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Antibacterial Activity of Aqueous and Ethanol Fruit Extracts of *Cucumis sativus* Linn. Against Selected Microorganisms at the University of Maiduguri Teaching Hospital, Maiduguri

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ABSTRACT

Cucumber (*Cucumis sativus* Linn) [Cucurbitaceae] is a famous vegetable crop used for food since ancient times but little is known of its antibacterial potential. This study investigated the phytochemical constituents and antibacterial activities of the aqueous and ethanol fruit extracts of the plant against some clinical isolates (*Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis* and *Corynebacterium spp*) using the disc diffusion method. The qualitative phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, terpenoids, cardenolites and flavonoids. *Corynebacterium spp* was the most susceptible (with maximum inhibition zone of 30.00±0.00 mm). The two extracts had varied antibacterial activity at the same level. At the concentration of 400mg/ml, aqueous extract inhibited *S. aureus, Corynebacterium spp, E. coli* and *K. pneumoniae,* while ethanol extract inhibited *S. aureus, S. pyogenes, S. typhi* and *Corynebacterium spp*. *B. subtilis* was inhibited only at the highest (significant) concentration of 600mg/ml (7.00±0.00 mm) [$p<0.05$]. The MIC value for both extracts against *Corynebacterium spp* was 25mg/ml. The MBC value against *Corynebacterium spp* was observed at 50mg/ml and 25mg/ml for the aqueous and ethanol extract respectively. The results suggest that the ethanol extract was bactericidal at low concentration while the aqueous extract was bacteriostatic at low concentration and bactericidal at high concentration against *Corynebacterium spp*. The difference between the MBC for the two extracts was significant ($p<0.05$). In conclusion, the results of this study showed that extract of *C. sativus* could be a potential source of natural antibacterial agent.

Keywords: Antibacterial activity; *Cucumis sativus* Linn; Ethanol extract; Phytochemical screening

INTRODUCTION

Virtually in all cultures, medicinal plants have been used as important source of medicine to prevent and treat microbial infections (Dar *et al.,* 2017). Medicinal plants are employed either directly as folk remedies, in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines (Dhama *et al*., 2014). Cucumber (*Cucumis sativus* Linn.), is an annual plant which belongs to the gourd family Cucurbitaceae (Fapohunda *et al*., 2018). It is one of the most important vegetables used in Traditional Chinese Medicine as a result of their anti-inflammatory activity (Fapohunda *et al*., 2018). *C. sativus* fruit has been reported with various activities such as cytotoxic, antifungal, antacid, carminative, hepatoprotective, hypoglycemic, hypolipidemic and healing activities (Gopalakrishnan and Kalaiarasi, 2014; Sahu and Sahu, 2015; Mandey *et al*., 2020; Saeedi *et al*., 2020). The spread of resistant organisms in the clinical setting presents a considerable public health concern. In considering the antibiotic resistance as a major problem in the treatment of bacterial infections, there is need to find alternative treatment to infectious diseases using plants which are found in almost every part of our environment. The extracts of these plants may overcome the antibiotic resistance and serve as a source of novel drugs for the treatment of these diseases. Hence, this study investigated the antibacterial activity of *C. sativus* against some selected clinical isolates collected from University of Maiduguri Teaching Hospital in Maiduguri.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh fruit of *C. sativus* Linn. was collected from Mohammed Lawal College of Agriculture Maiduguri, Borno State. Identification and authentication of the plant was done by plant Taxonomist, Professor S. S. Sanusi of Department of

Biological Science, Faculty of Sciences, University of Maiduguri and voucher specimen (DCPT: 017) was deposited in Pharmacology Laboratory, Department of Clinical Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, University of Maiduguri, Maiduguri, Borno State.

Preparation of Fruit Extracts

The fruits of *C. Sativus* were cut into pieces, shade-dried at room temperature and powdered using a wooden mortar and pestle. Two hundred and fifty grams (250 g) of the dried fruit powder was weighed accurately and extracted using distilled water and ethanol respectively with the aid of reflux condenser. It was filtered rapidly and the filtrate was transferred to a flat bottom flask (dish), then evaporated to dryness on a water bath and stored in desiccator. The percentage yield was determined for each solvent using the formula below:

Percentage yield (%) = Final weight (g) / Initial weight (g) × 100

Phytochemical Analysis of the Fruit Extracts

Each of the extracts was subjected to qualitative phytochemical screening to test for the presence of the following constituents: alkaloids, tannins, flavonoids, terpenoids, carbohydrates, soluble starch, cardenolites, cardiac glycosides, saponin glycosides, phlobatannins and anthraquinones, as described by Brain and Turner (1975), Vishnoi (1979), Markham (1987), Silver *et al* (1998), Sofowora (2008) and Evans (2009).

