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Antimicrobial activities of four varieties of *Capsicum annuum* fruits cultivated in Southeast Nigeria against multidrug-resistant and susceptible organisms

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Abstract

Due to increasing drug resistance in microbial pathogens and side effects of certain antimicrobial agents, there is a need to search for cheaper, effective and less toxic novel antimicrobial agents that would overcome these disadvantages. Our study evaluated the antimicrobial activities of ethanolic extracts from four different varieties of *Capsicum annuum* cultivated at Nsukka viz. *Capsicum annuum* var. shombo, *Capsicum annuum* var. tatashi, *Capsicum annuum* var. Nsukka yellow pepper and *Capsicum annuum* var. atarugu, against multidrug-resistant (MDR) *Listeria monocytogenes* and MDR *Escherichia coli, Candida albicans, Cryptococcus neoformans* and *Aspergillus flavus.* All the varieties of *Capsicum annuum* fruits showed variable antimicrobial activities and were bactericidal as well as fungicidal. However, they showed fungistatic activity against *Aspergillus flavus.* The minimum inhibitory concentration (MIC) for MDR *E. coli* isolates was between 9.4 - 37.5 mg/ml, while the minimum bactericidal concentration (MBC) ranged from 18 - 37.5 mg/ml. Antibiotic susceptible *E. coli* isolates had MIC values between 4.7 and 37 mg/ml and MBC range between 9.4 and 37 mg/ml. Similar results were obtained for *C. annuum* var. tatashi and *C. annuum* var. shombo with MIC range of 9.4 - 37 mg/ml and MBC range of 18 - 37 mg/ml for MDR *E. coli* isolates while multidrug-susceptible (MDS) *E. coli* isolates had MIC range of 7.8 – 18 mg/ml and MBC range of 9.4 - 31 mg/ml. The range of MIC values for *L. monocytogenes* observed for all the four extracts was 9.4 - 37.5 mg/ml while the MBC range was between 14.1 - 37.5 mg/ml. This study shows that *Capsicum annuum* has both antibacterial and antifungal activities and might be a useful source of antibiotics and food preservatives.

Key words: Capsicum, antimicrobial activity, bacteria, fungi, Nsukka, Nigeria

1. Introduction

Human infections caused by pathogenic microbes constitute a huge public health burden all over the world [1, 2]. However, developing countries appear to be disproportionately affected because of a variety of reasons which includes lack of appropriate/quality conventional drugs, the expensive nature of quality conventional drugs, drug abuse/incomplete dosing etc. High cost of drugs may lead to inconsistent use of antibiotics which can possibly result in microbial drug resistance. The cost of medication is a major factor in deciding how infected patients obtain appropriate treatment. The level of poverty is high in many developing countries and cost of medications sometimes make sick persons to look for cheap and available alternative medications to treat their ailments. Incidentally, medicinal plants are very abundant in these areas and many of these plants have been shown to contain different types of chemical constituents/compounds with therapeutic properties which are useful in the treatment of many human diseases [3, 4]. However, many of these medicinal plants have not been scientifically validated in the laboratory thereby creating some doubts on their usefulness.

The genus *Capsicum* is a member of family Solanaceae and has five species that are commonly recognized as domesticated: *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, and *Capsicum pubescens*. *Capsicum* is an important tropical agricultural crop and one of the popular vegetables, not only because of its economic value, but also for the combination of color, taste and nutritional values of its fruit [5]. *Capsicum* species has been a part of human diet since 7500 BC and can be eaten raw or cooked. Those used in cooking are generally varieties of *Capsicum annuum* and *Capsicum frutescens* species [6]. Both species contain a wide range of nutritional components and pharmacologically active metabolites.

