

Original Research Article

# *In vitro* Antimicrobial Activity of *Cucumis metuliferus* E. Mey. Ex. Naudin Fruit Extracts against *Salmonella gallinarum*

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## Abstract

The fruit of *Cucumis metuliferus* was collected at Vom, Jos South Local Government Area of Plateau State, this was cleaned, sliced, air dried, pulverised and cold extracted with solvents of different polarities. The (n-hexane, chloroform, methanol and water) extracts of *C. metuliferus* were tested for *in vitro* antimicrobial assay against *Salmonella gallinarum* at varying concentrations, 200 mg/ml to 1000 mg/ml using the disc diffusion method. There was no zone of inhibition by the n-hexane and chloroform extracts at 1000 mg/ml, it was resistant. The methanol extract showed zones of inhibition of  $8.33 \pm 0.52$ ,  $9.67 \pm 0.52$ ,  $11.67 \pm 1.03$ ,  $13.67 \pm 0.52$  and  $14.67 \pm 0.52$  mm at 200, 400, 600, 800 and 1000 mg/ml respectively. The water extract showed zones of inhibition of  $7.50 \pm 0.55$ ,  $8.50 \pm 0.55$  and  $9.67 \pm 0.52$  mm at 600, 800 and 1000 mg/ml respectively. The zones of inhibition for the standard antibiotics ciprofloxacin 5 µg, chloramphenicol 10 µg and augmentin® 30 µg was  $19.33 \pm 1.03$ ,  $10.66 \pm 0.52$ ,  $7.33 \pm 0.52$ , mm respectively, while tetracycline 30 µg, erythromycin 5 µg, ceftazidime 30 µg and oxacillin 1µg were resistant. The MIC and MBC of 50 mg/ml was recorded for the methanol extract. This study therefore showed that the fruit extracts of *Cucumis metuliferus* has antimicrobial activity and may probably provide the basis for its use in traditional medicine.

**Keywords:** Antimicrobial activity, *Cucumis metuliferus*, *Salmonella gallinarum*, zone of inhibition.

## Introduction

Salmonella remains a primary cause of food poisoning worldwide and massive outbreaks have been witnessed in recent years. The Center for Disease Control and Prevention (CDCP) estimated that approximately 1.4 million cases of salmonellosis were annually reported in the United States [1] and the European Union (EU) also reported more than 100,000 cases [2]. Salmonellae are the leading cause of morbidity and mortality in poultry and lead to significant economic losses [3, 4]. Fowl typhoid is a disease of poultry and it should be a notifiable disease [5]. Recent studies have shown that the prevalence of antibiotic resistant Salmonella in humans and animals is increasing [6, 7], thus, novel, efficient and safe remedies for salmonellosis are necessary and these have necessitated a search for new antimicrobial substances from other sources including plants [8]. The plant *Cucumis metuliferus* (Cucurbitaceae) is a monoecious annual herb with staminate flowers that grows wild [9]. It flowers and fruits from July to September and the fruits ripen from October to December [10]. It was documented that the highest inhibitory effect of *Guiera senegalensis* and *Zizyphus mauritania* on *Salmonella gallinarum* was seen with the methanolic extracts than with the aqueous extract [11].

Much work has been reported on the antiviral properties (especially, Newcastle and Gumboro diseases) of *C. metuliferus*, but no work has been documented on the antibacterial activity of *C.*

*metuliferus* fruits, therefore, this study is designed to find out the *in vitro* antibacterial activity of various extracts of *C. metuliferus* fruits against *Salmonella gallinarum*.

## Materials and Method

### Plant Collection and Identification

The fruits of *C. metuliferus* were collected in Vom village in Jos South Local Government Area, Plateau State, Nigeria in Nov. 2012. The plant was identified and authenticated by a plant taxonomist Prof. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Maiduguri.

