JOURNAL OF BASIC PHARMACOLOGY AND TOXICOLOGY
Original Research Article

Antibacterial activity of *Cyperus esculentus* (Tiger nut) and *Cucumis sativus* (Cucumber) against multiresistant bacteria isolates

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Abstract

The increase in antimicrobial resistance has become a great threat in the treatment of common infections. Antimicrobial resistance has direct and severe consequences on both morbidity and mortality rates of humans and animals: healing process is delayed; surgical operations and other medical procedures are endangered due to microbial infections and treatment costs increase. The ethanolic and aqueous extracts of *Cyperus esculentus* and *Cucumis sativus* were investigated against *Staphylococcus aureus*, *Listeria ivanovii*, *Bacillus cereus*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Escherichia coli*. The results revealed that the ethanolic and aqueous extracts of *Cucumis sativus* contain more bioactive components than ethanolic and aqueous extracts of *Cyperus esculentus*. The phytochemical analysis of the *Cucumis sativus* extracts showed the presence of flavonoids, saponins, alkaloids and steroids, while *Cyperus esculentus* showed the presence of alkaloids, resins, saponins, tannins and steroids. The aqueous extract of *Cucumis sativus* pulp had a high antibacterial activity against *Staphylococcus aureus* (IZD = 27 ± 0.27 mm) and *Bacillus cereus* (IZD = 26.5 ± 0.41 mm) at the 50 mg/ml concentration. Also, the ethanolic extract of *Cucumis sativus* pulp showed good activity at the same concentration against *Bacillus cereus* (IZD = 18.5 ± 0.49 mm) and *Serratia marcescens* (IZD = 16 ± 0.00 mm). In contrast, both the ethanolic and aqueous extracts of *C. sativus* and *C. esculentus* peels only showed very negligible activities against the test bacterial isolates. Also, the minimum inhibitory concentration (MIC) of the ethanolic and aqueous extracts of *C. sativus* pulp was lower (1.53 mg/ml) against *Bacilius cereus* and *Listeria ivanovii* than other bacteria. The minimum bactericidal concentration (MBC) of the extracts was only observed against *Staphylococcus aureus* (6.13 mg/ml) and *Listeria ivanovii* (3.06 mg/ml). These findings revealed that the ethanolic and aqueous extracts of *Cucumis sativus* pulp may have potential applications in the phytomedicine for treating human infections.

Keywords: *Cucumis sativus*, *Cyperus esculentus*, Antibacterial activity, Multi-resistant bacteria

1 Introduction

The increase in antimicrobial resistance has become a great threat in the current treatment of common infections [1]. The most remarkable is the fast-spreading multi-resistant bacteria species which cause different kinds of human and animal infections [2]. The World Health Organization recently declared antimicrobial resistance as one of the top 10 global public health threats [3].

Antimicrobial resistance has direct and severe consequences on both morbidity and mortality rates of humans and animals [4].

In effect, healing process is delayed, surgical operations and other medical procedures are at the risk of microbial infections, with the attendant surge in treatment cost [5].

According to the final report and recommendations of Jim O'Neill on tackling drug-resistance infections globally, about 700, 000 people die annually due to infections caused by drug-resistant microorganisms. This figure could rise to 10 million per year by 2050, if no proactive measure is taken [6]. Looking further into recent yearly accounts, about 33, 000 deaths with 1.1 billion Euros health care expenditures are recorded for European countries [7], while more than 35, 000 lives have been lost in the United States [8]. Again, the United States Centre for Medicare and Medicaid Services also reported that the spending rate on drug prescriptions increases to 3.7% in 2020. Meanwhile, the national health care estimate of the United State for 2020 is projected for $4.01 Trillion, to reach $6.19 Trillion by 2028. This will contribute to 19.7% increase in the United States National Gross Domestic Product (GDP) when compared to 2018 account [9]. Obviously, these negative impacts have kept spreading beyond the health sector and would frustrate the present global sustainable development agenda of the United Nations over 2030 if nothing is done now [10]. Considering the current dryness in the supply of new antibiotics to combat the list of priority pathogens [11] and the recommendations on the Global Action Plan issued by World Health Assembly [12], there is immediate need for quality-assured and cost-effective novel antibiotics remedies. The exploration of orthodox phytomedicine and a careful choice of innovative plants extracts for this demand is essential [13]. The knowledge of medicinal herbs is as old as man, whom in search of food and shelter discovered that the plants used as food could also cure them of certain