Antibacterial Studies of *C. sativus* Aqueous and Ethanol Extracts

Test Microorganism

Clinical isolates of *Staphylococccus aureus, Streptococcus pyogene, Bacillus subtilis*, and *Corynebacterium spp*., *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were obtained from the Department of Medical Microbiology, University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Borno State.

Antibacterial Sensitivity Test of the Extracts

The antimicrobial sensitivity test was carried out using the agar plate disc diffusion technique as described by Usman & Osuji (2007). The stock concentration of 1000 mg/ml of the aqueous extract and 500 mg/ml of the ethanol extract by dissolving 10 g and 5 g respectively into 20 ml and 10 ml sterile distilled water was used in carrying out the tests. From the standard suspension of the test organism prepared, 0.5 ml volume of each of the concentrations prepared was dispensed into each of the 9 mm bored holes to afford 200 mg, 400 mg and 600 mg hole of both aqueous and ethanol extracts, respectively. After incubation at 37^0 C for 24 hours, the average diameter of three readings of the clear zone around the hole was recorded as the measure of inhibitory level of the extract against the test bacteria and reported as Mean±SEM. The dilution ratio for gram-positive bacteria and gram-negative bacteria was 1:1000 and 1:5000 respectively using peptone water (Usman & Osuji, 2007). The plates were inoculated with the same standardized inoculum to check for the activities of standard drugs against the tested organisms using standard antimicrobial disc Ciprofloxacin (5 µg) and Tetracycline (30 µg).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation (Andrews, 2001). In this method the broth dilution test was utilized, the concentration ranging from 12.5 mg /ml to 200 mg/ml for both aqueous and ethanol extracts using nutrient broth and later inoculated with 0.2 ml suspension of the test organisms. After 18 hours of incubation at 37^0C, the test tubes were observed for the presence of turbidity. The least concentration where no turbidity was observed was determined and recorded as the minimum inhibitory concentration (MIC). The minimal bactericidal concentration was determined from the broth dilution test resulting from the MIC test tubes as described by Usman and Osuji (2007) by inoculating the content of each test tube on a nutrient agar plate. The plates were then incubated at 37^oC for 24 hours. The minimum bactericidal concentration (MBC) is the lowest concentration of antimicrobial that will prevent the growth of an organism after subculturing into antibiotic free media (Andrews, 2001).

Statistical Analysis

Data obtained was subjected to statistical analyses using suitable statistical software (GraphPad InStat version 5.01, 2007). The zones of inhibition of the extracts were expressed as means and standard error of mean and were compared with that of the standard drugs. Significance was inferred at $p<0.05$.

RESULTS

Extracts Yield

The yield of the aqueous and ethanol extracts of *C. sativus* appeared dark brown to coffee brown colour, gummy mass with pungent smell. The percentage yield of the aqueous and ethanol extracts was 25.03 % and 23.73 % respectively as shown in Figure 1.

Phytochemical Studies

The summary of the phytochemical screening for both aqueous and ethanol extracts are presented in Table 1. Carbohydrates, terpenoids, cardiac glycosides and cardenolites were present in both aqueous and ethanol extracts of *C. sativus* while flavonoids were present only in ethanol extract.

Table 1: Phytochemical Screening of the Aqueous and ethanol Extracts of *C. sativus*

	Results	
Plant Constituents/Test	**Aqueous**	**Ethanol**
Cabohydrates		
i. General test (Molisch's Test)	+	+
ii. Test for monosaccharide (Barfoed's Test)	+	+
iii. Test for free reducing sugar (Fehling's test)	+	+
iv. Test for combined reducing sugar	+	+
v. Test for ketoses	+	+
Test for soluble starch	+	-
Test for Anthraquinones		
i. Test for free anthraquinone	-	-
ii. Test for combined anthraquinone	-	-
Test for cardiac glycosides		
i. Salkowski's test	-	-
ii. Lieberman-Burchard's test	-	+
Test for Terpenoids	-	+
Test for Flavonoids		
i. Shinoda's test	+	+
ii. Ferric chloride	-	-
iii. Lead acetate	-	-
iv. Sodium hydroxide	-	-
Test for saponins		
i. Frothing test	-	-
Test for phlobatannins	-	-
Test for tannins		
i. Ferric chloride	-	-
ii. Lead acetate	-	-
Test for alkaloids		
i. Dragendoff's reagent	-	-
ii. Mayer's reagent	-	-
Test for cardenolides		
i. Keller-Killianis test	+	+

