



STUDY ON THE PHYTOCHEMICAL AND INVITRO ANTIBACTERIAL ACTIVITY OF SOME SPICE EXTRACTS ON SOME BACTERIA ISOLATED FROM MEAT PRODUCTS

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ABSTRACT

The invitro activity of ethanolic and water extracts of some spices on some bacteria isolated from kilishi (a traditional meat snack) was investigated by disc diffusion method. Disc concentrations of 10, 100, 1000, 2000 and 3000µg were used. The result showed that the ethanolic extract of ginger was active against Salmonella at disc concentrations of 100, 1000, 2000 and 3000µg, with zone diameters of 8, 19, 26 and 30mm respectively. The ethanolic extract of cloves was active against Salmonella spp at disc concentrations of 10, 100, 1000, 2000 and 3000µg, with zone diameters of 13, 24, 25, 26 and 26mm respectively. The ethanolic extract of West African Black pepper was active against Salmonella spp at disc concentrations of 10, 100, 1000, 2000 and 3000µg, with zone diameters of 12, 14, 20, 22 and 23mm respectively. The ethanolic extract of pepper was active against Staph aureus at disc concentrations of 10, 100, 1000, 2000 and 3000µg, with zone diameters of 11, 12, 16, 22 and 34mm respectively. The ethanolic extract of cloves was active against Staph aureus at disc concentrations of 10, 100, 1000, 2000 and 3000µg, with zone diameters of 12, 13, 13, 17 and 22mm respectively. There was no activity on E. coli. The mixture of all the extracts however was not active on all the test organisms at the above mentioned disc concentrations. Each of the extracts contains atleast three of such secondary metabolites as alkaloids, tannins, flavonoids, saponins, reducing sugars and steroids. It could be recommended that bioactivity of spices should not be relied upon in the reduction of microbial populations of meat products. The ethanolic fractions of the spices should be put to further pharmacological in vivo tests to elucidate their potency, toxicity and efficacy.

Key words: Phytochemical, bioactivity, spices, bacterial isolates, kilishi.

INTRODUCTION

Production and consumption of traditionally prepared meat products is a common practice in many parts of the world (Vilar *et al* 2000). Kilishi is one of these products. It is a sun dried spiced and grilled meat snack that can be kept for considerably long time without getting spoiled due to its dry nature. During the production of the various traditional meat products, spices are applied at one point or the other.

Spices could be defined as the natural vegetable products or mixtures thereof, without any extraneous matter that is used for flavoring, seasoning and imparting aroma to foods (International Standard Organisation, 1972). These spices used in the production of *Kilishi*, are ginger, pepper, cloves and West African black pepper. These spice ingredients are reduced to powder and mixed in water, so that the dried meat is dipped into the liquid spice mixture, which also consists of groundnut, salt and seasoning.

Spice ingredients are thought to have some antimicrobial activities, and yet meat treated with spices have high microbial load (Shamsuddeen, 2009). There is therefore the need to investigate the antibacterial activities of extracts from the various

spice ingredients, on bacteria isolated from the meat products.

MATERIALS AND METHODS

Isolation of the test organisms from meat snack (*kilishi*)

This was carried out according to Food and Agriculture Organization (1979). For *E. coli*, EMB plates were streaked with inoculum from from gas positive tube of lactose broth. Colonies that showed green metallic sheen were streaked on to agar slant and kept for biochemical characterization (indole and methyl red test). For *Staphylococcus*, Plates of mannitol salt agar were inoculated and incubated at 35°C for 24hrs. Following incubation, mannitol fermenting organisms which showed a yellow zone surrounding their growth were isolated on to agar slants for biochemical tests (coagulase and catalase tests). For *Salmonella* spp a loopful of inoculum from the enrichment medium (selenite cystine medium) was streaked onto brilliant green agar plates and incubated at 37°C for 24hrs. The colonies were gram-stained and tested for motility. The gramnegative motile rods were inoculated on to nutrient agar slant for subsequent biochemical test (Kligler's iron agar test).

Collection and Identification of the spices

Up to 1000g of the spices were bought from Kurmi market of Dala Local Government, Kano State, Nigeria. These are confirmed at the herbarium section of Department of Biological Sciences by herbarium curator. They were reduced to powder using pestle and mortar.

Extraction of ethanolic and water fractions from the spices.

Three hundred grams (300g) of each spice ingredient powder was suspended in water, and in 99% ethanol (500ml) in separate flasks. The suspensions were kept at room temperature and left 14 days with regular shaking. The suspensions were then filtered, and the solvents were removed (both water and ethanol) by evaporation to dryness at room temperature (Fatope *et. al.* 1993). These gave the extract used in preparing the stock solutions for further analysis.

