WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 6, 722-732. Research Article ISSN 2277– 7105

ANTIMICROBIAL PROPERTIES OF SPICES: Ginger *(Zingiber officinale);* Bayleaf *(Laurus nobilis)* AND THEIR FORMULATION AS MIXED SPICE

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Article Received on
04 March 2019,

Revised on 24 March 2019,
Accepted on 15 April 2019,

DOI: 10.20959/wjpr20196-14770

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ABSTRACT

Aqueous extracts of two spices namely *Zingiber officinale* (Ginger) and *Laurus nobilis* (Bayleaf) used primarily in food flavoring in homes were studied for their antimicrobial activities against some selected Gram positive (*Staphylococcus aureus)* and Gram negative *(Salmonella sp and Escherichia coli)* pathogenic microorganisms as well as fungi*(Candida albican*). The antimicrobial activity of the spice extracts was determined using the agar disc diffusion method in which their zones of inhibition was measured after 18hours of incubation. The extracts were impregnated into the discs at different concentrations (10, 5 and 2.5mg/dl). The spice extracts showed varied levels of antimicrobial activities against the microorganisms. Extracts of Ginger and Bayleaf showed good activity against *E.coli* and *Staph. aureus* at all concentrations, little activity on *C. albican* and no activity on *Salmonella sp.* The formulation as mixed spice showed good activity on *E.coli*, it also showed activity on *S.aureus* and *Salmonella sp* at concentration 10mg/dl and 5mg/dl and no activity on *C. albican*. The plant extracts showed varing MIC values against the microbes with the minimum value of 1.25mg/ml on *E. coli* for Ginger and the formulated mixed spice. The MBC value was recorded within the range of 1.25mg/ml for *E. coli* to 5.0mg/ml. This present study shows that Ginger and Bay leaf possess antimicrobial components which could be used as substitutes for antibiotics.

KEYWORDS: Antimicrobial activity, Bayleaf, Ginger, mixed spice.

1.0 INTRODUCTION

Spice is a seed, fruit, root, barinfectious disease and why the use of spice is prominent in meat which is particularlysusceptible to spoiling (Thomas et al, 2012). Spices are sometimes use in medicine or other plant substance primarily used for flavoring, coloring or preserving food. Spices are distinguished from herbs which are leaves, flower or stems from plants used for flavoring or garnish. Sometimes, spices may beground into powder for convenience. Many spices have antimicrobial properties. This may explain why spices are mostly used in warmer climate which have moreinfectious disease and why the use of spice is prominent in meat. Spices are sometimes used in medicine, religious rituals, cosmetics or perfumes production or as vegetables.

However the desire to obtain these products was all cultural, social and economic phenomenon known as the **spicetrade.** The manufacture of spices made from mixture of several of them with other ingredients begen in the 19th century(Regencyspices.hk/spicetrade, 2014). One issue with spice today is the dilution, where spices are blended to make inferior quality powdered spices by including roots, stems and other admixture In the production of spice powder(Regencyspices.hk/spicetrade, 2014).

Recently, most spices have been identified as sources of various phytochemicals of which many possess powerful medicinal properties(Larson, 1988; Vehoghi et al,1998; Kahkoren et al, 1999; Dragland et al, 2003). Spices have tried, tested, and trusted medicinal values and a profound effect on general health. Traditional spices used as part of diet have holistic effect on human health. Spices are mostly consumed by man unawares of its medicinal importance. The benefits of spices have been ignored by scientists if not up to recently.

Several scientific reports describes the inhibitory effects of spices on a variety of microorganism (Akgul and Kivanc, 1988). Gould (1995), has emphasized the possible use of spices and its derivatives in a new perspective of food conservation called “Natural Antimicrobial System”. Microbial resistance is influenced by the constituents of this spices. Study in the past decade has confirmed that the growth of both gram positive and gram negative foodborne bacteria, yeast, and mold can be inhibited by garlic, onion, cinnamon, cloves, thyme, sage, and other spiceshence this study is aimed atdetermining the antimicrobial properties of Ginger*(Zingiber officinale),* Bayleaf*(Laura nobilis)* and their dilution as mixed spice on some selected microorganisms with these objectives: To Isolate the microorganisms of interest in the microbiological laboratory, determine the antimicrobial

effectiveness of the four spices and their dilution as mixed spice against strains of *Staphylococcus aureus, Salmonella*sp, *Candida albican, Escherichia coli,* to study the inhibitory effects of the four spices on the microorganism of concern**.**

2.0 MATERIALS AND METHODS

Laboratory facilities and reagents were sourced from the Science Technology laboratory, Federal polytechnic Nekede Owerri, Imo State.

