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Evaluation of a Traditional Decoction Made from *Psidium guajava* and *Zingiber officinale* for Anti Bacterial Activity

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Abstract: A traditional decoction made from the leaves of *Psidium guajava* (guava) and rootstalk of *Zingiber officinale* (ginger) prepared in schnapps (42% v/v ethanol: water) that is widely used in Akoko North community in Ondo State, Nigeria to treat many diseases was evaluated for antibacterial activity on some common bacteria that cause diarrhea in south-west Nigeria. Its activity was compared with that of *Psidium guajava* leaf extract in schnapps without *Zingiber officinale* and *Psidium guajava* leaf extract in water with or without *Zingiber officinale*. The effect of storage temperature on the antibacterial activity of the extracts was also investigated. All the extracts inhibited the growth of all the test organisms which include Enterotoxigenic *Escherichia coli* (ETEC), *Shigella dysenteriae*, *Staphylococcus aureus*, *Salmonella tyhpi* but the leaf extract in schnapps without *Zingiber officinale* gave the highest inhibition on the growth of the test organisms with diameter ranging from 7.0-16.0 mm. These values were comparable with the inhibition mediated by most of the antibiotics used. The inhibitory effect reached climax by the third day and declined to zero level for most of the test organisms by the fifth day. The storage temperatures used (4, 20, 28±2°C) did not have significant effect ($p \geq 0.05$) on the potency of the prepared extracts on the growth of the test organisms. It is being suggested that in the absence of immediate medical attention when one is having running stomach, this decoction may be used as first aid in the treatment of bacterial diarrhea caused by these organisms.

Key words: Guava leaves, ginger rootstalks, schnapps, antibacterial effects, diarrheal bacteria

INTRODUCTION

Diarrhea is a major cause of death in developing nations, especially among pre-school children (Walderman, 1998; Lutterodt *et al*., 1999). Children under three years of age may experience as many as ten episodes of diarrhea yearly (Kosek *et al*., 2002). Although most episodes of diarrhea last less than 7 days and can be effectively controlled with oral rehydration therapy combined with an appropriate diet, often time, the disease kills if there is no prompt attention.

Diarrhea is spread through the faeco-oral route and is particularly favored by poor sanitary conditions, poor storage temperatures of food items and improper cooking of food items (Cheesbrough, 1994; Walderman, 1998).The disease is caused by a number of pathogens such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Clostridium difficile*, *Campylobacter jejuni*, among others (Prescott, 2005).

Uptill today, diarrhea is still a serious problem in most of the rural communities in countries of the developing world. Lack of primary health centers in most of these communities compound this problem. Not only this, some children do not like the taste of oral rehydration solution (unpublished information), so it becomes imperative to search for alternative therapies in these rural communities.

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Actually in most of these communities, many plant species are used as elixirs for treating diarrhea and other disease conditions. The type that is being used in a community depends on oral traditions. In the south-western part of Nigeria, several plants are locally employed of which many are reported to be highly potent, individually and in synergy. For example, Ilori *et al.* (1996) and Adebolu and Salau (2005) reported the growth inhibitory activities of the leaf extracts of *Ocimum gratissimum* on common diarrheal bacteria. In the study done by the latter, the oil was reported to exert greater inhibitory activity on the growth of these organisms. Iwu *et al.* (1999) reported that *Xylopia aethiopica* has anti dysentery effect. *Cryptolepis sanguinolenta* is another plant that has been reported to have marked effectiveness against enteric pathogens (Sawer *et al.*, 1995). So also the methanolic extract of the leaves of *Psidium guajava* (Lutterodt *et al.*, 1999). The plant, *Zingiber officinale* has also been reported to relive travel sickness (Ernst and Pittler, 2000). This plant is majorly used in conjunction with other plants, for example, in Akoko North area of Ondo State, Nigeria, it is added to decoctions of guava in water or local gin or schnapps in treating all kinds of gastrointestinal disturbances.