PHYTOCHEMICAL SCREENING OF THE SPICE EXTRACTS

Test for alkaloids

This was carried out according to the method described by Ciulci, (1994). To 1.0ml of each extract in two separate test tubes, 3 drops of Dragendoff's reagent was added. An orange red precipitate/turbidity with Dragendoff's reagent, would indicate the presence of alkaloids.

Test for flavonoids

This was carried out according to the method of Sofowora, (1993). A piece of magnesium ribbon was added to 4mg/ml of each extract. This was followed by the addition of concentrated hydrochloric acid (HCl), drop wise. Crimson to magenta colour indicated the presence of flavonoids.

Test for saponins

This was carried out according to the method of Brain and Turner (1975). Half gram (0.5g) of each extract was placed in a test tube and then 0.5ml of distilled water was added. The tube was then shaken vigorously. A persistent froth that lasted for at least 15mins indicated the presence of saponins.

Test for reducing sugars

This was carried out according to the method of Brain and Turner (1975). One milliliter (1ml) of stock solution of each extract was diluted with 2ml of distilled water, followed by the addition of Fehling's solution (A+B) and the mixtures warmed. Brick red precipitate at the bottom of the test tubes indicated the presence of reducing sugars.

Test for steroids

This was carried out according to the method described by Ciulci (1994). Two grams (2g) of each

extract was evaporated to dryness. The residues were dissolved in acetic anhydride, and chloroform was then added. Concentrated sulphuric acid was then added by the side of the test tube. A brown ring at the interphase of the two liquids and the appearance of violet colour in the supernatant layer indicated the presence of steroids.

Test for tannins

This was carried out according to the method described by Ciulci, (1994). Solutions of the extracts were made with distilled water and 3 drops of 5% ferric chloride solution was added. A green-black or blue-black colouration indicated the presence of tannins.

BIOASSAY

Preparation of extracts impregnated paper discs.

Two grams (2g) of each of the extracts were dissolved in 2ml of appropriate diluent (water for water extract and dimethyl sulfoxide (DMSO) for ethanolic extract), to yield 1.0g/ml (1,000,000µg) solution. This was labeled as the stock solution. From the stock solution 0.1ml was transferred in to a bijou bottle containing 0.9ml diluent, to effect 10 times dilution which gave a concentration of (100,000µg/ml). Subsequently 0.1ml was transferred in to another bottle containing 0.9ml diluent which gave a concentration of 10,000µg/ml and this was further diluted to yield 1,000µg/ml, 100µg/ml and 10.0µg/ml, on pro-rata basis. One hundred discs (100) discs, 6.0mm diameter of Whatman No. 1 filter paper were impregnated with the extracts to arrive at concentrations 1000, 100 and 10µg/disc respectively. Greater disc potencies of 2,000, and 3,000µg/disc were also prepared and stored in refrigerator before use (Deeni and Hussein, 1991)

Growth Media

Plates of Mueller Hinton agar (Oxoid) were prepared according to the manufacturer's instructions. Excess moisture was removed by drying in agar dryer for 15 minutes.

Preparation of Turbidity Standard

One per cent (1% v/v) solution of sulphuric acid was prepared by adding 1ml of concentrated (H₂SO₄) into 99ml of water. One per cent (1% w/v) solution of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride in 50ml distilled water. Barium chloride solution (0.6ml) was added to 99.4ml of sulphuric acid solution to yield 1.0% w/v barium sulphate suspension. The turbid solution formed was transferred into a test tube as the standard for comparison (Cheesebrough, 2000).

Standardization of inoculum

Using inoculation loop, enough material from an overnight culture of test organisms that is *E. coli*, *Salmonella* and *Staphylococcus aureus* (isolated from meat) were transferred into a tube containing about 2.0 ml normal saline, until the turbidity of the suspension matched the turbidity of the standard (1% barium sulphate) (Cheesebrough 2000).

Sensitivity testing of the isolates to the extracts

Two loopfuls of the standard inoculum were evenly streaked on to the plates in duplicates. Discs of different concentrations as well as the control discs (impregnated with only diluent) were placed firmly on the surface of the medium (Mueller Hinton agar) by means of sterile syringe needle at about 40mm apart. The plates were incubated at 37°C for 24 hours. Diameters of the zones of inhibitions were measured with ruler and the mean recorded to the nearest (mm).

RESULTS

Results of the physical characteristics and phytochemical analysis of the spice ingredients are as

presented in Tables 1 and 2 below. In Table 1, the extracts have different colours ranging from red, brown to dark brown, with some oily and some gummy in texture, while all have a spicy smell. In Table 2, all the extracts except water extract of West African black pepper contained alkaloids, flavonoids and reducing sugars. Saponins were detected in all the extracts except ethanolic extract of West African black pepper. Steroids were detected in water extracts of pepper, ethanolic and water extract of ginger, and water extract of cloves, while in the others no steroids was detected. Tannins were detected in pepper water extract, Ginger water extract, cloves ethanolic and water extract then West African black pepper water extract.

