



# Phytochemical, Proximate Analysis, and Antibacterial Activity of *Cucumis sativus* On Hypervirulent *Klebsiella pneumoniae*

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## Abstract

**Background:** *Cucumber* (*Cucumis sativus* L.) are cultivated worldwide belonging to *Cucurbitaceae* family that carries cylindrical fruits, which are used as vegetables **Aim:** The objective of the study is to detect the phytochemical compounds, conduct proximate analysis and test the antibacterial efficacy of *C. sativus* **Method:** A qualitative method using Soxhlet extractions for both phytochemical and proximate analysis were used. Confirmatory biochemicals and string tests were conducted for identification of hypervirulent *Klebsiella pneumoniae*. Agar-well diffusion test was used to determine the efficacy of the extract. **Results:** Results indicated that Phytochemicals show that steroids (+++), terpenoids (+++), were found at relatively high concentrations; saponins (++) , alkaloids (++) , and flavonoids (++) are present in moderate concentrations, while tannins (+) are present in mild levels. Out of the total 69 isolates obtained and confirmed using the biochemical test, 24 (35%) were Hypervirulent *Klebsiella pneumoniae* while 45 (65%) were classic *Klebsiella pneumoniae*. Antibiotic susceptibility profile of ethyl acetate extracts at variable concentrations shows that hypervirulent *Klebsiella pneumoniae* was susceptible at 100ul/ml (21mm) and 75ul/ml (19mm) but resistant at 50ul/ml (18mm). *Cucumber peels* are a source of secondary metabolites that is alkaloids, flavonoids, terpenoids, tannins, and saponins steroids which may reduce blood sugars and prevent bacterial infection. *Cucumber peels* may play a vital role in preventing various diseases and conditions such as inflammation, bacterial infection, lipid peroxidation, fever, and constipation. Further studies should be conducted to advance methods such as bioassay-guided fractionation to measure the constituent of the *Cucumis sativus* that is responsible for antibacterial activity

**Keywords:** *Klebsiella pneumoniae*, MDR, XDR, PDR.

## 1. Introduction

Phytochemicals are secondary metabolites produced by plants. These products are biologically and chemically active naturally in various parts of the plant, providing human health benefits beyond those attributed to macronutrients and micronutrients. They have several functions that include providing strength to plants, and attracting insects for pollination and feeding, while some are simply waste products (Lekha *et al.*, (2022). The deflection system protects plant cells from environmental hazards such as pollution stress, drought, exposure to ultraviolet rays, and pathogens Hamedalla *et al.*, (2022). This *C. sativus* is implicated in human health by helping to protect against degenerative diseases (Okeke *et al.*, (2021). Phytochemicals found in plants are used as important components in human and animal nutrition, this includes all the constituents of the plant. Phytochemicals that can be used as chemotherapeutic or chemotherapeutic agents are formed during the normal metabolism of plants (Dario *et al.*, (2022).

The most important of these phytochemicals are alkaloids, tannins, flavonoids are phenolic compounds. The component of the fruit are widely used as antimicrobial agents (Insanu *et al.*, 2022). The increasing development of resistance to conventional antibiotics due to misuse contributed to a high prevalence of pathogenic strains in both



community and hospital settings. Many bacterial species developed the ability to tolerate antibiotics long before humans started to mass-produce them to prevent and treat infectious diseases, some for industrial and agricultural uses (Larsson and Flach, 2022). This human misuse has created bacterial superbugs that are virtually resistant to almost all frequently used antibacterial agents. However, to find susceptible antibiotics, researchers are resorting to phytochemical constituents that are naturally available in almost all our domestic plants and fruits.

*C. Sativus* have been reported to exhibit much antibacterial activity (Larsson and Flach, 2022), and anticancer (Khan *et al.*, 2021). It also represents a wide range of pharmacological activities such as antitumor, anthelmintic, anticoagulant, analgesic, and antioxidant (Wahid *et al.*, (2021). Therefore, the study aimed to analyse the phytochemicals in the ethyl acetate extract of cucumber peel and to test the efficacy of the extract on hypervirulent *Klebsiella pneumoniae*. The study was limited to extracting cucumber peels with ethyl acetate and determining the phytochemicals present in the extract.

