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# Antibacterial Activity of *Myristica fragrans* and Curry Powder against Selected Organisms

**Anibijuwon, I. I., Omojasola, P. F., Olayiwole, A., Abioye, J. A. and Odaibo, D. O.**
Public Health Laboratory Unit, Department of Microbiology, Faculty of Science, University of Ilorin, P.M.B 1515 Ilorin, Kwara State, Nigeria

**ABSTRACT:** The *in vitro* antibacterial activity of ethanolic and methanolic extracts of two spices; *Myristica fragrans* (nutmeg) and Curry powder was investigated using agar well diffusion method. The sensitivity of five food-borne pathogens (*Pseudomonas aeruginnosa, Staphylococcus aureus, E. coli, Salmonella typhi* and *vibrio spp*) to the extracts was investigated using agar well diffusion method. Both spices exhibited antibacterial activity against all the tested organisms *Pseudomonas aeruginnosa* was the most susceptible organisms to both spices, while *Vibrio spp* showed the most resistance to both spices The Minimum Inhibitory Concentration (MIC) was determined for each of the spice extract. The lowest and most effective MIC value was that of the ethanolic extract of Nutmeg against *Pseudomonas aeruginosa* and highest was that of methanolic extracts of against *Staphylococcus aureus , Salmonella typhi* and the ethanolic extract of nutmeg against *Vibrio spp*. Most of the extracts were merely inhibitory against the organisms except the ethanolic extract of Curry and Nutmeg which were bactericidal against *Pseudomonas aeruginnosa,* Comparative studies with antibiotics showed that some bacteria that showed resistance to certain antibiotics were sensitive to the extracts of the spices. Such observation was comparatively significant and demonstrated the potential use of these spices as antimicrobial in the treatment food-borne illnesses.

**KEYWORDS:** Antimicrobial activity, Food-borne pathogens, spices, Antibiotics resistance

## 1.0 Introduction

A medicinal plant is any plant in which one or more of its organ, contain substances that can be used for therapeutic purposes or which is a precursor for the synthesis of useful drugs (WHO, 2012). They range from plants which are used in the production of mainstream pharmaceutical products to plants used in herbal medicine preparation which could either be in the form of infusion or decoction. A spice is a dried seed, fruit, root, bark, or flower of a plant or an herb used as food additives, for flavor, colour or as preservative, many spices are used for other purposes, such as medicine, religious rituals, cosmetics and perfumery (Linda, 2007).

Today as throughout history, spices are used for their fragrance, flavor, preservation, colour and medicinal properties, they serve a very important biological function. They are extensively used, particularly in many Asian, African and other countries. In recent years, in view of their beneficial effects, use of spices has been gradually increasing in developed countries also. Spices have been shown to be indispensible for daily human health. Besides adding flavor and taste to dishes, they help prevent and alleviate various health problems. Over the last few years several bioactive compounds have been isolated from spices, providing a scientific basis for the use of spices in our diet (Suekawa *et al*., 2006). Spices have a unique aroma and flavor which are derived from compounds known as phytochemicals or secondary metabolites (Avato *et al.*, 2000).

Food-borne diseases are an increasing concern, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreak caused by some pathogenic and spoilage microorganisms in foods. Outbreaks of food-borne diseases have been associated with the consumption of foods like poultry, beef,

**Corresponding Author**
Tel.: +2348036115296;
E-mail: kunledoexploit@gmail.com

processed meat, cheese, sea foods and other related foods. Foods can become contaminated through raw material, cross contamination, poor personal hygiene and the immediate environment in the kitchen. The increasing antibiotic resistance of some pathogens that are associated with food-borne illness is another concern (Stermite *et al*., 2000). There has been resurgence of interest in quest of newer molecules with antimicrobial property of biological origin among scientific community due to development of resistance by pathogens (Wallace *et al*., 2005), expensive treatment regime of synthetic drugs already in practice and their gross side effect due to indiscriminate use (Hostettmann, 2007). One major impact of antibiotics resistant is the emergence of microbes which are difficult to treat, which may eventually lead to increase cost of disease management and in the long run lead to.