Key: - = Absent
+= Present

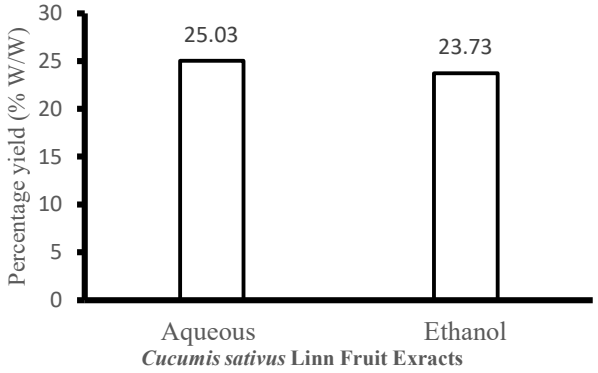

Figure 1: Percentage yields of the aqueous and ethanol fruit extracts of *Cucumis sativa* Linn

Antibacterial Susceptibility of the Aqueous and Ethanol Extracts of *C. sativus*

The results of the antibacterial susceptibility of the extracts on the microorganisms are shown in Table 2 and Table 3. Aqueous extract inhibited the growth of gram positive bacteria *Corynebacterium spp.* at all concentrations whereas *S. aureus* was only inhibited at concentration of 400 mg/ml. Gram negative bacteria *E. coli* and *K. pneumonia* were susceptible at 400 mg/ml. The ethanol extract inhibited the growth of gram positive bacteria *Corynebacterium spp.* and *S. pyogene* at all concentrations, while *S. aureus* and *B. subtilis* were susceptible at 400 mg/ml and 200 mg/ml respectively. The only gram negative bacteria that was inhibited by the ethanol extract was *S. typhi* at 400 mg/ml. While the fungal strain (*C. albicans*) was resistant at various concentrations used. *Corynebacterium spp.* recorded the largest zone of inhibition of 30.00 ± 0.00 mm at 600 mg/ml out of the sensitive microorganisms tested using the ethanol extract.

MIC Determination

The result of the MIC assay is presented in Tables 4 (Aqueous extract) and 5 (Ethanol extract). It shows the concentration of the extract which was bacteriostatic to the organism (*Corynebacterium spp.*). The MIC value of *Corynebacterium spp.* was 50 and 25 mg/ml for both aqueous and ethanol extracts, respectively.

MBC Determination

The result of the MBC assay is presented in Tables 6 (Aqueous extract) and 7 (Ethanol extract). It shows the concentration of the extract which was bactericidal to the

organism (*Corynebacterium spp.*). The aqueous extract had MBC value at 50mg/ml while ethanol extract MBC value is at 25 mg/ml.

DISCUSSION

The emergence of bacterial resistance to most of the available antibacterial agents has led the researchers to systematically look for new agents with novel mechanism of action in order to reduce or eliminate the problem of antibacterial resistance (Jackson *et al*., 2018; Serweci´nska, 2020). Plant extracts with antibacterial properties can be of great value in the treatments of infection caused by these pathogenic microorganisms (Ventola, 2015).

Table 2: The zone of inhibition produced by the aqueous extract of *Cucumis sativus*

	Diameter of zones of Inhibition (mm)/Resistance				
Organism used	200 mg/ml	400 mg/ml	600 mg/ml	Ciprofloxacin	Tetracycline
Escherichia coli	R	7.00±0.00	10.00±0.00	18.00±0.00	9.00±0.00
Salmonella typhi	R	R	R	28.00±0.00	19.67±0.33
Klebsiella pneumonia	R	7.00±0.00	9.00±0.00	17.00±0.00	R
Pseudomonas aeruginosa	R	R	R	35.00±0.00	R
Staphylococccus aureus	R	8.00±0.00	10.00±0.00	20.00±0.00	10.00±0.00
Streptococcus pyogene	R	R	R	35.00±0.00	15.00±0.00
Bacillus subtitis	R	R	R	25.00±0.00	11.00±0.00
Corynebacterium spp	19.67±0.33	25.00±0.00	30.00±0.00	35.00±0.00	R

The results are expressed as mean ± SEM, n=3 per group
Key: R= Resistant

Table 3: The zone of inhibition produced by the ethanol extract of *Cucumis sativus*