## 2.0 Materials and method

### 2.1 Study Area

The federal polytechnic Bauchi is located in Bauchi state Nigeria on latitudes  $11^{\circ}.4027^{\circ}$ N,  $10^{\circ}11^{\circ}28^{\circ}$ E and longitude  $11.67417^{\circ}$ N,  $10.191110^{\circ}$ E. The institution has a laboratory unit with microbiology, Biology and chemistry laboratory saddle with many assays.

### 2.2 Sample Collection and preparation

Freshly ripped cucumbers were obtained using stratified sampling from a farm along Gubi Dam on February 23-2021. Cucumbers are thoroughly washed under running water and manually peeled using a sterilized peeler. The pods were then dried in the shade at room temperature for 5 days. The shade-dried cucumber rind is pulverized in a laboratory blender and kept in an airtight bottle until later use. Powdered cucumber peels were soaked in methanol and chloroform for 72 h using the water immersion technique. The supernatant was filtered through Whatman No.1 filter paper and concentrated using a rotary evaporator and the dry residue was stored at  $5^{\circ}\text{C}$  in sealed bottles until further use.

### 2.3 Ethyl acetate extraction of *Cucumis sativus*

The prepared Cucumber peel extract was mixed with 300ml of ethyl acetate in a round-bottomed flask. The mixture was placed in a Soxhlet extractor and heated on a coated condenser. The heated mixture was then filtrated using number 4 Wartman filter paper inside the sterile conical flask. The sidearm is covered with glass wool, then the solvent was reheated again, the vapor was collected through the condenser, is reintroduced into the flask and the cycle begins again.

## 2.4 Determination of Phytochemicals Constituent of *Cucumis sativus*

### 2.4.1 Determination of Terpenoids

Five (5) ml of the extract with 2 ml of chloroform was carefully added to 3 ml of concentrated  $\text{H}_2\text{SO}_4$ . The reddish-brown color at the surface indicates the presence of terpenoid

### 2.4.2 Determination of Steroid

Half a millstone (0.5ml) of acetic anhydride and a few drops of concentrated  $\text{H}_2\text{SO}_4$  was added to 1ml of the extract, and a red-green precipitate indicates the presence of steroids.

### 2.4.3 Flavonoid determination

One (1) ml of the extract was transferred to a 5 ml test tube. Two (2) ml of 10% NaOH were then added. Then colourless, adding dilute hydrochloric acid shows us that there are no flavonoids.

### 2.4.4 Determination of saponins

Add 1ml of extract to 5ml of distilled water in a test tube and stir continuously to indicate the presence of saponins.



#### **2.4.5 Determination of tannins**

One (1) ml of the extract was transferred to a 5ml test tube. Three drops of 5% iron III chlorides were added to the extract. The greenish-black precipitate indicates the presence of tannins.

#### **2.4.6 Alkaloid determination**

One (1) ml of the extract was transferred to a 5 ml test tube. Two (2) ml of chloroform and a few drops of Wagner reagent were added. The reddish-brown precipitate is evidence of alkaloids.

#### **2.5 Proximate analysis**

The proximate analysis of the homogenate of *C. sativus* fruits for moisture, ash, fat, and carbohydrate was determined as described by AOAC (2000). The concentration of crude protein and fiber was determined using methods described by Pearson (1976). All determinations were done in triplicates and the results were expressed as means of percent values on a dry weight basis.

#### **2.6 Confirmation of Hypervirulent *Klebsiella pneumoniae***

A total of 69 pure isolates of *Klebsiella pneumoniae* obtained from the hospital were subcultured on MacConkey agar for 24hours followed by a comparative biochemical test as described below:

##### **2.6.1 Indole test**

Using a sterile cord loop in a single day natural subculture turned into inoculated on Tryptone broth and incubated at 37°C for 24hours. After incubation 5 drops of Kovac's reagent had been positioned into the check tube, Indole manufacturing is indicated through the manufacturing of purple to pink color.