Apart from the general use of pepper as a food ingredient, it has been reportedly used as a remedy for treating different diseases including gastroenteritis, stomach ache, diabetes, arthritis, pain etc. One of the added advantages of using pepper as a potential therapeutic remedy is because of the

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unlikelihood of side effects associated with many plants and conventional antibiotics. The reason is because it is part of virtually every meal prepared and consumed in many places. The study was therefore carried out to investigate the antimicrobial activity of pepper (*Capsicum annum* of different varieties) cultivated in Nsukka, Nigeria against some pathogenic microorganisms such as MDR and MDS *Escherichia coli,* MDR and MDS *Listeria monocytogenes, Cryptococcus neoformans, Candida albicans and Aspergillus flavus.*

2. Materials and Methods

2.1. Plant Material

Four different varieties of *Capsicum annuum* fruits mostly unique to Nsukka, *Capsicum annuum* var. shombo, *Capsicum annuum* var. tatashi, *Capsicum annuum* var. Nsukka yellow pepper and *Capsicum annuum* var. atarugu were collected from Ogige market in Nsukka, Enugu State Nigeria. Taxonomic identification of the *Capsicum* fruits was carried out and confirmed by an experienced plant taxonomist in the Department of Plant science and Biotechnology, University of Nigeria, Nsukka, Nigeria. All the *Capsicum* samples were fresh, ripe and firm.

2.2. Test Microorganisms

The microorganisms used in the study included, multidrug-resistant (MDR) *Listeria monocytogenes* and multidrug-susceptible *Listeria monocytogenes,* three MDR *Escherichia coli* and three multidrug-susceptible (MDS) *Escherichia coli,* two clinical isolates of *Candida albicans* and *Cryptococcus neoformans* and a mould isolate of *Aspergillus flavus.* These organisms were selected based on their roles in the etiology of many diseases.

2.3. Plant Extracts Preparation

Fresh, unblemished *Capsicum* fruits were washed with sterile water and then oven dried for 48 hr at 60 °C, stored at room temperature, and ground to a fine powder. The ethanolic extract was obtained by mixing 10 g of powder in 100 mL of 70% ethanol for 48 hr. The mixture was agitated at 8 hr intervals. The mixture was filtered through Whatman filter paper No. 2 (Whatman International, Maidstone, England). It was then evaporated at 50 °C until dry in an oven. The concentrated crude extract was stored in the refrigerator until further use [7].

2.4. Preparation of inocula

Bacterial and fungal cultures were grown in Muller-Hinton broth overnight, then diluted with sterilized Muller-Hinton broth to obtain 0.5 McFarland. The diluted cultures were inoculated in Mueller-Hinton agar and allowed to dry for 10 min at 22 °C ± 2 °C. Excess moisture was blotted/removed.

2.5. Determination of the antimicrobial activity of Capsicum annuum ethanolic extracts

One gram of the extract was measured into a sterile test tube and 10 ml of 20% v/v dimethyl sulfoxide (DMSO) was added to dissolve it. This gave a 100 mg/ml concentration of the extract and this was diluted in two-folds to obtain four different dilutions of the extract: 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml, in addition to the 100 mg/ml concentration. The standardized broth form of different organisms were poured in the solid media namely, Mueller Hinton agar for bacteria and Sabouraud dextrose agar (SDA) for fungi and spread with glass rod to achieve an even distribution of the organisms on the plates. The excess was removed and allowed to dry. With the aid of a sterile standard 6 mm cork borer, 6 wells were bored at equidistant positions.

Each well was then filled with about 100 µl of different concentrations of the crude extract and the sixth well contained the diluent, dimethyl sulfoxide (DMSO) which was used as the control. This procedure was repeated for all the test organisms and allowed for 30 minutes on the bench and then incubated for 24 hr at 37 °C. In the second set of culture plates having the same set of organisms, the antibiotic discs were aseptically placed on them and incubated for 24 hr at 37 °C. Fluconazole was used for fungi and gentamycin for bacteria as positive controls. After 24 hr, the resulting zones of inhibition were measured using a ruler calibrated in millimeters (mm). The average of the two readings was taken to be the zones of inhibition of the bacterial or fungal isolate tested at that particular concentration. The standard error of mean was calculated.

2.6. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for bacterial isolates

The broth dilution technique described previously [3] was utilized where the plant extract was prepared to the highest concentration of 200 mg/ml (stock concentration) in dimethyl sulfoxide (DMSO) and serially diluted (two-fold) to a working concentration ranging from 0.780 mg/ml to 100 mg/ml using Mueller Hinton broth and later inoculated with 0.1 ml suspension of the test organisms. After 18 hr of incubation at 37 °C, the tubes were observed for turbidity. The least concentration where no turbidity was observed, was noted as the minimum inhibitory concentration (MIC) value [8].