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ailments. Despite this primordial insight, identification of herbs appropriate for the treatment of a specific infection is apparently elusive [14]. Before now, several studies on the importance and therapeutic uses of plants have been done, which have continued to suggest a diverse plant species with such potentials [14]. Phytocompounds have a wide range of medicinal applications including anti-proliferative and anti-parasitic effects. For example, artemisinin isolated from *Artemisia annua* showed activity against malaria and was proven to be effective against multi-resistant strains of *Plasmodium falciparum* which causes malignant cerebral malaria in human [15]. Shadia *et al*, [16] reported that some herbs have been used as food additives, flavouring agents and natural food preservatives such as: rosemary, garlic, pepper, ginger, thyme etc. Herbs can also be used to treat asthma, eczema, premenstrual syndrome, rheumatoid arthritis etc. The herbs are considered safe to use as an antimicrobial agent, because they have been used for long time in the local traditions and their use is associated with minimal complications.

Cyperus esculentus has been described as a root tuber. It is from the family *Cyperaceae*. It produces a sweet almond-like tuber which is highly appreciated for their health benefits and nutritive value [14]. Also, *Cyperus esculentus* contain alkaloid, resins, tannins, sterols and saponins [17]. Furthermore, *Cyperus esculentus* was reported to contain high content of fiber, protein, sugar, oleic acid, glucose, phosphorous, potassium, and vitamin C and E [18]. On the other hand, *Cucumis sativus* have been described as a vining plant. It belongs to the family *Cucurbitaceae* and is a widely cultivated plant which is eaten in the unripe, green form in template and tropic zones. Also, *Cucumis sativus* contains many important components such as: glycosides, flavones, terpenoids, phytosterol, saponins, tannins etc. [19]. Furthermore, *Cucumis sativus* has been used in the past to treat diseases such as headaches, bleeding; dizziness and pale skin [19]. Therefore, this study investigated the *in vitro* antibacterial activities of *Cyperus esculentus* tubers and *Cucumis sativus* fruit extracts against multi-resistant bacteria (*Staphylococcus aureus, Bacillus cereus, Listeria ivanovii, Serratia marcescens, Pseudomonas aeruginosa* and *Escherichia coli*) isolates.

2 Materials and methods

2.1 Plant material

The fresh Tiger nut (*Cyperus esculentus* L) and Cucumber (*Cucumis sativus* L) were bought from Ogige Market in Nsukka, Enugu State, Nigeria. The samples in sealed plastic bags were conveyed to the Microbiology Laboratory, University of Nigeria, Nsukka, Nigeria.

2.2 Test microorganisms

The pure bacterial cultures (*Staphylococcus aureus*, *Listeria ivanovii*, *Bacillus cereus*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Salmonella* Wangata and *Escherichia coli*) were obtained from the stock cultures maintained in the Department of Microbiology Laboratory, University of Nigeria, Nsukka. The subcultures were maintained at 4 ^{o}C on Nutrient Agar (NA) slants.

2.3 Preparation of extracts

Cyperus esculentus tubers were thoroughly washed, macerated using mortar, and air-dried at room temperature for 72 hr. The dried *Cyperus esculentus* was ground into fine powder. Also, the *Cucumis sativus* was washed thoroughly. Then, the back was peeled off and-air dried with the pulp separately at room temperature for 72 hr. Both the peel and pulp were ground into fine powder. The extracts were stored in an airtight container.

Ethanol and aqueous extraction were carried out using the method described by Alo *et al* [20]., by immersing the sample in solvent in the ratio of 1:5 w/v in a conical flask. The set up was left for 72 hr with occasional shaking of the flask. Thereafter, the respective sample-solvent mixture were filtered out with a sieve and allowed to evaporate at room temperature. Afterwards, the extracts were scrapped using spatula and stored in the sample bottle. About 1g of each extract was weighed and dispensed into a sterile test tube. Then, 4 ml of 10% v/v dimethyl sulphoxide (DMSO) was used to dissolve the extract with vortexing to give a stock solution.

2.4 Phytochemical analysis

The phytochemical analysis of the extracts was carried out based on procedures outlined by Harbourne [21].

2.5 Standardization of the test microorganisms

The isolates were sub-cultured into Mueller Hinton broth overnight. The inoculum was prepared by dispensing the pure isolates into test tubes containing normal saline solution using sterile micropipette tips. The turbidity of this suspension was compared to a 0.5 McFarland standard equivalent to 1.5×10^8 cfu/ml [22].