Based on the interesting nature of traditional medical practice among the local people especially in the south western part of Nigeria in the treatment of diarrhea with herbs and the surprising outcome gotten a times from those practices, it is imperative to look into and explore those ethnomedical practices for knowledge, refinement and possible new discoveries. The objectives of this study therefore, are to assay the traditional decoction made from guava leaves and ginger that is commonly used in some parts of southwest, Nigeria to treat gastrointestinal problems whether it's efficacy is due to antibacterial activity on diarrheal bacteria, to determine the effect of storage temperature on the efficacy of the decoction and to determine the best method of preparation.

MATERIALS AND METHODS

This study was carried out between January and June, 2006 at the Microbiology Research Laboratory, Federal University of Technology, Akure, Nigeria.

Bacterial Strains

The bacterial isolates used were collected from Microbiology Laboratory, University College Hospital, Ibadan; Nigerian Institute for Medical Research (NIMR); Lagos State University Teaching Hospital (LASUTH) and Lagos University Teaching Hospital (LUTH). The organisms include *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella enteritidis*, Enterohemorrhagic *Escherichia coli*, Enterotoxigenic *Escherichia coli*, *Shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus*.

Guava Leaves and Ginger Roots

The guava leaves that were used were gotten from the vicinity of the University while the ginger rootstalks were bought at the local market in Akure. The schnapps that was used for soaking the leaves was bought from a departmental shop in town.

Preparation of Guava Leaf Decoctions Containing Ginger

Freshly plucked leaves of *Psidium guajava* were washed with clean water and sun dried for three days. One hundred grams of the crushed leaves was introduced each into three different sterile conical flasks (500 mL) respectively and the flasks were each filled with 350 mL schnapps (42% v/v ethanol: water). Ginger (20 g) that was washed with hypochlorite solution and crushed by using a sterile pestle and mortar was then added to each of the flasks containing the guava decoction at the ratio 1:5. The flasks were kept at three different temperatures, the refrigerator (4°C), water (20°C) and room temperature (28±2°C). Another batch was prepared following the same procedure but instead of using schnapps, sterile distilled water was used. These were also kept at the different temperature regimen.

Preparation of Guava Leaf Extracts

Exactly as above, a second set of guava leaves extract was prepared but without the addition of ginger. These were also kept at the chosen storage temperatures.

Assay for Antibacterial Activity of the Different Guava Extracts Prepared

One milliliter of each of the test organism grown overnight in peptone water was transferred into different sterile petridishes. Nutrient agar (20 mL) was then poured on these inocula and the plates swirled for even dispersion of the organisms in the agar. After the plates had solidified, a 6 mm diameter cork borer was used to make 6 wells into each plate and 0.3 mL of the different extracts prepared was introduced, different one per different well. Schnapps alone which served as one of the controls was put in one of the wells so also is sterile distilled water which served as the second control was put in one of the wells. All the plates were incubated at 37°C for 24 h. This assay was conducted at regular intervals of 24 h until a marked decline was noticed in the potency of the extracts in inhibiting the growth of the test organisms. Zones of clearance round each well means inhibition and the diameter of such zones was measured with a ruler.

Antibiotic Sensitivity Assay

The test organisms were prepared as above but instead of using the prepared guava leaves extracts, standard commercial antibiotic disks were used.

RESULTS

Antibacterial Activity of the Decoctions

All the prepared *Psidium guajava* decoctions with or without *Zingiber officinale* inhibited the growth of all the test organisms used, namely; *Bacillus cereus*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella enteritidis*, *Shigella dysenteriae*, *Staphylococcus aureus*, enterohemorrhagic *Escherichia coli* and enterotoxigenic *Escherichia coli*. The highest growth inhibitory activity was achieved with the extract prepared from the dried leaves of *Psidium guajava* in schnapps without the addition of *Zingiber officinale*. The zones of inhibition ranged from 7.0 to 16.0 mm as compared to the one mediated by the combination of *Psidium guajava* leaves and crushed *Zingiber officinale* soaked in schnapps which ranged from 4.0 to 12.0 mm. The addition of ginger to the guava leaf extract caused a decline in potency of the extract on all the test organisms except *Staphylococcus aureus* and *Salmonella typhi* whose growth inhibition remained the same (Table 1).