The MBC was determined from the broth dilution resulting from the MIC tubes by subculturing the tubes used for the MIC which did not show any visible growth on antimicrobial free agar as previously described [4]. Briefly, a sterile wire loop was used to streak a loopful of the contents of each test tube with no visible growth on agar plate free of bacteria and incubated at 37 °C for 18 hr. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC. The experiment was carried out in duplicate.

2.7. Determination of minimum inhibitory concentration and minimum fungicidal concentration for fungal isolates

Broth dilution protocols based on Clinical Laboratory Standard Institute (CLSI) approved reference document [9] as previously described by Josep et al. [10] was used to determine MIC values for *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus flavus.* A two-fold serial dilution in broth was prepared, with final test concentrations ranging from 0.78 to 50 mg/ml for *Capsicum annuum*, the MIC was obtained using standardized inoculum. Results were read after 48 hr, and MICs were defined as the lowest test concentrations causing complete growth inhibition. Fluconazole was used as positive control, determinations of the MIC values of fluconazole was performed by using concentrations of 0.97 to 500 µg/ml. To determine minimum fungicidal concentration (MFC) values, after reading the corresponding MIC values, the clear tubes and the last one showing growth were subcultured on Sabouraud dextrose agar (SDA) plates. The agar plates were incubated at 35 °C for 48 hr and MFC values were determined as the lowest concentration with no visible growth. This was done in duplicate and results were presented as minimum fungicidal concentration (MFC) ± standard error of mean.

3. Results

The different varieties of *Capsicum* extracts were similar, having a range of 4.7 – 50 mg/ml. The least MIC value for *L. monocytogenes was* 9.4 mg/ml obtained from *C. annuum* var. tatashi and *C. annuum* var. shombo, while the highest MIC value was 50 mg/ml obtained from *C. annuum* var. atarugu. The lowest MIC for the MDR and MDS isolates of *E. coli* was observed in *C. annuum* var. atarugu with values of 9.4 mg/ml and 7.4 mg/ml respectively. The negative controls containing the extracts in broth without the test organism showed no growth. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Capsicum annuum* var. atarugu and var. Nsukka yellow pepper fruits against test organisms are shown in Table 1**.**

The MIC and MBC of *Capsicum annuum* var. tatashi and var. shombo against tested microbial isolates is shown in Table 2. The least MIC for *L. monocytogenes* was 9.4 mg/ml observed for *C. annuum* var. tatashi while the highest MIC was 37.5 mg/ml by *C. annuum* var. shombo. This observation shows that *C. annuum* var. tatashi may be more active against *C. annuum* var. shombo. All the four varieties of *C. annuum* extracts were active against *Candida albicans* and *Cryptococcus neoformans* but lowest activity was observed for *Aspergillus flavus* which has the highest MIC values. *C. annuum* var. tatashi and *C. annuum* var. shombo were found to have the least values of MIC but the MBC of *C. annuum* var. shombo was lower. MIC and MBC values of 28.1 mg/ml and 5.5 mg/ml were respectively observed for *C. annuum* var. shombo and against *A. flavus*, while it was 18.8 mg/ml and 31.25 mg/ml respectively for *C. annuum* var. atarugu.

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extracts of *Capsicum annuum* var. atarugu and var. Nsukka yellow pepper fruits against test organisms; Data is presented as data ± S.E.M. (n=2).

	***C. annuum* var. atarugu**		***C. annuum* var. Nsukka yellow pepper**	
	MIC (mg/ml)	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
Bacterial isolates				
MDR *L. monocytogenes*	18.8 ± 6.25	18.75 ± 9.0	37.5 ± 12.5	18.8 ± 6.25
MDS *L. monocytogenes*	13.3 ± 11.7	50 ± 0.00	37.5 ± 12.5	37.5 ± 12.5
MDR *E. coli* 1	37.5 ± 3.1	18.8 ± 6.3	31.25 ± 6.3	37.5 ± 12.5
MDR *E. coli* 2	9.4 ± 3.1	18.8 ± 6.3	18.8 ± 6.3	25 ± 0.0
MDR *E. coli* 3	18.8 ± 6.3	31.3 ±18.8	15.6 ± 9.4	18.8 ± 6.3
MDS *E.coli* 1	4.7 ±1.6	7.8 ± 4.7	4.7 ±1.6	18.8 ± 6.3
MDS *E.coli* 2	15.6 ± 9.3	28.1±3.1	37.5 ± 12.5	37.5 ± 12.5
MDS *E.coli* 3	4.7 ± 1.6	9.4 ± 3.1	18.8 ± 6.3	25 ± 0.0