	Diameter of zones of Inhibition (mm)/Resistance				
Organism used	200 mg/ml	400 mg/ml	600 mg/ml	Ciprofloxacin	Tetracycline
Escherichia coli	R	R	R	18.00±0.00	9.00±0.00
Salmonella typhi	R	7.67±0.33	10.00±0.00	28.00±0.00	19.67±0.33
Klebsiella pneumonia	R	R	R	17.00±0.00	R
Pseudomonas aeruginosa	R	R	R	35.00±0.00	R
Staphylococccus aureus	R	7.00±0.00	9.00±0.00	20.00±0.00	10.00±0.00
Streptococcus pyogene	7.00±0.00	9.00±0.00	12.00±0.00	35.00±0.00	15.00±0.00
Bacillus subtitis	R	R	7.00±0.00	25.00±0.00	11.00±0.00
Corynebacterium spp	18.00±0.00	23.00±0.00	28.00±0.00	35.00±0.00	R

The results are expressed as mean ± SEM, n=3 per group
Key: R= Resistant

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for *Corynebacterium spp* Isolate against Aqueous and Ethanolic Extracts of *Cucumis sativus*

Test	**Extract**	**Concentration of extract used in mg/ml**				
		12.5	**25**	**50**	**100**	**200**
MIC	Aqueous	+	+	α	-	-
	Ethanol	+	α	-	-	-
MBC	Aqueous	+	+	β	-	-
	Ethanol	+	+	β	-	-

KEY:
+ = Growth
- = No growth
α = MIC i.e The lowest concentration at which there was no growth
β = MBC i.e The lowest concentration at which there was death

In this study, phytochemical screening of the fruit extracts of *C. sativus* showed the presence of carbohydrates, cardiac glycosides, terpenoids, cardenolites and flavonoids which have been reported with antibacterial activity (Akanmu *et al.,* 2019). This differs with the report of Mallik and Akhter (2012) which showed the presence of saponins, tannins and alkaloids while flavonoids were absent in the *C. sativus* ethanol extract. This might be as a result of difference in methods of extraction employed and geographical location.

The *in vitro* antibacterial screening of aqueous extract of *C. sativus* showed a considerable inhibition against *E. coli, K. pneumonia, S. aureus* and *Corynebacterium spp.* while ethanol extract was active against *S. typhi*, *S. aureus, S. pyrogenes* and *Corynebacterium spp*. The inhibitory action

was found to increase with increase in concentration against susceptible bacterial strains. Similar result was obtained by Georgios *et al.* (2010) in which the extract of *C. sativus* peel inhibited the growth of *E. coli, S. aureus* and *K. pneumonia.* The result of the ethanol extract is in agreement with the previous work done by Ankita *et al.* (2012) in which the pathogens tested were highly sensitive to the methanol extract with exception of *E. coli* and *P. aeruginosa*.

The result of the minimum inhibitory concentration (MIC) showed that the extracts were active against the tested microorganism (*Corynebacterium specie)*. This is indicated with MIC of 25 and 50 mg/ml for both aqueous and ethanol extracts respectively.

The minimum bactericidal concentration (MBC) data obtained showed that ethanol extract has MBC value of 25 mg/ml, while the aqueous extract MBC value was 50 mg/ml. This difference in MBC value may be attributed to the difference in the degree of polarity of the solvents used. The MBC data obtained from the evaluation of *Corynebacterium spp.* against aqueous extract was found to be higher than the MIC suggesting that it is bacteriostatic at low concentration and bactericidal at high concentration.

The ability of the extracts to inhibit the growth of these organisms *in-vitro* may be due to the presence of flavonoids which are in both aqueous and ethanol extracts. Flavonoids, terpenoids and cardiac glycoside are known to have antibacterial activities and curative properties against bacterial pathogen (Atanasov *et al*., 2015; Gadisa *et al*., 2019; Gadisa and Tadesse, 2021). The plant studied here can be seen as a potential source of useful antimicrobial drugs.

In conclusion, the fruit extracts of *C. sativus* contain; carbohydrates, cardiac glycosides, terpenoids, cardenolites and flavonoids which could be responsible for its antibacterial activity. The fruit extracts of *C. sativus* showed antibacterial activity against some selected organisms which justify its ethnomedicinal use. The ethanol fruit extract of *C. sativus* was found to be more sensitive to the tested gram positive bacteria at different concentrations recorded than aqueous extract. However, further studies need to be done in order to establish the mechanism of action and isolate, identify, characterize and elucidate the structure of the bioactive compounds and to clarify the *in-vivo* potential of this plant in the management of human disease resulting from bacterial infections.

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Authors' Contributions

The study was designed by OAS, AOA and HHY. The plant and seeds collection and the experimental procedure were done by OAS, AOA, HHY and IG. STB, AOA, IG and HHY helped in collation of data, analysis of data and interpretation of data. OAS, LMP and AOA wrote the first draft of the manuscript, while STB, LMP and AOA managed the literature search. OAS, STB, AOA and LMP helped to review and edit the manuscript.

Conflict of Interests

The authors have no conflict of interest to declare.

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