Antibiotic Sensitivity Pattern of the Test Organisms

The result of the antibiotic sensitivity pattern of the test organisms to conventional antibiotics showed that only ofloxacin and ciprofloxacin inhibited the growth of all the test organisms. Antibiotics like ampiclox and cotrimoxazole did not inhibit the growth of any of the test organisms while the other antibiotics used inhibited the growth of only a few of the organisms (Table 2). When these results were compared with that observed with the prepared guava leaves extracts for each test organism, ciprofloxacin gave the highest growth inhibition for organisms such as *Bacillus cereus* (21.0 mm), *Staphylococcus aureus* (24.0 mm), *Salmonella enteritidis* (22.0 mm), *Salmonella typhi* (22.0 mm), EHEC (14.0 mm) and ETEC (21.0 mm). For *Salmonella paratyphi* A however, guava leaves extract in schnapps gave the highest growth inhibition (16.0 mm) while for *Shigella dysenteriae*, ofloxacin gave the highest growth inhibition (21.0 mm).

Effect of Storage Temperature on the Antibacterial Activity of the Leaf Extracts

The different storage temperatures used, room temperature (28±2°C), water bath (20°C) and refrigeration (4°C) did not prolong the days of efficacy of the prepared guava leaves extract in

Table 1: The antibacterial activity of different types of *Psidium guajava* leaf extracts prepared on the test organisms

	Diameter of zones of inhibition (mm)							
Types	A	B	C	D	E	F	G	H
1	8.0	12.0	12.0	8.0	10.0	8.0	4.0	4.0
2	13.0	13.0	13.0	9.0	10.0	16.0	7.0	8.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	5.0	3.0	4.0	4.0	6.0	4.0	4.0	4.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

A = *Bacillus cereus*, B = *Staphylococcus aureus*, C = *Salmonella enteritidis*, D = *Shigella dysenteriae*, E = *Salmonella typhi*, F = *Salmonella paratyphi* A, G = Enterohemarrhagic *E. coli*, H = Enterotoxigenic *E. coli*, 1 = Guava leaves in schnapps + ginger, 2 = Guava leaves in schnapps alone, 3 = Guava leaves in water + ginger, 4 = Guava leaves in water alone, 5 = Control 1, 6 = Control 2

Table 2: Antibiotic sensitivity patterns of the test organisms to common antibiotics

	Diameter of zones of inhibition (mm)							
Antibiotics	A	B	C	D	E	F	G	H
Ciprofloxacin	21.0	24.0	22.0	12.0	22.0	13.0	14.0	21.0
Ofloxacin	19.0	13.0	6.0	21.0	9.0	7.0	11.0	2.0
Norfloxacin	0.0	0.0	17.0	11.0	21.0	0.0	8.0	7.0
Tetracycline	0.0	0.0	0.0	0.0	15.0	0.0	7.0	7.0
Amoxycillin	0.0	0.0	0.0	0.0	18.0	0.0	8.0	11.0
Chloramphenicol	0.0	0.0	6.0	0.0	9.0	0.0	7.0	0.0
Cephalexin	4.0	2.0	0.0	0.0	0.0	0.0	6.0	2.0
Ampicillin	0.0	0.0	0.0	0.0	0.0	0.0	9.0	0.0
Gentamycin	0.0	6.0	0.0	0.0	0.0	0.0	9.0	4.0
Nitrofurantoin	0.0	0.0	0.0	0.0	0.0	0.0	7.0	7.0
Floxapen	4.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0
Augmentin	0.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0
Erythromycin	3.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0
Clindamycin	0.0	16.0	0.0	0.0	0.0	0.0	0.0	0.0
Ampliclox	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cotrimoxazole	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

A = *Bacillus cereus*, B = *Staphylococcus aureus*, C = *Salmonella enteritidis*, D = *Shigella dysenteriae*, E = *Salmonella typhi*, F = *Salmonella paratyphi* A, G = Enterohemarrhagic *E. coli*, H = Enterotoxigenic *E. coli*

Table 3: Effect of storage temperature on the antibacterial activity of the prepared extracts on the test organisms