2.1 The Study Area

This study was conducted in the Science Technology laboratory, Federal polytechnic Nekede Owerri, Imo State.

METHODOLOGY

2.2 Collection And Preparation Of Test Samples

The fresh forms of Ginger*(Zingiber officinale)*; Bayleaf*(Laurusnobilis)* was purchased from Relief market Owerri. They were washed in one litre of distilled water, sliced and air dried at 50^0C in a hot air oven. Dried samples were ground to powder using a mechanical grounder. The powdered samples obtained were stored in a clean brown bottles at room temperature until needed for use.

25g of each of the fine grounded powder of the test samples was measured with an electronic weighing balance. The measured out samples was mixed together. This mixture served as the mixed spice. The mixture was stored in a clean brown bottle at room temperature until needed.

2.3 Collection of Test Organisms

The test organisms used in this research consist of *Staphylococcus aureus, Candida albica, Salmonella specie, Escherichia coli,* isolates was collected from the Department of Microbiology/Biochemistry Federal Polytechnic Nekede, Owerri.

2.3.1 Identification of The Test Organisms

The test organisms obtained on the incubated plates were identified on the basis of cultural, morphological and biochemical characteristics.

2.4 Preparation of Aqueous Extract of Test Samples

The method of Olayemi and Opaleya (1999) was adopted for the extraction of the spice. This was carried out by measuring 1.5g of each of the fine grounded powder of the Test samples: Ginger*(Zingiber officinale);* Bayleaf*(Laurusnobilis)* and their formulation as mixed spice on an electronic weighing balance. This was dispensed into separate beakers containing 13.5ml of distilled water. This was soaked for 72 hours after which the solution was carefully filtered with filter paper into a sterilized conical flask. The filtrates obtained was stored in the refrigerator at the temperature of 4^0C until use.

2.5 Preparation of Antimicrobial Disc Using Aqueous Extract of The Test Sample

The disc 6mm in diameter was punched out from a sheet of good quality filter paper(Whatman No 1).This disc was matched with numbers or letters or color codes for easy identification. The disc was placed in a petri dish, space of 2-4mm was left between them and was sterilized in the hot air oven at 70^0C for 1hour. The solution of the tests was prepared in the desired concentration(100ug/ml). Each disc contained 20ul(0.02ml) of the prepared solution. The solution was sterilized by filtration. After cooling, 20ul of the solution was pipetted onto each disc aseptically. The discs were dried in a hot air oven at temperature of 40^0C. The disc (prepared)were placed in an airtight container and was storedat 2-8^0C until neede use.

2.6 Inhibitory Test For Aqueous Extract of Test Samples

The Agar disc diffusion method was used. The test organism was standardized with peptone broth and was incubated for 2-5 hours in water bath until turbidity occurs. Samples of the test organisms were spread unto the surface of the Nutrient Agar medium. This was left for few minutes for surface absorption into the agar, the prepared antimicrobial discs were applied. The plates were incubated at 37^0C for 24hours and zones of inhibition will be observed.

2.7 Inoculation of Plates

About 7g of Nutrient Agar was weighed and dissolved in a conical flasks containing 250ml of distilled water. The solution was sterilized in an autoclave at temperature of 121^0C for 15minutes at 15psi. The sterile agar was cooled to about 45^0C before pouring into different sterile petri dishes. The agar plates were seeded by placing a loopful of the inoculum on each of the agar plate as grouped and spreaded all over with a glass rod spreader. The appropriate test sample impregnanted disc was placed on the surface of the agar. The discs were placed at least 22mm(2cm) apart from each other and at least 14mm away from the edge of the plate.