### Preparation and Extraction of Plant Material

The ripe fruits of *C. metuliferus* were collected, cleaned, sliced, air dried and pulverised in the laboratory at National Veterinary Research Institute, Vom, Plateau State and this was kept in an air tight container until used. The powder (1.5 kg) was weighed and stored at room temperature in an air tight bottle, prior to use. The dried powder was extracted using solvents of different polarities (n-hexane, chloroform, methanol and distilled water) after maceration for 24 h and then filtered according to the method of [12].

### n-Hexane Extraction (CHE)

1500 g of the fine powder of *C. metuliferus* was weighed and divided equally into two round bottom flasks, macerated in 2.5 L of n-hexane, shaken and allowed to stand for 24 hr. The supernatant was filtered using Whatman No. 1 filter paper and the filtrate was poured onto a tray and allowed to evaporate under a constant flow of air. The final crude extract obtained was stored at 4 C. The yield of the crude n-hexane extract (CHE) was then calculated.

### Chloroform Extract (CCE)

To 1318.91 g of the air-dried marc obtained from n-hexane extraction was added 2.5 L of chloroform and allowed to stand for 24 hr, after which the sample was shaken vigorously before filtration using Whatman No.1 filter paper. The yield of the crude chloroform extract (CCE) was calculated.

### Methanol Extract (CME)

To 1315.20 g of the air-dried marc obtained from chloroform extraction was added 2.5 L of methanol was added and shaken vigorously and allowed to stand for 24 hr before filtering with Whatman No. 1 filter paper. The yield of the crude methanol extract (CME) was calculated.

### Crude Aqueous Extract (CAE)

To 1312.20g of the air-dried marc obtained from methanol extraction was added 2.5 L of distilled water and allowed to stand for 24 hr, after which the sample was shaken vigorously before filtration using Whatman No.1 filter paper. The yield of the crude aqueous extract (CAE) was calculated.

## Antimicrobial Studies

### Antimicrobial Agents

Standard antibacterial agents ciprofloxacin (CIP) 5 µg/disc, chloramphenicol (CHL) 10 µg/disc, augmentin® (AUG) (amoxicillin and clavulanic acid) 30 µg/disc, tetracycline (TET) 30 µg/disc, erythromycin (ERY) 5 µg/disc, fortum® (ceftazidime) (CEF) 30 µg/disc and oxacillin (OXA) 1 µg/disc (Oxoid Ltd, Basingstoke, Hampshire, England) were applied in the test and their zones of inhibition were compared with those of the extracts.

### Preparation of Concentrations of the Extract

Stock solution of each of the extracts was prepared by weighing 0.2 g, 0.4 g, 0.6 g, 0.8 g and 1.0 g of each of the extracts (CHE, CCE, CME and CAE) using a digital scale and to each of the extracts was added 1 ml of distilled water to obtain the following concentrations respectively 200, 400, 600, 800 and 1000 mgml<sup>-1</sup> from the fruit extracts.

### Antimicrobial Sensitivity Tests

Antimicrobial susceptibility testing was determined using a modification of the Kirby-Bauer Disk diffusion method as recommended by the National Committee of Clinical Laboratory Standards [13] and Clinical and Laboratory Standard Institute [14]

to determine the antibacterial activity of all the extracts of *C. metuliferus* fruit. Discs containing different concentrations of dissolved extracts were prepared with sterilized filter papers (Whatman No.1; 6 mm in diameter using a paper punch) soaked in different concentrations (200, 400, 600, 800 and 1000 mgml<sup>-1</sup>) of the extracts. The discs were dried at 50°C.

### Standardization of Inoculum

Laboratory isolates of pure culture of *Salmonella gallinarum* from an 18-hour plate culture were obtained from the National Veterinary Research Institute, Vom. A sterile wire loop was used to pick 2 to 3 colonies of *Salmonella* isolate and emulsified in a tube containing 5ml of sterile physiological saline. The tube containing the bacterial suspension was inserted into a sensititre nephelometer (TREK Diagnostic Systems, UK) after calibration with a standard. Adjustment was made with extra inoculums or diluents, where necessary until 0.5 Mcfarland standards was obtained [14].