		Diameter of zones of inhibition (mm)							
Storage temperature	Day	A	B	C	D	E	F	G	H
4°C	1	5.0	10.0	6.0	5.0	9.0	13.0	5.0	8.0
	2	9.0	10.0	17.0	14.0	10.0	10.0	6.0	6.0
	3	14.0	10.0	10.0	4.0	4.0	4.0	14.0	5.0
	4	7.0	4.0	4.0	0.0	0.0	1.0	8.0	1.0
	5	1.0	1.0	0.0	0.0	0.0	0.0	2.0	0.0
20°C	1	4.0	3.0	4.0	11.0	15.0	13.0	6.0	6.0
	2	12.0	8.0	6.0	8.0	8.0	6.0	14.0	8.0
	3	15.0	5.0	10.0	7.0	4.0	4.0	6.0	7.0
	4	6.0	1.0	5.0	3.0	0.0	0.0	2.0	4.0
	5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
28±2°C	1	13.0	13.0	4.0	4.0	8.0	16.0	6.0	8.0
	2	11.0	8.0	14.0	6.0	10.0	10.0	8.0	10.0
	3	4.0	11.0	10.0	7.0	17.0	4.0	14.0	5.0
	4	0.0	6.0	6.0	4.0	8.0	0.0	9.0	1.0
	5	0.0	2.0	1.0	0.0	1.0	0.0	1.0	0.0

A = *Bacillus cereus*, B = *Staphylococcus aureus*, C = *Salmonella enteritidis*, D = *Shigella dysenteriae*, E = *Salmonella typhi*, F = *Salmonella paratyphi* A, G = Enterohemarrhagic *E. coli*, H = Enterotoxigenic *E. coli*

inhibiting the growth of the test organisms used, the efficacy waned by day 4 (Table 3). For some of the organisms however, for example *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella paratyphi* A

and EHEC, guava leaves extract that was kept at room temperature of 28±2°C gave the highest growth inhibition after 24 h of preparation while for *Salmonella enteritidis* and *Shigella dysenteriae* it was the extract stored at 20°C that gave the highest inhibition after 24 h of production.

DISCUSSION

In this study, the antibacterial activity of a traditional decoction made from the dried leaves of *Psidium guajava* and rootstalk of *Zingiber officinale*, an important decoction being used by traditional healers especially in Akoko land, Ondo State, Nigeria to treat various ailments was investigated for antibacterial activity on the growth of common bacteria that cause diarrhea in south western part of Nigeria. The effectiveness was compared with that of conventional antibiotics, guava leaves extract alone prepared with schnapps and guava leaves extract prepared in water with or without the addition of ginger. It was discovered that all the prepared guava leaves extracts used in this investigation inhibited the growth of all the test bacteria used except the guava leaves extract in water to which ginger was added. The growth inhibition mediated by the guava leaves extracts without ginger agrees with the findings of Lutterodt *et al.* (1999) that guava leaves in 75% methanol was effective in inhibiting the growth of *Salmonella typhi*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Vibrio cholerae* and *Staphylococcus aureus*. The choice of schnapps in this study instead of 75% methanol is because the local people use schnapps or local gin to prepare the decoction.

The growth inhibition that was observed with the prepared guava leaves extract on the selected organisms when compared with that of standard antibiotics showed that the extract effectively compete with most of the antibiotics used and in some cases gave superior inhibition (Table 1 and 2). For instance, the extract gave the highest inhibition on the growth of *Salmonella paratyphi A* (16.0 mm) compared to that of all the antibiotics used including ciprofloxacin which mediated an inhibition of only 13.0 mm, although this waned as the day of preparation increased and by day 4, the extract was no longer effective against the organism. So it is being suggested that the extract should be prepared only when needed and should not be kept for more than 4 days. The short-term effectiveness might likely have been due to the easy degradable nature of the bioactive components of guava leaf extract as suggested by Sanches *et al.* (2005).

This research has been able to show that the traditional decoction produced from dried leaves of *Psidium guajava* and rootstalk of *Zingiber officinale* has antibacterial activity against the test bacteria used. However to obtain greater activity, the extract from the dried leaves of *Psidium guajava* alone in schnapps should be used.

Based on these observations therefore, it is being suggested that ginger should not be added to the decoction because it reduced the antibacterial activity of the decoction and guava leaves decoction that has exceeded 4 days of preparation should not be used.

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