The antimicrobial efficacy attributed to some spices in treating diseases has been beyond belief. It is estimated that the local communities have used about 10% of all flowering plants on earth to treat various, infections, although only 1% have gained recognition by modern scientist (Lewis and Ausubel, 2006). With no doubt, medicinal plant based antimicrobials represent a vast untapped source of pharmaceuticals.

There is therefore a need to verify these claims scientifically in order to allow for better understanding of the use of these spices in the management of infections and diseases, which could also solve the problems antibiotics has created.

The objective of our study was to determine the in vitro antibacterial property of the ethanolic and methanolic extract of curry powder and *Myristica fragrans* (nutmeg) against the above listed food-borne pathogens for future application as natural antibacterial agents and then compare the inhibitory effect of these spices with that of already known and purified antibiotics.

## 2.0 Materials and Methods

### *2.1 Collection of spices*

Samples of curry powder and *Myristica fragrans* (nutmeg) were obtained from Yoruba road market Ilorin, Kwara state and taken to the laboratory for processing and analysis.

### *2.2. Collection and maintenance of test organism*

Pure culture of selected organisms were obtained from the microbiology laboratory of university of Ilorin Teaching Hospital (UITH) they were collected in sterile nutrient agar slants and were incubated at $37^0$C. They were then kept in the refrigerator to serve as stock culture. They were selected on the bases of their occurrence in the kitchen and ability to cause food-borne illness. They were routinely sub-cultured for the purpose of purity during storage on suitable media and stored at $4^0$C until required for use.

### *2.3 Preparation of extract*

The method described by Anibijuwon and Abioye (2011) was adopted in the preparation of the extract. The powdered spices were soaked in 70% ethanol and methanol. This was placed on a shaker for about 120hours (5days) at about 28± 2°c. The resulting extract was passed through Muslin cloth and filtered through a Whatman No 1 filter paper. The resulting extract was evaporated to dryness at 60°C in a hot air oven. The stock concentration was stored in sterile covered sample bottles to prevent evaporation and then stored in the refrigerator until use. The extract of each spice was reconstituted back in their respective solvent. The varying concentration obtained were 200, 150, 100 50mg/ml.

### *2.4 Antimicrobial sensitivity test of the extracts*

The agar well diffusion method as described by Ogundipe *et al*. (2000), was used to determine the sensitivity of the test organisms to the extract. Overnight broth culture of the test organisms were then swabbed on the surface of sterile Sensitivity test agar using sterile swab sticks to obtain a lawn swab, plates were labeled appropriately. Sterile cork borer was used to bore five well of equidistantance with the fifth

well in the centre serving as the control. Each well was labeled appropriately on the reverse side of the media plate. A sterile syringe was used to dispense 0.2ml of the different concentration of the extracts (curry and nutmeg) into respective well in the agar plates. The fifth well contained the solvent used to extract each spice. The inoculated plates were allowed to stand 30minutes for the extracts to diffuse into the agar. The plates were then incubated in an upright position at 37°C for 24hours. After 24-48 hours. After incubation the plates were examined for clear appearance around the well (zone of inhibition). The zones were measured in milliliters and recorded.

*2.5 Antimicrobial assay of antibiotics*

The method described by Bauer *et al.* (1966) was employed. Aseptically sterile forcep was use to place antibiotics disc depending on the gram property of the organism on sensitivity test agar already inoculated with the test organism. The plates were incubated uprightly at 37ºC for 24 hours. After incubation the zones of inhibition around each antibiotic disc were measured in milliliters and recorded.