### Inoculation of Test Plates

Optimally, within 5 to 10 minutes after adjusting the turbidity of the inoculums suspension, the inocula were spread on the surface of dried nutrient agar plates with sterile cotton wool swabs, which had been dipped in the diluted suspension of the organism. The plates were allowed to stand for absorption, incubated at 37°C for 30 minutes before applying the drug impregnated discs.

### Application of Discs to Inoculated Agar Plates

The extract discs were applied aseptically and evenly dispensed onto the surface of the inoculated agar plates. The treated plates were inverted and incubated at 37°C for 24 hours. The same procedure was carried out using the standard drugs (ciprofloxacin, chloramphenicol, augmentin®, tetracycline, erythromycin, ceftazidime and oxacillin as the positive control. A plate without the antibiotic or extract disc was set up as the negative control experiment.

### Examination of Plates and Interpretation of Results

Each plate was examined after 24h of incubation. The zone of inhibition above 6 mm diameter of each isolate was used as a measure of susceptibility to the extracts and this was compared to that of the standard antibiotics [14].

### Determination of Minimum Inhibitory Concentration (MIC) of Plant Extracts

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the drug which will inhibit growth as measured by observed turbidity in the test tube [15]. The MIC was determined using the method described by Greenwood (16). Six sterile test tubes were arranged in three rows in a test tube rack, each row of each extract was determined against pure culture of *S. gallinarum* in triplicates at varying concentrations. The test utilizes the lowest concentration of an antimicrobial extract to inhibit the

visible growth of a micro-organism after overnight inoculation. Each potential extract was determined by micro-broth dilution technique. These concentrations were obtained by first making serial dilution of the stock concentration of the extracts in double fold and from there a two-fold dilution of the extracts was carried out to obtain the dilutions required. Exactly 0.5 MacFarland standard suspensions of the test organism was inoculated in a sterile tube of nutrient broth containing different two fold dilutions of each plant extract. This was incubated at 37 C for 18 to 24 h and MIC was determined by observing for growth or no growth in each of the test tubes with different concentrations of the extracts by observing for turbidity. Range of MIC for each extract was determined by observing the lowest concentration of each extract that inhibited growth of the organism. The results are presented in Table 3

### Determination of Minimum Bactericidal Concentration (MBC) of Plant Extracts

The MBC is defined as the lowest concentration that kills the organisms completely, where no bacterial growth is observed [16]. This was determined using the broth dilution technique described by [17] as adopted by [18] by assaying the test tubes resulting from MIC determinations. A loopful of the content of each test tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37°C for 24h and observed for bacterial growth. The lowest concentration of the extract that showed no bacterial growth was noted and recorded as the MBC in Table 4.

### Calculation of Activity Index

This was estimated as diameters zone of inhibition of extract divided by diameters zone of inhibition of the standard antibiotics multiplied by 100 (expressed as %) [19, 20].

## Result

### Percentage Yield and Texture of the Extracts

The result of the percentage yield of the various extracts; crude n-hexane (CHE), crude chloroform (CCE), crude methanol (CME) and crude aqueous (CAE) of the fruit of *Cucumis metuliferus* was calculated and the texture were represented in Table 1.

**Table 1: The yield and texture of crude extracts of the fruit of *Cucumis metuliferus***

S/N	Extract	Yield (%)	Texture of Extract
1	CHE	3.75	Oily
2	CCE	3.16	Oily
3	CME	7.11	gel-like
4	CAE	16.35	gum-like

Key:

CHE = Crude n-Hexane Extract  
 CCE = Crude Chloroform Extract  
 CME = Crude Methanol Extract  
 CAE = Crude Aqueous Extract

### Zone of Inhibition of Methanol Extract (CME) of *C. metuliferus*

Table 2 show the result of the zone of inhibition of methanolic extract of the fruit of *C. metuliferus*. The extract showed zone of inhibitions of  $8.33 \pm 0.52$ ,  $9.67 \pm 0.52$ ,  $11.67 \pm 1.03$ ,  $13.67 \pm 0.52$  and  $14.67 \pm 0.52$  mm at 200, 400, 600, 800 and 1000 mg/ml respectively. The minimum and maximum zones of inhibition of  $8.33 \pm 0.52$  and  $14.67 \pm 0.52$  milimetre was seen at 200 and 1000 mg/ml respectively.