Each disc was gently pressed on the agar surface using forceps to provide uniform contact with the surface. The plates were incubated at 37^0Cfor 16-18hours. Appearance of clear zones around the disc, is indicative of inhibition.

2.7.1 Measuring Zones of Inhibition

The zones of inhibition is a clear zone that is free of life seen on the agar surface indicating that the antimicrobial agent is effective.

After overnight incubation, the plates were examined for zones of inhibition. The zones of inhibition were measured with metre rule. The diameter of the various zones of inhibition obtained were measured by placing and holding the ruler on the underside of the petridish. A direct reading in millimetres was made. The size of the antimicrobial sample was included in the measurement. Readings of multiple zones were taken to obtain an average size.

2.8 Determination of Minimum Inhibitory Concentration of The Extracts of *Zingiber Officinale, Laura Nobilis* And The Formulated Mixed Spice

Prior to testing, each isolate was subcultured from the stock cultures by inoculating into 9mlpeptone broth and incubating at 16hours at 37^0C. Cultures obtained were diluted to10^5 cfu/ml in correspondence to Mc farlands standard; the maximum to produce confluent growth at inoculation positions. The mic of active extracts was evaluated by the tube dilution method. The MIC's of all the extract to various concentrations ranging from 5.0 -1.25mg/ml. Controls were included. After overnight incubation at 37^0C, the tubes were examined for turbidity indicating the growth of microorganisms.

The lowest solution of the extract that inhibited the growth of the microorganismas detected by the lack of visual turbidity matching the negative control) was designated as the minimum inhibitory concentrations

3.9 Determination of The Minimum Bactericidal Concentrations of The Spice Extracts

The minimum bactericidal concentration was determined by subculturing the test dilution which showed no visible growth in MIC onto a freshly prepared nutrient agar media. The plates were incubated further for 18hours at 37^0C. The highest dilution that yielded no single bacteria colony on the nutrient agar plates was taken as the MBC's.

3.0 RESULT

The result of the antimicrobial properties of Ginger*(Zingiber officinale)*, Bayleaf*(Laura nobilis)* and their dilution as mixed spice on some selected microorganisms is as represented in the tables and figures below:

Table 1A: The colonial and morphological characteristics of the pure cultures of the test organisms isolated.

Organism	Colonial and Morphological characteristics
Escherichia coli	Round, Large, Raised, Entire, Cream, Slimy, Translucent small growth.
Staphylococcus aureus	Round, Flat, Entire, Yellow, Translucent, Puntiform Growth.
Salmonella sp.	Small cream, Round, Flat, Entire, Translucent, Growth
Candida albican	Creamy Ovoid Colonies

Table 1B: The biochemical reactions of the pure cultures of the test organisms isolated. *Candida albican* was identified through Microscopy.

Gram reaction	Catalase	Coagulase	Motility	Citrate	Indole	Vogues proskauer	Methyl Red	Probable organism
Negative rod	+	ND	+	+	+	-	+	E. coli
Positive Cocci	+	+	-	+	-	+	-	S. aureus
Negative Rod	+	ND	+	+	-	-	+	Salmonella sp.

KEYS:

+ = Positive

- = Negative

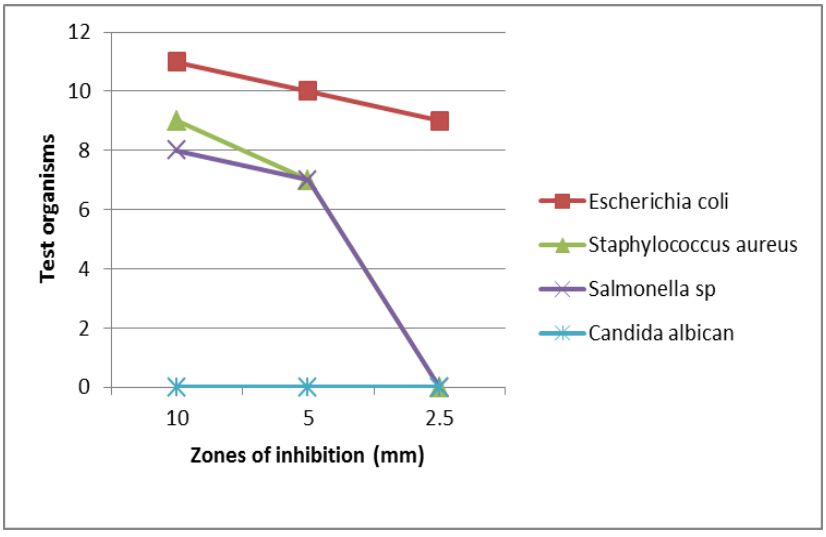

Fig 1: Zones of inhibition of aqueous extracts of *zingiber officinale* on test organisms.