*2.5 Determination of minimum inhibitory concentration*

The MIC of both ethanolic and methanolic extracts of the spices was determined using the test tube-tube dilution method Usman *et al.* (2007). 1ml of each extract with concentration ranging from 50mg/ml to 200g/ml of the same volume was dispensed into each test-tube already containing 9ml of sterile Nutrient broth. A loopful of test organisms culture was inoculated into each test-tube and incubated at 37ºC or 24hours. Control test-tubes were set up which contained the extract and Nutrient broth. After incubation, each of the test-tube was examined for the presence or absence of growth by checking its turbidity against the control tubes using a spectrophotometer.

**3.0 Results and Discussion**

The result of agar well test indicated that the extracts of nutmeg and curry powder were effective against the test bacteria and showed different degrees of growth inhibition, depending on the bacteria as indicated in Table 1. Curry powder showed zones of inhibition, ranging from 0-22mm, the ethanolic extract showed the highest antibacterial activity against *Staphylococcus aureus* (ZI, 22mm), followed by *Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli* with equal zones of inhibition (ZI, 20mm). *Vibrio spp* had the lowest zone of inhibition (ZI, 8mm). The methanolic extract showed less antibacterial activity when compared with the ethanolic extract. It inhibited the growth of four bacteria, with zones of inhibition (ZI) ranging from 0-15mm, except for *Salmonella typhi*. The highest activity was against *Escherichia coli* having a ZI of 15mm, followed by *Pseudomonas aeruginosa*(ZI, 10mm), *Vibrio spp* (ZI, 6mm) and lowest activity against *Staphylococcus aureus* with ZI of 5mm.

Nutmeg showed zones of inhibition, ranging from 0-25 mm, the ethanolic extract showed the highest antibacterial activity, with *Pseudomonas aeruginosa* being the most susceptible organism to the extract, having a zone of inhibition of 25 mm, followed by *Staphylococcus aureus* (ZI, 17 mm) and *Escherichia coli* (ZI, 15 mm). *Vibrio spp* showed the least susceptibility to the extract with ZI of 5 mm. Nutmeg ethanolic extract was inactive against *Salmonella typhi*. The methanolic extract showed less antibacterial activity when compared to the ethanolic extract, with ZI ranging from 0-23 mm; it inhibited the growth of all test bacteria except *Escherichia coli* (ZI, 0). The highest activity was shown against *Pseudomonas aeruginosa* (ZI, 23 mm), followed by *Salmonella typhi* (ZI, 18 mm), *Staphylococcus aureus* (ZI, 15 mm) and *Vibrio spp* (ZI, 7 mm).

Overall the ethanolic extract of curry powder showed the broadest antibacterial activity by inhibiting the growth of all the bacteria tested,

Table 1: Antibacterial activity of the spices

| **Test Organisms** | **Diameter of the Zones of Inhibition(mm)** | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nutmeg | | | | | | | | Curry Powder | | | | | | | |
| | Ethanol Extract(mg/ml) | | | | Methanol Extract(mg/ml) | | | | Ethanol Extract(mg/ml) | | | | Methanol Extract(mg/ml) | | | |
| | 50 | 100 | 150 | 200 | 50 | 100 | 150 | 200 | 50 | 100 | 150 | 200 | 50 | 100 | 150 | 200 |
| *Pseudomonas aeruginosa* | 6 | 10 | 24 | 25 | 0 | 10 | 18 | 23 | 5 | 10 | 20 | 20 | 0 | 5 | 6 | 10 |
| *Staphylococcus aureus* | 0 | 4 | 10 | 17 | 0 | 0 | 7 | 15 | 1 | 6 | 15 | 22 | 0 | 0 | 0 | 5 |
| *Escherichia coli* | 0 | 0 | 7 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 20 | 2 | 4 | 10 | 15 |
| *Salmonella typhi* | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 18 | 0 | 0 | 19 | 20 | 0 | 0 | 0 | 0 |
| *Vibrio spp* | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 7 | 0 | 0 | 1 | 8 | 0 | 0 | 0 | 6 |