**Table 2: Zones of Inhibition of Methanol Extract of *Cucumis metuliferus* Against *Salmonella gallinarum***

Concentration of Extract (mg/ml)	Zone of Inhibition (mm)
200	$8.33 \pm 0.52$
400	$9.67 \pm 0.52$
600	$11.67 \pm 1.03$
800	$13.67 \pm 0.52$
1000	$14.67 \pm 0.52$

### Minimum Inhibitory Concentration (MIC) of Methanol Extract of *C. metuliferus*

Table 3 shows the MIC of the methanol extract of the fruit of *C. metuliferus*. The extract had a minimum inhibitory effect against *Salmonella gallinarum* at 50 mg/ml.

**Table 3: Minimum Inhibitory Concentration (MIC) of Methanol Extract of *Cucumis metuliferus* Against *Salmonella gallinarum***

Test organism	Concentration of extract (mg/ml)					
	6.25	12.5	25	50	100	200
<i>Salmonella gallinarum</i>	+	+	+	β	-	-

Key:

β = Minimum concentration at which no turbidity was observed (MIC)

= Negative, meaning 'No turbidity seen'

+ = Positive, meaning 'there was turbidity'

### Minimum Bactericidal Concentration of Methanol (MBC) Extract of *C. metuliferus*

Table 4 shows the MBC of the methanol extract of the fruit of *C. metuliferus*. The extract had a minimum bactericidal effect against *Salmonella gallinarum* at 50 mg/ml.

**Table 4: Minimum Bactericidal Concentration (MBC) of Methanol Extract of *Cucumis metuliferus* Against *Salmonella gallinarum***

Test organism	Concentration of extract (mg/ml)					
	6.25	12.5	25	50	100	200
<i>Salmonella gallinarum</i>	+	+	+	β	-	-

Key:

β = Minimum concentration at which no growth was observed (MBC)

= Negative, meaning 'No bacterial growth seen'

+ = Positive, meaning 'there was bacterial growth'

### Zone of Inhibition of Water Extracts of *C. metuliferus*

Table 5 showed the result of antimicrobial activity of the aqueous extract of the fruit of *C. metuliferus*. The extract showed zone of inhibitions of  $7.50 \pm 0.55$ ,  $8.50 \pm 0.55$  and  $9.67 \pm 0.52$  mm at 600, 800 and 1000 mg/ml respectively. At the minimal concentration of 200mg/ml there was resistance, meaning the extract was not able to inhibit the growth of *Salmonella gallinarum*. Hence no MIC and MBC studies were carried out.

**Table 5: Zone of Inhibition of Water Extract of *Cucumis metuliferus* Against *Salmonella gallinarum***

Concentration of extract (mg/ml)	Zone of Inhibition (mm)
200	R
400	R
600	$7.50 \pm 0.55$
800	$8.50 \pm 0.55$
1000	$9.67 \pm 0.52$

Key:

R = Resistant, meaning 'no zone of inhibition seen at the concentration'

### Zone of Inhibition of n-Hexane Extract of *C. metuliferus*

Plate 1 shows there was no zone of inhibition by the n-hexane extract at 1000 mg/ml, it was resistant.

**Plate 1**



Zone of inhibition of hexane extract (1000mg/ml) of *Cucumis metuliferus* against *Salmonella gallinarum*

### Zone of Inhibition of Chloroform Extract of *C. metuliferus*

Plate 2 there was no zone of inhibition by the extract at 1000 mg/ml it was resistant.

**Plate 2**



Zone of inhibition of chloroform extract of *Cucumis metuliferus* at 1000mg/ml

### Antimicrobial Studies of Standard Drug (Antibiotics)

Table 6 shows the result of antimicrobial activity of standard drug ciprofloxacin 5µg against *S. gallinarum*. The zone of inhibition of  $19.33 \pm 1.03$  mm was recorded.