##### **2.6.2 Methyl Red and Voges-Proskauer Test Procedure**

Two check tubes containing MR-VP Broth had been inoculated with natural subculture and incubate at 35 °C for as much as 24hours. Five drops of the methyl pink indicator solution (C.I. Acid Red 2 or methyl pink reagent) turned into brought to the primary tube for the Methyl Red check whilst (Five% alpha-naphthol, accompanied through 0.2 mL of 40% KOH) turned into brought to the second one tube for Voges-Proskauer check. A superb response is indicated if the color of the medium adjustments to pink inside a couple of minutes for the methyl pink check and pink to brown for the Voges-Proskauer check.

##### **2.6.3 Citrate Utilization Test procedure**

Tubes containing Simmons citrate agar had been inoculated within a single day natural subculture the use of sterile cord loop gently at the slant through touching the top of a needle to a colony this is 24 hours old. It turned into Incubate at 37°C for twenty-four hours. The improvement of the blue color suggests a superb result.

#### **2.7 String test for Hyper-virulent *Klebsiella pneumoniae* (HvKp)**

Sterile inoculation loop turned into used to generate a vicious string of pinkish mucoid colonies on agar plates of showed *K. pneumoniae* through stretching for distance, a period much less than 5mm of *K. pneumoniae* lines turned into taken into consideration as a superb string check a good way to layout as hypervirulent *Klebsiella pneumoniae*, whilst something much less 5mm turned into taken into consideration as non-hypervirulent *Klebsiella pneumoniae*

#### **2.8 Agar-well diffusion method**

The densities of the hyper-virulent *Klebsiella pneumoniae* strain were standardized to achieve a final concentration of  $1.5 \times 10^8$  CFU/ml using the growth method. Approximately 0.5 ml of  $1 \times 10^8$  inoculum was grown in 5 ml nutrient agar and incubated at 37°C until cloudy (0.5 McFarland standard) is achieved according to the method of Jorgensen & Turnidge, (2007). Using the pout plate method, a mixture of HvKp and nutrient agar was poured into a Petri dish. Five (5) 6 mm wells were generated by perforating the gel on prepared nutrient agar, and these wells were moderately filled with a drop of ethyl acetate extract of *C. sativus* of 50µl, 75µl, 100µl, using a micropipette. The prepared plates were incubated at 37 °C for 24 h, and the zones of inhibition were measured and recorded.

### 3.0 Results and Discussion

As shown in Table 1, bioactive compounds such as steroids, and terpenoids, were found at relatively (+++) concentrations; Saponins, alkaloids, and flavonoids are present in (++) concentrations, while tannins are present in (+) levels. The proximate analyses of the homogenate of *C. sativus* showed the presence of moisture, crude protein, ash, and crude fiber with values shown in Table II. The homogenate of *C. sativus* had high concentrations of moisture ( $96.4 \pm 0.08\%$ ) followed by crude protein concentration with  $4.21 \pm 0.07$ , crude fiber  $2.02 \pm 0.01$ , Ash  $0.99 \pm 0.24\%$ , Lipid  $0.75 \pm 0.13$  and the lowest carbohydrate concentration  $0.48 \pm 0.09$ . Following the standard confirmatory biochemical test, the results show that Indole, MethylRed negative, Voges-Proskauer, and citrate utilization positive. Out of the total 69 isolate obtained and confirmed using the biochemical test, 24 (35%) were Hypervirulent *Klebsiella pneumoniae* while 45 (65%) were classic *Klebsiella pneumoniae* as characterized using the string test presented in Figure I.