zone of inhibition (ZI) ranging from 0-22mm, while that of nutmeg inhibited the growth of almost all organisms, ZI ranging from 0-24mm. *Pseudomonas aeruginosa* showed the least resistance to the methanolic and ethanolic extracts of both spices and hence the highest sensitivity, making it the most susceptible organism. *Vibrio spp* showed the least susceptibility and hence the highest resistance to methanolic and ethanolic extracts of both spices. Activity was a linear function of concentration, different organisms responded differently to the extract at different concentration. At the lowest concentration (50mg/ml), the organisms were least sensitive, while at the highest concentration (200g/ml) the organisms were most sensitive.

The result of the antibiotic sensitivity analysis of the test organisms is shown in Table 2, of the 5 organisms, it was observed that *Pseudomonas aeruginosa* was the most resistant organisms to the antibiotics. Of the twelve antibiotics tested, only four showed activity, Ofloxacin was most effective, as it inhibited the growth of all the organisms it was tested against, it was followed by Gentamycin, Cotrimoxazole and Nitrofurantoin. However Nitrofurantoin showed the highest activity. The low MIC value is indicative of the effectiveness of the spices extract against the microbes. The lowest and hence the most effective MIC was that of the ethanolic extracts of Nutmeg and Curry powder against *Pseudomonas aeruginosa* (50mg/ml).

Growth of organisms at different concentration was depicted in Figures 1-4. Figures 1 and 3 showed the growth of organisms against concentration of ethanolic extract of Nutmeg and curry powder respectively while Figures 2 and 4 showed organisms against concentrations of methanolic extract of Nutmeg and curry powder respectively. Furthermore Figure 1 showed that *Pseudomonas aeruginosa* had highest resistance by growing on all the tested concentration while *Vibrio spp* grow only at highest dilution of 200mg/ml of the ethanolic extract of nutmeg. These was similar to Figure 2 which showed the growth of *Pseudomonas aeruginosa* at some concentration except the

Table 2: Antibiotics susceptibility of test organisms

| **ANTIBIOTICS** | **Test Organisms** | | | | |
|---|---|---|---|---|---|
| | ***Pseudomonas aeruginosa*** | ***Staphylococcus aureus*** | ***E. coli*** | ***Salmonella typhi*** | ***Vibrio*** |
| Oflxaxin | 12 | No growth | 14 | 19 | 13 |
| Nitrofurantoin | No growth | No growth | 10 | 20 | No growth |
| Gentamycin | No growth | 9 | 15 | 10 | No growth |
| Co-trimazole | No growth | 13 | No growth | 15 | No growth |

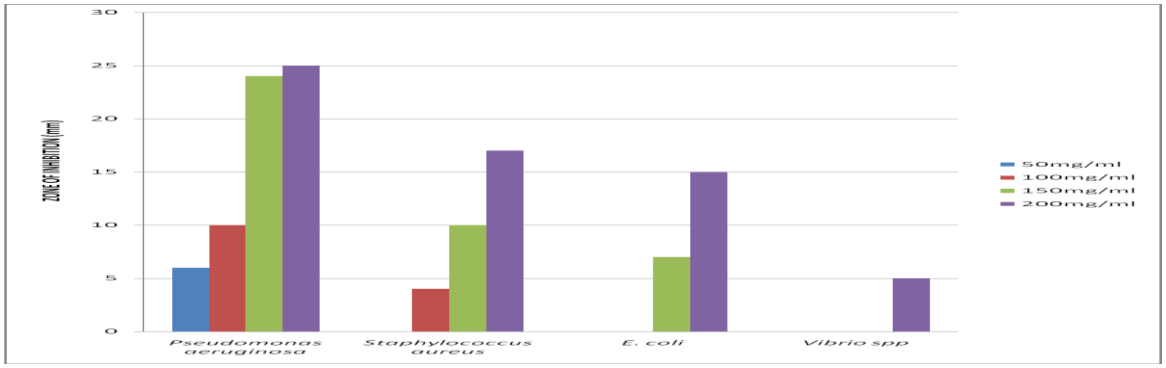


Figure 1: Growth of organisms at different concentrations of ethanolic extract of nutmeg