Table 6 shows the result of antimicrobial activity of standard drug chloramphenicol 10µg against *S. gallinarum*. The zone of inhibition was noted as  $10.66 \pm 0.52$  mm.

Table 6 shows the antimicrobial activity of standard drug augmentin® (amoxycillin and clavulanic acid) 30µg against *S. gallinarum*. The zone of inhibition of  $7.33 \pm 0.52$  mm was recorded.

Table 6 shows the result of antimicrobial activity of standard drugs tetracycline 30 µg, erythromycin 5 µg, ceftazidime 30 µg and oxacillin 1µg against *S. gallinarum*, the organism showed resistant to these antimicrobial agents.

**Table 6: Zone of Inhibition of Standard Antimicrobial Agents**

Standard Antibiotic Discs	Zone of Inhibition (mm)
Ciprofloxacin 5µg	$19.33 \pm 1.03$
Chloramphenicol 10µg	$10.66 \pm 0.52$
Augmentin® 30µg	$7.33 \pm 0.52$
Tetracycline 30µg	R
Erythromycin 5µg	R
Ceftazidime 30µg	R
Oxacillin 1µg	R

Key:

R= Resitant



Plate 3 shows the picture of the negative control, which is the disc that was not impregnated with either an antibiotics or extract. There was no growth.

**Plate 3**



Negative control plate showing discs that were not impregnated either with antibiotics or extracts.

**Activity Index (%) of Methanol and Water Extracts of *C. metuliferus* against Standard Drugs Ciprofloxacin, Chloramphenicol and Augmentin**

The result of the activity index (AI) of crude methanol (CME) and crude aqueous (CAE) extracts as shown in Table 7 showed that the activity of CME at 200, 400, 600, 800 and 1000 mg/ml concentrations against the standard drug ciprofloxacin (5µg) was 43.09, 50.03, 60.37, 70.72 and 75.89 respectively. The result of the activity of CME at 200, 400, 600, 800 and 1000 mg/ml when compared to the standard drug chloramphenicol (10µg) was shown to be 78.14, 90.71, 109.47, 128.24 and 137.62 % respectively (Table 7). The result of the activity of CME against the standard drug augmentin® (30µg) as shown in Table 7 showed that the extract activity at 200, 400, 600, 800 and 1000 mg/ml was 113.64, 131.92, 159.21, 186.49 and 200.14 % respectively.

**Table 7: Activity Index (%) of Methanol and Water Extracts of *C. metuliferus* against Standard Drugs Ciprofloxacin, Chloramphenicol and Augmentin®**

Extract	Concentration of Extract (mg/ml)	Standard Antimicrobial Drugs		
		CIP (5µg) %	CHL(10µg) %	AUG (30µg) %
CME	200	43.09	78.14	113.64
	400	50.03	90.71	131.92
	600	60.37	109.47	159.21
	800	70.72	128.24	186.49
	1000	75.89	137.62	200.14
CAE	200	R	R	R
	400	R	R	R
	600	38.80	70.36	102.32
	800	43.97	79.74	115.96
	1000	50.03	90.71	131.92

Key

R = Resistance, "meaning organism was resistant to the concentrations, so no zones of inhibition recorded"

CIP = Ciprofloxacin

CHL = Chloramphenicol

AUG = Augmentin

CME = Crude methanol extract

CAE = crude aqueous extract

The result of the activity index of CAE at 200 and 400 mg/ml concentrations was not calculated because the organism (*S. gallinarum*) was resistant. However, at 600, 800 and 1000 mg/ml, the AI against ciprofloxacin (5µg) was 38.80, 43.97 and 50.03 % respectively (Table 7).The activity of CAE at 600, 800 and 1000

mg/ml was 70.36, 79.74 and 90.71 respectively against the standard drug chloramphenicol (10µg).The result of AI of CAE at 600, 800 and 1000 mg/ml against augmentin® (30µg) was 102.32, 115.96 and 131.92 % respectively (Table 7).