The results of the bioactivity of the extracts on the test organisms is as presented in Tables 3 and 4, where the ethanolic extracts of cloves, ginger and WABP of the spices were active on *Salmonella* spp and also the ethanolic extracts of pepper and cloves were active on *Staphylococcus aureus*. The mixture of extracts from all the spices how ever was not active against the test organisms at the disc concentrations used.

Table 1: Some physical characteristics of the extracts from spices

| Extract | Colour | Texture | Solubility | Smell |
|----------------|------------|---------|------------|-------|
| Pepper ethanol | Red | Oily | DMSO | Spicy |
| Pepper water | Dark brown | Oily | Water | Spicy |
| Ginger ethanol | Brown | Gummy | DMSO | Spicy |
| Ginger water | Dark brown | Gummy | Water | Spicy |
| Cloves ethanol | Brown | Gummy | DMSO | Spicy |
| Cloves water | Dark brown | Gummy | Water | Spicy |
| WABP ethanol | Brown | Oily | DMSO | Spicy |

Key: WABP = West African black pepper

Table 2: Phytochemical composition of spices used in *kilishi* processing

| Extract | Alkaloids | Flavonoids | Saponins | Red. sugars | steroids | Tanins |
|----------------|-----------|------------|----------|-------------|----------|--------|
| Pepper ethanol | + | + | + | + | - | - |
| Pepper water | + | + | + | + | + | + |
| Ginger ethanol | + | + | + | + | + | - |
| Ginger water | + | + | + | + | + | + |
| Cloves ethanol | + | + | + | + | - | + |
| Cloves water | + | + | + | + | + | + |
| WABP ethanol | + | + | - | + | - | - |
| WABP water | - | + | + | + | - | + |

Key: WABP = West African black pepper, + = detected, - = not detected

Table 3: Bioactivity of spice extracts from spice, on *Salmonella* spp isolated from *kilishi*

| Extract | Diameter of zone of inhibition (mm) | | | | |
|----------------|-------------------------------------|--------|---------|---------|---------|
| | 10µg | 100 µg | 1000 µg | 2000 µg | 3000 µg |
| Pepper ethanol | 00 | 00 | 00 | 00 | 00 |
| Pepper water | 00 | 00 | 00 | 00 | 00 |
| Ginger ethanol | 00 | 08 | 19 | 26 | 30 |
| Ginger water | 00 | 00 | 00 | 00 | 00 |
| Cloves ethanol | 13 | 24 | 25 | 26 | 26 |
| Cloves water | 00 | 00 | 00 | 00 | 08 |
| WABP ethanol | 12 | 14 | 20 | 22 | 23 |
| WABP water | 00 | 00 | 00 | 00 | 00 |

Key : WABP = West African black pepper, µg = microgram

Table 4: Bioactivity of extracts from spice, on *Staph. aureus* isolated from meat

| Extract | Diameter of zone of inhibition (mm) | | | | |
|----------------|-------------------------------------|--------|---------|---------|---------|
| | 10µg | 100 µg | 1000 µg | 2000 µg | 3000 µg |
| Pepper ethanol | 12 | 11 | 16 | 22 | 34 |
| Pepper water | 00 | 00 | 00 | 00 | 00 |
| Ginger ethanol | 00 | 00 | 00 | 00 | 00 |
| Ginger water | 00 | 00 | 00 | 00 | 00 |
| Cloves ethanol | 12 | 13 | 13 | 17 | 22 |
| Cloves water | 00 | 00 | 00 | 00 | 00 |
| WABP ethanol | 00 | 00 | 00 | 00 | 00 |
| WABP water | 00 | 00 | 00 | 00 | 00 |

Key: WABP = West African black pepper, µg = microgram

DISCUSSION

According to Pruthi (1980), properties of spices include bactericidal, bacteriostatic, fungistatic, anthelmintic, medicinal and flavouring. From the result of this study, it is clear that not all the spices extracts were active against the test organisms. Even those that were active were the ethanolic extracts the water extracts were not active. Considering the fact that the spices are used in combination during the production of the traditional meat product (*kilishi*) and the mixture of all the extracts of the spices was not active against the test organisms at the disc concentrations used, the effect of the spices on the

microbial loads of the meat product would not be remarkable. This also signifies that, individually the extracts of the spices are more active against the test organisms than when they were combined. According to Frazier and Westhoff (2006), spices and condiments do not have any marked bacteriostatic effect in the concentrations normally used but may help other agents in preventing the growth of microorganisms. They also showed that unless the spices are treated to reduce their microbial content, they may add high numbers and undesirable kinds of microorganisms as observed from this study.

Conclusion and Recommendations

It could be concluded that the ethanolic extract of the spices have potential for use in the development of antimicrobial drugs. The spices are active against the test organisms individually but in combination the

activity is lost. It could therefore be recommended that spices should be treated to reduce their microbial loads, and potable water should always be used in their preparation.

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