	Concentration (ml)									
	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$	$5x10^3$	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$	$5x10^3$
S. mutants	0	0	0	0	0	0.48	0.49	0.53	0.53	0.57
L. acidophilus	0	0	0	0	0	0.20	0.23	0.32	0.36	0.41

Table 5: Zones of Inhibition (mm) of Bacterial Growth of the Rhizome extract of *Zingiber officinale*: Disk Diffusion Technique

Test org.	Petroleum Spirit					Water				
	Concentration (ml)									
	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$	$5x10^3$	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$	$5x10^3$
S. mutants	0	0	0	0	0	4.80	4.90	5.10	5.50	5.70
L. acidophilus	0	0	0	0	0	2.00	2.30	3.20	3.60	4.10

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

The results obtained for the effect of crude ginger extract on cariogenic organisms with special emphasis on *S. mutants* and *L. acidophilus* are recorded in Tables 1, 2, 3, 4 and 5with Table 1 showing the results of the phytochemical analysis of the ginger rhizome extract. The results shows that carbohydrate (sugar), alkaloid, tannins, flavonoids, sterols and glycosides are present in the extract. These classes of compounds are known to have antimicrobial effect against several enteric organisms amongst which are *E. coli, S. faecalis,* a *typhimurim, S. aurens, P. aeruginosa, B. cereus* and *C. perfringes,* Dott, (2008).

Table 2 and 3 shows the average zones of inhibition of the bacterial growth at various concentration in centimetres, and millimetres respectively using cup-plate method.

Tables 4 and 5 shows the average zone of inhibition (cm) and (mm) from Disk diffusion technique. From the tables, water extract showed a minimal amount of inhibition against the test organisms which had been known to be causative agents of dental caries, Nilcole and Hamilton, (2016); Soames and Southam, 2013).

However, the results of petroleum spirit extract did not have any effect on the test organisms which could be trace to the fact that ginger rhizome has both pungent and aromatic components. The pungent components are primarily made up of volatile oil, which has sesquiterpen hydrocarbons and three (3) active compounds *ginger shogoal and zingerone*. Apart from the fact that these three compounds are known to be non-microbial agent, they are also immiscible with water, been gummy and sticky, and so could not have much effect on the test organism as observed by Naveena and Mendritta, (2014).

Furthermore, a cursory glance at the tables depicted a progressive increase in the zones of inhibition of the extract against the test organism, except for the values of 5mls used against *Lactobacillus acidophilus* which recorded 0.82cm and 8.20mm respectively. This discrepancy could be attributed to presence of microbial contamination observed in the plate.

As for the minimum inhibitory concentration, the test organism exhibited slow lag phase when the concentration of the extract was only 1ml. In table 3 *S. mutants* exhibited a semi-exponential lag-phase growth pattern, and this is due to the presence of carbohydrates (sugar) in the extract which is its required growth substrate (Soames and Southam, 2013), Zero, 2004). Another reason could be best on the fact that this organism can synthesize both extra-cellular and intracellular glycan. The implication is that while in the extract, it utilizes the extra-cellular and intracellular glycan to supports its growth; and that gives it the opportunity to formed mutants as its name implies Hanada, B.S. (2010). However, at 3m1, the growth of the organism recorded as sharp retardation.

On the other hand, the effect of the extract on *Lactobacillus* (figure 3) was at more reduced rate at 1ml, but assumed a rapid progression till it was hampered at 3ml concentration. Though it is not known to be complicated as the *S. mutants,* the success of this test organism could be due to catabolite repression normally observed by microorganisms.

Conclusion

This study shows that the extract of crude ginger have antibacterial activities at the range of 3.0 x 10^3ug/ml to 5.0 x 10^3ug/ml. on *S. mutants* and *L. acidophilus.* The results show that carbohydrate (sugar), alkaloid, tannins, flavonoids, sterols and glycosides are present in the extract of ginger.

Recommendation

Base on this, it is suggested that the traditional medicinal use of *Zingiber officinale* should be continue since the plant *Zingiber officinale* despite its antimicrobial properties also has spicy and antioxidant characteristics, which generally enhances salivary flow and viscosity.

That ginger extract should be fortified and incorporated in confectionaries and as a mouth wash as obtained with Trichloropherol (TCP) solutions.

Further research on the class of carbohydrate present in the ginger extract should be elucidated.

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