Figure II shows the antibiotic susceptibility profile of ethyl acetate extract at the variable concentration on Hypervirulent *Klebsiella pneumoniae*. At 50ul/ml, a zone of inhibition of 18mm was recorded, 75ul/ml (19mm), and at 100ul/ml (21mm). while sterile distilled water was 0mm and Ciprofloxacin was recorded at 20mm. Based on the Clinical Laboratory Standard Institution (CLSI) guideline zone of inhibition 19mm from 75ul/ml and 21mm from 100ul/ml is proving sensitive while 18mm from 50ul/ml is within the range of intermediate by CLSI standard. This implies that HvKp is sensitive to ethyl acetate at 75ul and 100ul but is intermediate resistant at 50ul. Table III shows a confirmatory biochemical test for the identification of *Klebsiella pneumoniae* and other gram-positive and enteric gram-negative bacteria.

Table I: Phytochemical constituents of the homogenate of *Cucumis sativus* fruit

Phytochemical	Relative presence
Alkaloids	++
Steroids	+++
Saponins	++
Tannins	+
Flavonoids	++
Terpenoids	+++

**Key:**

+ = slightly present;

++ = Moderately present;

+++ = Highly present.

Table II: Proximate composition of the *Cucumis sativus*

Constituent	Percentage
Moisture	$96.4 \pm 0.08$
Crude protein	$4.21 \pm 0.07$
Crude fibre	$2.02 \pm 0.01$
Ash	$0.99 \pm 0.24$
Lipid	$0.75 \pm 0.13$
Carbohydrate	$0.48 \pm 0.09$

Values indicate mean percentage  $\pm$  SD

Table III: Confirmatory Biochemical Test for *Klebsiella pneumoniae*

Biochemical Test	Result
Gram Staining	-ve
Catalase	+ve
Coagulase	-ve
Motility test	-ve
Indole test	-ve
Methyl Red	-ve
Voges – Proskeur	+ve
Oxidase	-ve
Citrate Utilisation	+ve

**Key: Positive result (+ve), Negative result (-ve)**

**Prevalence of Hypervirulent *Klebsiella pneumoniae* obtained from the isolates**

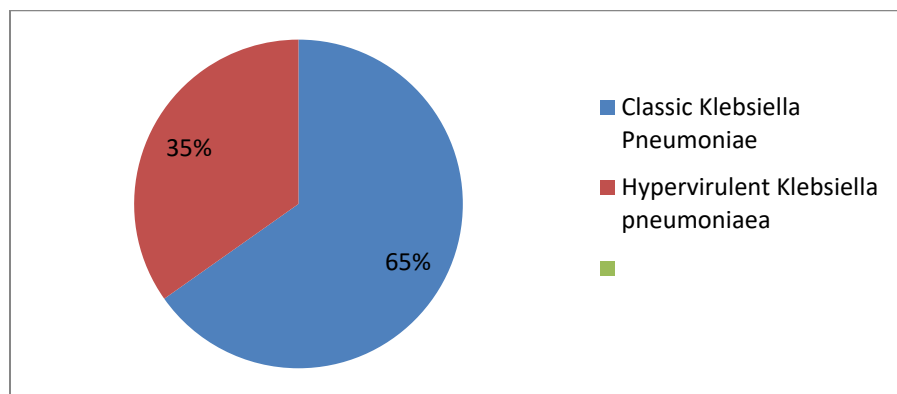


Figure I: Prevalence of classic *Klebsiella pneumoniae* and Hypervirulent *Klebsiella pneumoniae* among the isolates