2.6 Antibacterial susceptibility testing

The antibacterial susceptibility testing was done using standard antibiotic disk by Kirby-Bauer/ disk diffusion method. Using sterile swab sticks, the isolates were inoculated on the dried surface of MHA plates by streaking the swab over the entire agar surface. After about 15 min, the disks were placed over the agar plate using sterile forceps [23]. Thereafter, the plates were incubated at 37 ^{0}C for up to 18 hr. Zones of inhibition produced after incubation was measured in millimeter using meter rule. All the tested isolates showing resistance to three or more antibacterial drugs were further tested for their susceptibility to plant extracts.

2.7 Determination of antibacterial activity of ethanol and aqueous extracts

Agar well diffusion method according to Jorgensen and Turnidge [24], was used for antibacterial screening of the extracts. The *Cyperus esculentus* and *Cucumis sativus* (peel and pulp) extracts were serially diluted two-fold, to get the working concentrations of the samples. Also, 18 hr old cultures of bacteria isolates were inoculated in duplicates on Mueller Hinton Agar (MHA) plates. Thereafter, a sterile cork borer (6 mm diameter) was used to make wells on each plate, while 0.05 ml of the plant extracts were dispensed into each well. Gentamicin (10 mg/L) was dispensed into one of the wells which served as a positive control. The inoculated plates were left on the laboratory bench for about one hour to allow the extracts to diffuse properly into the agar. Thereafter, the plates were incubated at 37 ^{0}C for about 18 hr. Inhibition zone diameter (IZD) around each well after incubation was measured and recorded.

2.8 Determination of the minimum inhibitory concentration (MIC) of the Cucumis sativus pulp

Broth microdilution protocols according to the Clinical Laboratory Standard Institute (CLSI) approved reference document as previously described by Josep *et al.* [25], was used to determine minimum inhibitory concentration (MIC). Overnight cultures of the test bacterial isolates were prepared by sub-culturing the isolates in sterile Mueller-Hinton Broth (MHB) and incubated at 37 ^{0}C for 18 hr. Thereafter, the cultures were standardized to 0.5 MacFarland standard. The turbidity was adjusted to 0.5-1×10^6 cfu/ml. Each of the test tubes containing the serially – diluted extracts were inoculated with the standardized inocula. The set up was incubated at 37 ^{0}C for 18 hr. Thereafter, the tubes were observed for turbidity and the last tube without any turbidity or visible growth was recorded as the MIC.

2.9 Determination of minimum bactericidal concentration (MBC) of Cucumis sativus pulp

The sample from each Eppendorf tubes used in minimum inhibitory concentration that showed no visible growth after incubation were streaked over a Muller Hinton Agar surface and then incubated at 37 ^{0}C for 18 hr. The lowest concentration of the extracts that showed no growth on the MHA plate after 18 hr incubation was taken as minimum bactericidal concentration (MBC).

3 Results

The result of antibacterial resistance of Gram-positive and Gram-negative bacteria respectively revealed that all the bacterial isolates resisted three to four antibiotics except *Salmonella wangata* which was susceptible to all the drugs used. The result of the qualitative analysis of the phytochemical component revealed that the ethanolic and aqueous extracts of *Cucumis sativus* contain more bioactive components than ethanolic and aqueous extracts of *Cyperus esculentus*. The phytochemical analysis of the *Cucumis sativus* extracts showed the presence of flavonoids, saponins, alkaloids and steroids, while *Cyperus esculentus* showed the presence of alkaloids, resins, saponins, tannins and steroids. The aqueous extract of *Cucumis sativus* pulp showed more antibacterial activity with the highest against *Staphylococcus aureus* (inhibition zone diameter, IZD = 27 ± 0.27 mm) at 50 mg/ml concentration and *Bacillus cereus* (IZD = 26.5 ± 0.41 mm) at the same concentration. Also, the ethanolic extract of *Cucumis sativus* pulp showed good activity at 50 mg/ml against *Bacillus cereus* (IZD = 18.5 ± 0.49 mm) and *Serratia marcescens* (IZD = 16 ± 0.00 mm). However, both the ethanolic and aqueous extracts of *C. sativus* and *C. esculentus* peels showed little activities against the test bacterial isolates. More so, the minimum inhibitory concentration (MIC) of the ethanolic and aqueous extracts of *C. sativus* pulp was lower (1.53 mg/ml) against *Bacilius cereus* and *Listeria ivanovii* than other bacteria. The minimum bactericidal concentration (MBC) of the extracts was only observed against *Staphylococcus aureus* (6.13 mg/ml) and *Listeria ivanovii* (3.06 mg/ml).