Key: MDR = multi-drug resistant; MDS = multi-drug susceptible

effective in inhibiting the test cultures tested in the study. Though the response was not uniform, all *Capsicum* extracts showed an activity against both bacterial and fungal isolates. The MIC and MBC values of the four varieties of *Capsicum annuum* against *E. coli* and *L. monocytogenes* appeared

Gentamicin which was used as a positive control for comparative purposes was active against all the bacterial isolates and had MIC and MBC ranges of 0.5 - 128 µg/ml

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extracts of *C. annuum* var. tatashi and *C. annuum* var. shombo against test organisms; Data is presented as data ± S.E.M. (n=2).

	***C. annuum* var. tatashi**		***C. annuum* var. shombo**	
	MIC (mg/ml)	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
Bacterial Isolates				
MDR *L. monocytogenes*	9.4 ± 3.1	18.8 ± 6.3	37.5 ± 12.5	9.4 ± 3.2
MDS*L. monocytogenes*	31.3 ±18.8	18.8 ± 6.3	14.1 ± 10.9	26.6 ± 23.4
MDR *E. coli* 1	18.8 ± 6.3	37.5 ± 12.5	18.8 ± 6.3	27.0 ±21.9
MDR *E. coli* 2	18.8 ± 6.3	18.8 ± 6.3	9.4 ± 3.2	37.5 ± 12.5
MDR *E. coli* 3	18.8 ± 6.3	18.8 ± 6.3	13.0 ± 11.7	27±21.9
MDS *E.coli* 1	7.8 ± 4.7	18.8 ± 6.3	14.1 ±10.9	18.8 ± 6.3
MDS *E.coli* 2	7.8 ± 4.7	31.25 ± 6.3	18.8 ± 6.3	14.1 ± 10.8
MDS *E.coli* 3	12.5 ± 0.0	18.8 ± 6.3	18.8 ± 6.3	9.4 ± 3.1

Key: MDR = multi-drug resistant; MDS = multi-drug susceptible

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of gentamicin against test organisms; Data is presented as data ± S.E.M. (n=2).

Bacteria isolates tested	**Gentamycin**	
	MIC (µg/ml)	**MBC (µg/ml)**
MDR *Escherichia coli* 1	8	16
MDR *Escherichia coli* 2	16	32
MDR *Escherichia coli* 3	12	128
MDS *Escherichia coli* 1	32	128
MDS *Escherichia coli* 2	0.5	1
MDS *Escherichia coli* 3	32	64
MDR *L. monocytogenes*	0.5	16
MDS *L. monocytogenes*	64	64

Key: MDR= multi-drug resistant; MDS= multi-drug susceptible

Table 4. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of ethanolic extract of *Capsicum annuum* varieties against test organisms; Data is presented as data ± S.E.M. (n=2).

	***C. annuum* var. atarugu**		***C. annuum* var. Nsukka yellow pepper**		***C. annuum* var. tatashi**		***C. annuum* var. shombo**	
	MIC (mg/ml)	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
Fungi								
C. albicans	4.7 ± 1.5	15.6 ± 9.4	9.4 ± 3.1	18.8 ± 6.3	18.8 ± 6.3	37.5 ± 12.5	4.7 ± 1.5	7.0 ± 5.5
C. neoformans	7.0 ± 4.3	14.1 ± 10.8	18.8 ± 6.3	31.25 ± 18.8	9.375 ± 3.1	31.25 ± 18.8	14.1 ± 10.8	18.8 ± 6.3
A. flavus	37.5 ± 12.5	31.25 ± 18.8	37.5 ± 12.5	37.5 ± 12.5	18.8 ± 6.3	31.25 ± 18.8	28.1 ± 21.9	35.5 ± 12. 5

and 1-128 µg/ml respectively for *E. coli*. The lowest MIC value was observed in MDS *E. coli* (0.5 µg/ml) and the MBC value was 1 µg/ml. MDS *Listeria monocytogenes* isolates had the same MIC and MBC values (64 µg/ml), as seen in Table 3.