**Antibacterial Activity of Ethyl acetate Extract of *Cucumis sativus* on Hypervirulent *Klebsiella pneumoniae***

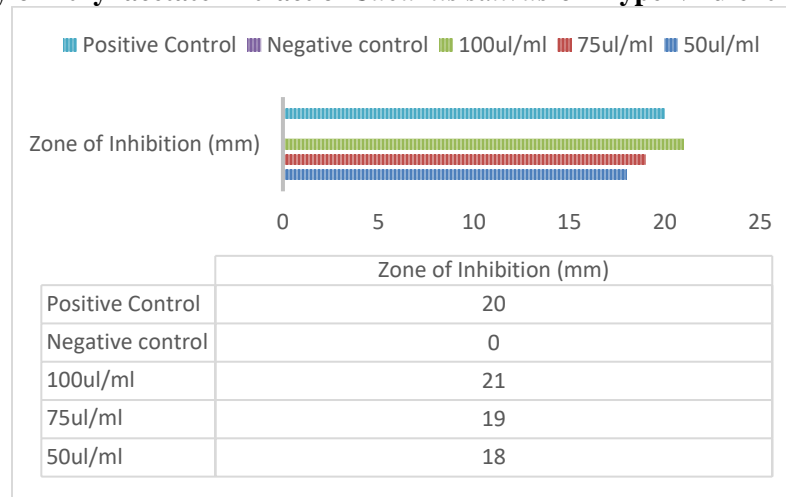


Figure II: Antibacterial Activity of Ethyl acetate Extract of *Cucumis sativus* on Hypervirulent *Klebsiella pneumoniae*

**Keys:**

Based on CLSI Guideline

- Resistance <14mm
- Intermediate 14-18mm
- Sensitive 19mm>



#### 4.0 Discussion

##### 4.1 Phytochemical constituents of the homogenate of *Cucumis sativus* fruit

The findings of this study revealed that *C. sativus* contains tannins, saponins, alkaloids, flavonoids, terpenoids, and steroids. The steroids and terpenoids were found to be slightly higher in concentration while saponins, alkaloids, and flavonoids were found to be moderately concentrated. This is similar to the result presented by Šeregelj *et al.*, (2022) but contrary to the work of Das *et al.*, (2022). Phytochemical analysis showed that *C. sativus* contain bioactive compounds which had been reported to have antibacterial potency and perhaps may have contributed to its antibacterial activity (Riaz *et al.*, 2022). Flavonoids are known to be synthesized by plants in response to microbial infection. They have effective antibacterial activities in vitro against a wide array of microorganisms. The presence of tannins could also show that it is an astringent, helps in wound healing, and is anti-parasitic.

##### 4.2 Proximate composition of the of *Cucumis sativus*

The proximate analysis of the homogenate of *C. sativus* fruit had high concentrations of moisture ( $96.4 \pm 0.08$ ) followed by crude protein ( $4.21 \pm 0.07$ ) and crude fiber ( $2.02 \pm 0.01$ ). Proximate analyses also revealed carbohydrates as being very low ( $0.48 \pm 0.09$ ). This finding is contrary to the work of Olowoake *et al.*, (2022). But similar to the work of Šeregelj *et al.*, (2022) **who reported a low Ash concentration in the homogenate extract of *C. sativus*.**

##### 4.3 Antibacterial Activity of Ethyl acetate Extract of *Cucumis sativus* on Hypervirulent *Klebsiella pneumoniae*

The findings of this study indicate the antibacterial activity of ethyl acetate extract on HvKp at 100ul/ml and 75ul/ml, (22mm and 19mm). However contrary to this result Hussan et al (2018) report that the crude extract of *C. sativus* was the most active against *Escherichia coli*, *Staphylococcus aureus* (22.5mm), and less against *Shigella flexneri* (17.0mm). Similarly, ethanol and methanol extracts of *P. emblica* reduce the growth of bacteria (de-Menezes *et al.*, 2022). The bacteria were susceptible to medicinal plant extracts because of the concentration of bioactive components (Elmongy *et al.*, 2022).

#### 5.0 Conclusion

Cucumber peels are a source of secondary metabolites that is alkaloids, flavonoids, terpenoids, tannins, and saponins steroids which may blood reducing sugars and prevent bacterial infection. Cucumber peels may play a vital role in preventing various diseases and conditions such as inflammation, bacterial infection, lipid peroxidation, fever, and constipation.

#### Recommendations

The present study suggests that the use of the plant *C sativus* is very beneficial in terms of providing a substitute to already resistant antibiotics which may be a good source of isolating novel drugs to improve human health. Further studies should be conducted to advance methods such as bioassay-guided fractionation to measure the constituent of the *C. sativus* that is responsible for antibacterial activity.

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