## Discussion

The results of Tables 2 and 5 show the zones of inhibition of methanol and water extracts, although no zones of inhibition were seen with the n-hexane and chloroform extracts (plate 1 and 2), nonetheless, the methanol and water extracts showed some antibacterial activity (Tables 2 and 5). This activity may be related to the various phytochemicals present in the fruit extract as reported by [21, 22]. The minimum inhibitory concentration of 50 mg/ml was recorded for the methanol extract and the same value was also recorded as the MBC (Tables 3 and 4). Because of the resistance of the organism at the minimum concentration of the water extract (Table 5), the MIC and MBC was not carried out. The lack of activity with the n-hexane and chloroform may be attributed to probably less phytochemicals present in the extract. The methanolic extract of *C. metuliferus* had shown antibacterial activity more than the other extract, this work tallies with the work of other researchers [23, 11], that the methanol extracts of various plants have greater antimicrobial activity than the aqueous extracts. It was reported that the biological activities of medicinal plants are not attributed to a single moiety but to the many kinds of chemical compound present in the plant [24].

The methanol extract showed a greater zone of inhibition ( $14.67 \pm 0.52$  mm) (Table 2) than the standard antibiotics chloramphenicol ( $10.66 \pm 0.52$  mm) and augmentin ( $7.33 \pm 0.52$  mm), however, ciprofloxacin showed a greater zone of inhibition ( $19.33 \pm 1.03$  mm) (Table 6) than both the methanol and water extracts (Tables 2 and 5). The water extract at 600, 800 and 1000 mg/ml also showed activity having zones of inhibition of  $7.50 \pm 0.55$  mm,  $8.50 \pm 0.55$  mm and  $9.67 \pm 0.52$  mm respectively. The antimicrobial activity of the water extract was more than the standard drug augmentin ( $7.33 \pm 0.52$  mm); even though the extract could not inhibit the growth of *S. gallinarum* at 400 mg/ml. It may be deduce that the fruit of *C. metuliferus* may probably be a promising antimicrobial agent to salmonellosis especially in the case of resistance to drugs as shown with tetracycline, erythromycin, ceftazidime and oxacillin (Table 6).

The result of the activity index (Table 7) which relates the activities of the test extracts against antibiotics showed that the standard drug ciprofloxacin has activity more than both the methanol and

water extract at all their concentrations. However, when CME and CAE were compared to chloramphenicol it was shown that the standard drug showed more activity than CAE at all concentrations and CME at 200 and 400 mg/ml only. But at 600, 800 and 1000 mg/ml CME showed more activity than chloramphenicol. The result of the activity of the extracts against augmentin® showed that both extracts (CME and CAE) showed greater activity than the standard drug augmentin®. CME at 200, 400, 600, 800 and 1000 mg/ml had activity index of 113.64, 131.92, 159.21, 186.49 and 200.14 % respectively, while CAE at 600, 800 and 1000 mg/ml had 102.32, 115.96 and 131.92 % AI. Since in activity index any value above 100 % shows that the extract has activity more than the standard drug(s) (19, 20). From the result of this activity it can be seen that the methanol extract of *C. metuliferus* showed greater activity than chloramphenicol and augmentin®, while the aqueous extract showed more activity than augmentin® only. Therefore, the plant *C. metuliferus* may probably be a potent antibiotic.

## Conclusion

In conclusion, this study had shown that the fruit extracts of *Cucumis metuliferus* possess some antimicrobial activity, this activity may be attributed to the various phytochemicals present and is seen with the methanolic extract, as the most active extract in this research. The use of *C. metuliferus* plant in traditional medicine is probably justified. However, further work needs to be carried out using more bacterial organisms (both Gram positive and Gram negative) as well as isolation of the active compound(s) responsible for the antimicrobial activity.

## Conflict of Interest

Authors have declared that no conflicting interests exist.

## Author's Contribution

This work was carried out in collaboration between all authors. UJG designed the study, wrote the protocol and wrote the first draft of the manuscript. UJG and OAS performed the antimicrobial work and literature searches of the study. UJG and SUK performed the statistical analyses. All authors read and approved the final manuscript.

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