Fluconazole was also used as the positive control for the three fungal isolates tested in this study. The MIC values observed for *C. neoformans, C. albicans* and *A. flavus* were 31.25 µg/ml, 62.5 µg/ml and 125 µg/ml respectively. Similarly, the MFC recorded for these same isolates were respectively 125 µg/ml, 250 µg/ml and 125 µg/ml (Table 4).

4. Discussion

Ethnomedicine is the oldest method used for curing diseases and infections. Various plants have been used in different parts of the world to treat human diseases and infections [3, 4]. Plants are used medicinally in different countries and are a source of many potential and powerful drugs. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world [11]. The interest in the consumption of capsicum, is to a large extent due to its content of bioactive compounds and their importance as dietary antioxidants. Peppers are used fresh, dried, fermented, or as an oleoresin extract. They are also used as a colourant, flavourant, and/or as a source of pungency. The main source of pungency in peppers is the chemical group of compounds called capsaicinoids (CAPS), which are produced in the fruit. Capsaicin ($C_{18}H_{27}NO_3$), trans-8-methyl-N-vanillyl-6-nonenamide), is the most abundant CAPS, followed by dihydrocapsaicin, with minor amounts of nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin and others. These compounds have both nutritional and nutraceutical importance [12].

Ethanolic extracts of various *Capsicum annuum* varieties were found to be active against all the clinical bacterial and fungal isolates tested. The antimicrobial activities of the ethanolic extracts of four varieties of *Capsicum annuum* fruits showed similar activities against all the test organisms. These findings tend to support the findings by Soetarno et al. [13] indicating that all types of capsicum fruits are useful as antibacterial and antifungal agents. However, we observed reduced activity of *Capsicum* species against *A. flavus* contrary to the findings of Harbant et al. [14] showing that capsicum extracts at very low concentrations completely inhibited the growth of *A. flavus*. The observed conflicting results in antimicrobial activity of *Capsicum annuum* could be due to the differences in their chemical components. It has also been reported that the samples collected from different geographic areas with different climates and vegetation show different antimicrobial activities [15]. Therefore, there is a need to test different varieties of *C. annuum* grown in different regions of the world against pathogenic microorganisms.

The least MIC value for *L. monocytogenes* was 9.4 mg/ml obtained from *C. annuum* var. tatashi and *C. annuum* var. shombo, while the highest MIC value was 50 mg/ml obtained from *C. annuum* var. atarugu, thus suggesting that *C. annuum* var. atarugu has lower activity against *L. monocytogenes*.

Dorantes et al. [16] found that capsicum species extracts exhibited antibacterial activity against *Listeria monocytogenes*. However, more isolates of *L. monocytogenes* may have to be tested before we can conclude since we tested only two isolates with this species. The lowest MIC values for multidrug-resistant and multidrug-susceptible isolates of *E. coli* was observed in *C. annuum* var. atarugu with values of 9.4 mg/ml and 7.4 mg/ml respectively, meaning that *C. annuum* var. atarugu had high antimicrobial activity against *E. coli*. Capsicum species may therefore serve as a potential source of novel antibacterial agents against infections caused by multidrug-resistant *E. coli*. Other authors have also made similar findings indicating that capsicum species have antimicrobial activities on a wide range of bacterial and fungal isolates [17, 18].

Compared to the antimicrobial activity of the conventional antibiotics, the activity of pepper fruit extracts was still much lower possibly because crude unpurified extracts were tested in this study. The MIC of gentamicin against *E. coli* and *L.* monocytogenes ranged from 0.5 to 128 µg/ml and 0.5 to 64 µg/ml respectively. The MIC of fluconazole against the fungal test organisms ranged from 6.25 to 125 µg/ml.

5. Conclusion

The indiscriminate use of commercial antimicrobial drugs has caused multiple drug resistance by pathogenic microorganisms. These resistant microorganisms have become a cause of major health concerns and require novel antimicrobial agents to tackle the problem. Our findings on the antimicrobial activity of *Capsicum annuum* against pathogenic organisms reveal that capsicum species may serve as a good source of antimicrobial agents against multidrug-resistant microorganisms known to cause various infections in humans and animals.

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