Table 1. Phytochemical composition of *Cyperus esculentus* and *Cucumis sativus*

Phytochemical content	*Cyperus esculentus*	*Cucumis sativus*
Alkaloid	+ + +	+
Glycoside	–	+ + +
Steroid	+ + +	+ + +
Saponins	+	+ +
Tannins	+	+ +
Terpenoids	–	+ + +
Flavonoids	–	+ + +
Resins	+ + +	+ + +

Key: - =not detected; + = slightly present;
++ = moderately present; +++ = highly present.

Table 2. Antibacterial susceptibility test (Gram-positive bacteria)

Inhibition zone diameter (mm) and concentration (µg/ml)									
Bacterial Isolates	**CAZ**	**CRX**	**GEN**	**CTR**	**ERY**	**CXC**	**OFL**	**AUG**	**MAR INDEX**
S. aureus	8.5 ± 0.34	0	19 ± 0.32	12.5 ± 3.54	19.5 ± 0.16	14 ± 0.38	24 ± 0.29	16.5 ± 0.17	0.38
B. cereus	0	0	21 ± 0.00	12 ± 0.00	13.5 ± 0.19	0	21 ± 0.00	11 ± 0.00	0.625
P. aeruginosa	22.5 ± 0.74	0	13 ± 0.39	18.5 ± 0.49	0	0	0	0	0.63
L. ivanovii	0	10 ± 0.00	18.5 ± 0.16	14 ± 0.38	21 ± 0.31	10 ± 0.00	21.5 ± 0.15	15 ± 0.00	0.5

Key: CAZ- 30 µg ceftazidime, CRX- 30 µg cefuroxime, GEN- 10 µg gentamycin, CTR- 30 µg ceftriaxone, ERY- 5 µg erythromycin, CXC- 5 µg cloxacillin, OFL- 5 µg ofloxacin, AUG- 30 µg augmentin.

Table 3. Antibacterial susceptibility test (Gram-negative bacteria)

Inhibition zone diameter (mm) and concentration (µg/ml)									
Bacterial isolates	**CAZ**	**CRX**	**GEN**	**CXM**	**OFL**	**AUG**	**NIT**	**CPR**	**MAR INDEX**
E. coli	16 ± 0.00	0	10±0.00	0	18±0.00	0	17.5±0.51	16.5±0.17	0.5
S. marcescens	0	0	21±0.31	0	20±0.00	11.5±0.3	25±0.00	20.5±0.16	0.5
S. wangata	18.5 ±0.16	20±0.00	22±0.00	19.5±0.16	24±0.29	17.5±0.7	23.5±0.44	19.5±0.16	0

Key: CAZ- 30 µg ceftazidime, CRX- 30 µg cefuroxime, GEN- 10 µg gentamicin, CXM- 5 µg cefixime, OFL- 5 µg ofloxacin, AUG- 30 µg augmentin, NIT- 30 µg nitrofurantoin, CPR- 5 µg ciprofloxacin

Table 4. Antibacterial activity of aqueous extract of *Cucumis sativus* pulp against bacteria isolates

Inhibition zone diameter (mm) and concentration (µg/ml)						
Bacterial isolates	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**3.125 mg/ml**	**Positive control (Gentamicin)**
P. aeruginosa	-	14 ± 0.00	17 ±2.40	10 ± 0.00	-	31.5 ± 0.38
S. aureus	27 ± 0.27	-	14 ± 0.38	10.5 ± 0.31	-	31.5 ± 0.38
B. cereus	26.5± 0.41	11.5± 0.21	18 ± 0.47	13.5 ± 0.19	-	34.5 ± 0.12
S. marcescens	-	-	12.5± 1.00	-	-	30 ± 0.00
L. ivanovii	-	-	17 ± 1.03	11 ± 0.00	-	34.5 ± 0.12

Key: - = no activity, SD = ±

Table 5. Antibacterial activity of ethanolic extracts of *Cucumis sativus* pulp against test bacterial isolates

Inhibition zone diameter (mm) and concentration (µg/ml)						
Bacterial isolates	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**	**3.125 mg/ml**	**Positive control (gentamicin)**
P. aeruginosa	-	15 ± 0.00	11 ± 0.00	10 ± 0.00	-	34 ± 0.00
S. aureus	13.5 ± 0.19	14.5 ± 0.93	12.25 ± 0.00	10 ± 0.00	10 ± 0.00	30 ± 0.00
B. cereus	18.5 ± 0.49	12.5 ± 0.20	13 ± 00	12 ± 0.00	13 ± 0.00	30 ± 0.00
S. marcescens	16 ± 00	13.5 ± 0.19	12.5 ± 0.00	-	10 ± 0.00	32.5 ± 0.62
L. ivanovii	13.5 ± 0.58	16.5 ± 1.22	11.5 ± 0.21	12.5 ± 0.00	12.5 ± 0.06	34 ± 0.17