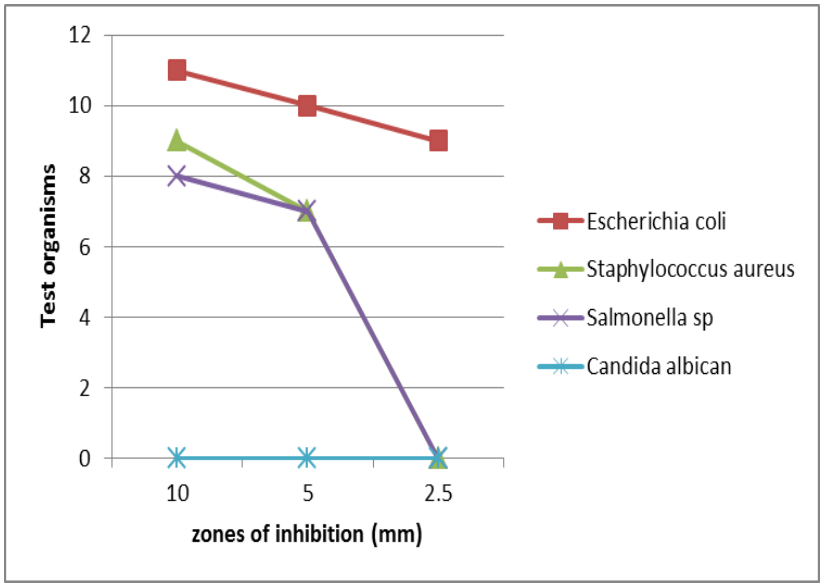

Fig 2: zones of inhibition of aqueous extract of *laurus nobilis* on test organisms.

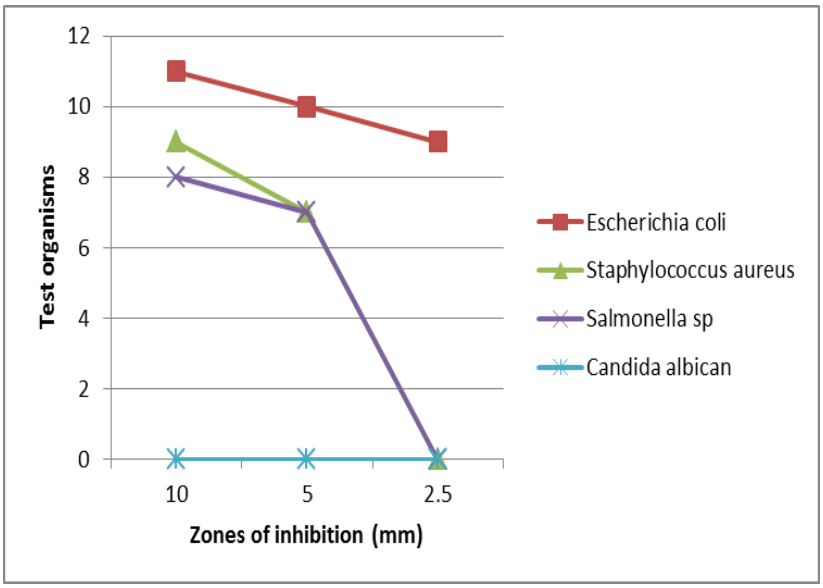

Fig 3: Zones of inhibition of the formulated mixed spice on test organisms at different concentrations.

4.3 Minimum Inhibitory Concentrations of The Spice Extracts

Table 4.3A Minimum Inhibitory Concentration of Extracts Of *Zingiber officinale* On Test Organisms.