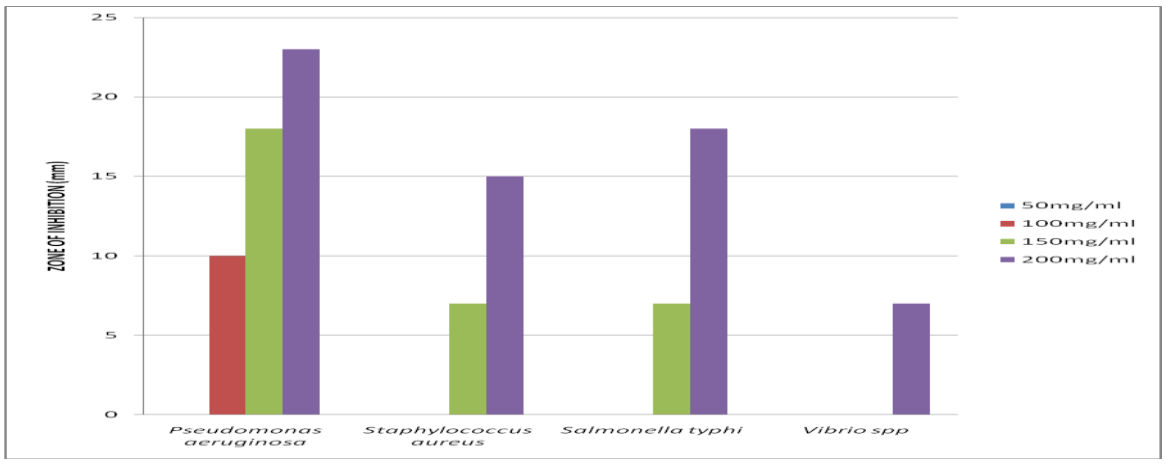


**Figure 2:** Growth of organisms at different concentration of methanolic extract of nutmeg

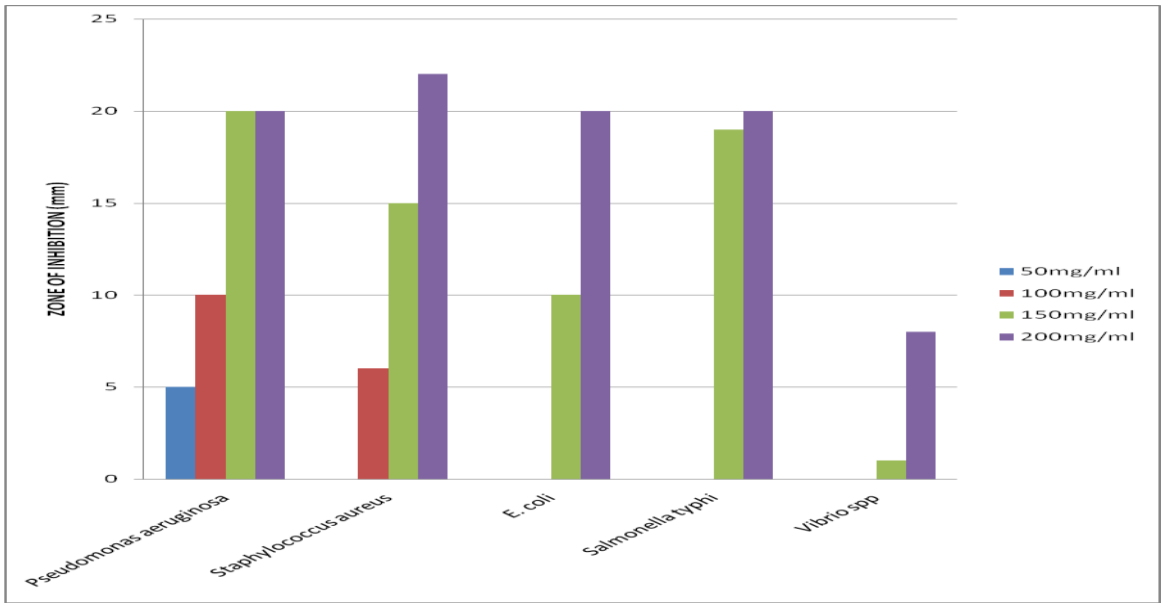


Figure 3: Growth of organisms at different concentrations of ethanolic extract of curry powder

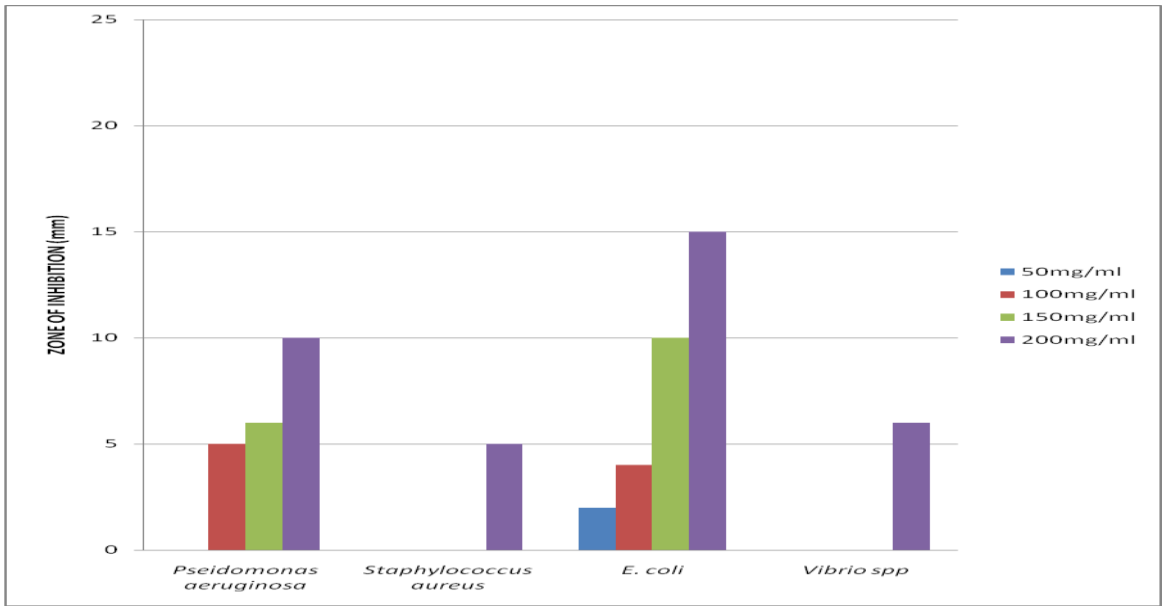


**Figure 4:** Growth of organisms at different concentrations of methanolic extract of curry powder

highest concentration of 50mg/ml concentration of methanolic extract of nutmeg.

It should be stated that Figure 3 showed the growth of the organisms i.e *E. coli, S. typhi and Vibrio spp* at concentration of 150mg/ml and 200mg/ml concentration of extract of curry powder. Both *Staphylococcus aureus* and *Vibrio spp* grow at highest dilution of 200mg/ml of concentration of methanolic extract of curry powder while *E. coli* grow against all the tested concentration