Key: - = no activity, SD = ±

Table 6. Antibacterial activity of ethanolic extract of *Cucumis sativus* peel against the test bacterial isolates

Inhibition zone diameter (mm) and concentration (µg/ml)						
Bacterial isolates	**200 mg/ml**	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**Positive control (Gentamicin)**
E. coli	5.00 ± 1.40	10.5 ± 0.43	-	-	-	32.5 ± 0.62
S. aureus	-	-	-	-	-	28.5 ± 0.39
S. aureus	-	-	-	-	-	28.5 ± 0.19
S. marcescens	7.5 ± 3.87	5.00 ± 0.00	-	-	-	23 ± 0.29
L. ivanovii	5.00 ± 0.00	-	6.00 ± 1.40	-	-	29.5 ± 0.13

Key: IZD, inhibition zone diameter (mm) ± Standard deviation (SD)

Table 7. Minimum inhibitory concentration (MIC) of *Cucumis sativus* pulp against test bacterial isolates

Bacterial isolates	**Aqueous (mg/ml)**	**Ethanolic (mg/ml)**
P. aeruginosa	3.07	12.50
S. aureus	25.00	12.50
B. cereus	3.06	1.53
S. marcescens	6.13	12.50
L. ivanovii	25.00	1.53

4 Discussion

The result of antibiotics susceptibility test of Gram-positive and Gram-negative bacteria revealed that *Salmonella wangata* was susceptible to all the drugs used. However, resistance to at least three antibiotics was observed in the other bacterial isolates. The result may reflect the misuse or overuse of antibiotics in medical, agricultural and livestock sectors which accelerates the spread of antimicrobial resistance according to the report of OECD [7]. The drugs that were once susceptible to bacteria became non-susceptible to two or three class of antibiotics overtime. In this study, the aqueous extract of *Cucumis sativus* pulp showed high antibacterial activity compared to the ethanolic extract of *Cucumis sativus* pulp. A previous work has shown that *Cucumis sativus* pulp contain bioactive components such as flavonoid and phenol [26, 27]. However, the ethanolic extract of *Cucumis sativus* peel showed slight antibacterial activity against the test bacterial isolates. While the aqueous peel extract did not show activity against the test bacterial isolates. This report was in agreement with the study of How *et al*, [28] which stated that the aqueous extracts of *Cucumis sativus* peel were inactive against all the microorganisms tested. In contrast, Sheila *et al*, [29] reported that *Cucumis sativus* peel extract inhibited all the tested organisms. Variation in results across different studies may however, depend on the type of solvents used which in this study are aqueous and ethanol.

In this study, the result of the antibacterial activity of aqueous extracts of *Cyperus esculentus* against test bacterial isolates showed slight antibacterial activity. The result correlates with the report of Kiran *et al*, [30] which stated that the aqueous extracts inhibited the test bacterial isolates at different concentrations of 10, 20, 30, 40 mg/L etc. The antibacterial activity of ethanolic extract of *Cyperus esculentus* showed no antibacterial effect against all the test bacterial isolates. This finding contradicts the reports of Arikpar and Alade [31] and Prakash and Ragavan [32], which showed that ethanolic extract of *Cyperus esculentus* has antibacterial activity against the bacterial isolates tested. The minimum inhibitory concentration (MIC) revealed that the ethanolic and aqueous extract of *Cucumis sativus* pulp all showed good inhibitory activity with the highest MIC of 1.53 mg/ml against *Bacillus cereus* and *Listeria ivanovii* respectively. This observation aligns with the study of Georgios *et al*, [33], that revealed interesting and promising antibacterial activity of *Cucumis sativus* pulp. Furthermore, the ethanolic and aqueous extracts of *Cucumis sativus* pulp showed minimum bactericidal concentration (MBC) against both *Staphylococcus aureus* (MBC = 6.13 mg/ml) and *Listeria ivanovii* (3.06 mg/ml). The findings in the study constitute further justification that many plant materials have good and proven therapeutic potentials as we have demonstrated in several other studies [34-43].

5 Conclusion

This study revealed that *Cucumis sativus* pulp extracts showed higher inhibitory activity against the test bacterial isolates. More so, this study showed that *Cucumis sativus* pulp could be an effective remedy for the treatment of human bacterial infections especially after purification of the bioactive components, and may be used in the production of phytomedicine.

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