Test Organisms	Concentrations (mg/ml)			
	5.0	2.5	1.25	MIC
Escherichia coli	-	-	-	1.25
Staphylococcus aureus	-	-	+	2.5
Candida albican	-	+	+	5.0

Table 4.3B Minimum inhibitory concentration of extracts of L*aurus nobilis* on test organisms.

Test Organisms	Concentrations (mg/ml)			
	5.0	2.5	1.25	MIC
Escherichia coli	-	-	-	1.25
Staphylococcus aureus	-	-	+	2.5
Candida albican	-	+	+	5.0

Table 4.3C: Minimum inhibitory concentration of the formulated mixed spice on test organisms.

Test Organisms	Concentrations (mg/ml)			
	5.0	2.5	1.25	MIC
Escherichia coli	-	-	-	1.25
Staphylococcus aureus	-	-	+	2.5
Candida albican	-	-	+	2.5

KEYS:

+ = Growth; - = No Growth

4.4 Minimum Bactericidal Concentrations Of The Spice Extracts

Table 4.4A: Minimum bactericidal concentration of *zingiber officinale* on test organisms.

Test Organisms	Concentrations (mg/ml)			
	5.0	2.5	1.25	MIC
Escherichia coli	-	-	+	2.5
Staphylococcus aureus	-	-	+	2.5
Candida albican	-	+	+	5.0

Table 4.4b: Minimum bactericidal concentration of *laurus nobilis* on test organisms.

Test Organisms	Concentrations (mg/ml)			
	5.0	2.5	1.25	MIC
Escherichia coli	-	-	+	2.5
Staphylococcus aureus	-	-	+	2.5
Candida albican	-	+	+	5.0

Table 4.4C: Minimum bactericidal concentration of the mixed spice extract on test organisms.

Test Organisms	Concentrations (mg/ml)			
	5.0	2.5	1.25	MIC
Escherichia coli	-	-	-	1.25
Staphylococcus aureus	-	-	+	2.5
Candida albican	-	+	+	5.0

KEYS: + = Growth, - = No Growth

DISCUSSION

The result of this study showed that isolates behaved differently in their sensitivities to the different extracts added to their growth medium.

Furthermore, in comparison of the result obtained from the two spices, bayleaf and ginger, it could be deduced that they both had a similar effect on the isolates. The mixed spice had an effect on *Salmonella* sp unlike the single spice. This could be as a result of enhancement of the constituents of this spices by each other on mixing thus achieving a positive synergitic effect.

The antimicrobial activity of ginger and bayleaf may be attributed to the presence of antimicrobial substances such as zingiberol, zingiberine, bisabolene and 1-8, cineole, sabinene, limonene, linalool (Michael derrida, 1999; Melvin et al., 2009; Said and Hussien, 2014).The rhizome of ginger contains pungent vanillyl ketones including gingerol and paradole, etc (Dougles and Miller, 1999; Melvin et al., 2009). Gingerol is a mixture of crystal gingerone and it is the major cause of acidity of ginger and plays a vital role in inhibiting bacteria such as *S. aureus, Trichomonas vasmalis* and help to cure bacterial vaginosis and skin diseases (Michael derrida, 1999; Melvin et al., 2009).

The results obtained in this study corroborate with the report of Roy et al (2006), which explains that bioactive compounds of ginger rendering antimicrobial activity are volatile in nature and that the antimicrobial actiity of ginger extract decreases upon storage. In addition to water, methanol and ethanol should also be used for extract preparation as de Boer et al, (2005) reported that bioactive compounds show better solubility in water miscible organic solvents.

According to earlier reports ginger and bayleaf have traditional dietary and medicinal applications as an anti-infective agent. In vitro evidence of the antimicrobial activity of fresh and freeze dried ginger and bayleaf extracts against many bacteria, fungi and viruses supports these applications. Organosulfur compounds and phenolic compounds have been reported to be involved in the antimicrobial activities of ginger and bayleaf.

CONCLUSION

This study emphasizes ginger and bayleaf extract as having antimicrobial activities on some pathogenic microbes isolated from human samples hence use of ginger and bayleaf as a natural supplement is considered a healthy choice.

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