Analysis of the result of the sensitivity and MIC testing indicated that the extract of both spices possess a significant amount of antibacterial activity against all five test organisms with *Vibrio spp* exhibiting the greatest amount of resistance against the different spice extract. This may probably be due to the inability of the organism to dissolve the active principle which is not enough to conclude that the spices are not so effective against this organism. Possible future investigation may reveal that if the concentrations of the extracts used are increased, the structural components of the organisms may be weakened inducing cellular malfuncting, inhibition and possible cell death (Elgahazali *et al.*, 1998). It was observed that the ethanolic extract of the spices were more effective than their methanolic counterpart, this may be due to the affinity of ethanol on the bioactive substances thereby exerting more inhibitory action, these findings as been reported by Nweeze *et al.*, (2004). Both spices in some instances demonstrated effectiveness greater than that of the antibiotics. The observation of such inhibition amongst the spices were comparatively significant, and demonstrated the potential use of these spices as antimicrobial agents with an efficacy that can be compared to that of the already recognized and widely used antibiotics. The tested organisms showed multi drug resistant, as only four out of the eleven antibiotics used were able to inhibit the growth of the organisms, this is an alarming finding, showing again the importance for the search of new antimicrobial to solve the menace antibiotics have created, and the spices used in this research have shown to have promising potential. An interesting observation is the resistance of *Pseudomonas aeruginosa* to 7 of the antibiotics it was tested against but the extract showed excellent activity against this organism.

The MIC of the extracts will make it easier for dosage determination and chemotherapeutic index of the extract if they were to be processed into drugs. In vitro activity of these spices extracts against pathogens justifies the folk medicinal use of these spices against certain illnesses. The result of this present study is quite encouraging as both spices exhibited antimicrobial activity. This study opens up the possibility for the search of new antimicrobials that can serve as an alternative to antibiotics. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and disseminated properly.

## Conclusion

Activity of the spices against pathogens that appear to be resistant to many antibiotics shows that spices have a glowing future in the treatment of illnesses caused by the food pathogen investigated in this study. However it is necessary to determine the toxicity of the active constituents, their side effect and pharmaco-kinetic properties.

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Table 3: Minimum Inhibitory Concentration

| **Test Organisms** | **MIC (mg/ml)** | | | |
|---|---|---|---|---|
| | **Nutmeg** | | **Curry Powder** | |
| | Ethanol | Methanol | Ethanol | Methanol |
| *Pseudomonas aeruginosa* | 50 | 100 | 50 | 100 |
| *Staphylococcus aureus* | 100 | 200 | 100 | _ |
| *Escherichia coli* | 150 | _ | 100 | 150 |
| *Salmonella typhi* | _ | 200 | 150 | _ |
| *Vibrio spp* | 200 | _ | _ | _ |

**Table 4:** Minimum Bactericidal Concentration

| **Test Organisms** | **MIC (mg/ml)** | | | |
|---|---|---|---|---|
| | **Nutmeg** | | **Curry Powder** | |
| | **Ethanol** | **Methanol** | **Ethanol** | **Methanol** |
| *Pseudomonas aeruginosa* | _ | + | _ | + |
| *Staphylococcus aureus* | + | + | + | + |
| *Escherichia coli* | + | + | + | + |
| *Salmonella typhi* | + | + | + | + |
| *Vibrio spp* | + | + | + | + |

Key: (-) no growth, (+) scanty growth

**Table 6**: Antibacterial activity of Antibiotics and Extract on Test Organisms

| Test Organisms | **Diameter of Zones of Inhibition** | | | | | |
|---|---|---|---|---|---|---|
| | **Antibiotics** | | | | **Spice Extract** | |
| | GEN | COT | OFL | NIT | Nutmeg Ethanolic Extract (200 mg/ml) | Curry Powder Ethanolic Extract (200 mg/ml) |
| *Pseudomonas aeruginosa* | - | - | 12 | - | 25 | 20 |
| *Staphylococcus aureus* | 9 | 3 | - | - | 17 | 22 |
| *Escherichia coli* | 15 | - | 14 | 10 | 15 | 20 |
| *Salmonella typhi* | 10 | 15 | 19 | 20 | - | 20 |
| *Vibrio spp* | - | - | 13 | - | 5 | 8 |