Studies the Preliminary Phytochemical Composition and Assessments of Antibacterial Effects of Aqueous- Methanol Extracts of Cucumis Ficifolius fruit, leaf, and seed parts in the University of Gondar, Gondar, Ethiopia

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Research Article

Keywords: antibacterial activity, Cucumis ficifolius, secondary metabolites, sensitive, resistant

Posted Date: March 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1451402/v1

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Abstract

Infectious diseases are the major problems of the world as a result of the emerging and reemerging of different antimicrobial-resistant microorganisms due to several reasons like missiles and repeating uses of antibiotics. As a result, searching for new treatment methods is essential from natural substances to against those infectious diseases in both human and animals’ prospects. Among those plants, *Cucumis ficifolius* has various roles against those infectious diseases via its different phytochemical components. The objectives of this study were assessing the antibacterial activity of the aqueous-methanol extract of the *Cucumis ficifolius* seed, leaf, and fruit parts and knowing the phytochemical constituents of the plant. Preliminary phytochemical screening revealed that the extract of *Cucumis ficifolius* seed, leaf, and fruit parts possesses flavonoids, quinone, phenols, saponins, tannins, and terpenoids. In addition the antibacterial activity of the plant extract was evaluated on seven pathogenic bacteria species using the agar well diffusion method at different concentrations of plant extracts. Minimum inhibition concentration and minimum bactericidal concentration determinations were done by tetrazolium chloride microtiter dilution assay. The inhibition zone of mean diameters ranging from 00.00 to 14.16 mm against all test bacteria was significantly (p < 0.05) much higher than that of the positive control oxytetracycline(30μg/disc) with the range of 4.45mm-13.45mm of inhibition zone of diameters. The inhibition zones of the tested bacteria at the concentration of 62.5mg/ml were much less than the higher concentration (500mg/ml) and significantly different (p < 0.05), whereas the MIC value ranges from 0.52mg/ml to 20.83mg/ml, and the MBC value ranges from 0.65 mg/ml to 25.00 mg/ml. The present study showed that *Cucumis ficifolius* have higher antibacterial activities with having potential for further study to serve as a source of antibacterial agents.

1 Background

In developing African countries like Ethiopia, livestock production remains essential and represents a major asset among resource-poor smallholder farmers by giving milk, meat, skin, manure, and traction. However, the economic benefits of livestock populations remain minimal due to prevailing livestock diseases, which are among the principal obstacles for livestock performance and cause of high economic losses of the resource-poor farmers (41).

The majority of traditional medicines used in developing countries have not been evaluated for quality, safety, and efficacy to some standards while in developed countries there are some remarkable claims made for their effectiveness (12). presently, many bacterial pathogens are becoming resistant to the existing antibiotics due to their misuse or repeated use of antibiotics in the treatment of infectious diseases; because of these, scientists advance in their research findings on the bacterial targets to attack the evolved bacteria and attention towards to the popular plant extracts and biologically active compounds isolated from the plant (38). This condition increases incidences of drug resistance and the emergence and re-emergence of deadly microorganisms are posing a great threat to society (48).

Currently, the emergence of resistant pathogens to many of the commonly used antibiotics has provided an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome the problems of resistance to currently available antimicrobial agents (5). Ethiopia is known for its high livestock population, being the first in Africa and tenth in the world (8). The current livestock population estimate stands at 59.5 million cattle, 30.70 million sheep, 30.20 million goats, and 1.21 million camels, considering only ruminant livestock species. In addition, 8.44 million donkeys, 2.16 million horses, 0.41 million miles, and 56.53 million poultry comprise the livestock resource of Ethiopia (9). Ethiopia is endowed with diverse biological resources including about 6, 500 species of higher plants, out of which more than 14% are said to have been used as traditional plant medicines to treat different human and livestock ailments, while more than 1,000 species have been documented at the Ethiopian National Herbarium database (44).

In Ethiopia, medicinal plants play important role in fulfilling the human and livestock health care needs of different communities. Traditional use of medicinal plants has remained as the main alternative solution for different human and livestock health problems largely due to shortage of pharmaceutical products and modern health service stations, unaffordable prices of conventional drugs, and drug resistance in Ethiopia (1). In Ethiopia, the animal disease remains one of the principal causes of poor livestock performance leading to an ever-increasing gap between the supply of, and the demand for livestock products. Conventional veterinary services, despite their paramount role, have limited coverage in developing countries due to this reason livestock keepers particularly in rural areas frequently visit traditional healers to get solutions for their ill-health animals; they complement modern medicine by developing a socially acceptable remedy from inexpensive resources (15).
*Cucumis Ficifolius* is a prostrate herb (21). This plant grows in grassland, wooded grassland, Acacia woodland, rocky slopes, secondary vegetation, and cultivated places and is known for its wide range of medicinal uses (27). The vernacular name of *C. ficifolius* is Yemidier embuay (Amharic language). Traditionally fruit part of *Cucumis ficifolium* is used as an abortifacient for women and hastens expulsion of the placenta for cows in Ethiopia. The fruit pars are also recognized in Ethiopia as highly toxic and are reported to treat rabies. In Nigeria and Ethiopia, the fresh fruit with an end cut-off is applied thimble-like as addressing for inflamed fingers. The fruit has veterinary use as a vermifuge with the addition of natron for horses by the Hausa. It is also used as a medicine for fowls. In some places, it is an ingredient of medicine for syphilis and as an emetic and in small doses with honey to relieve stomach ache for children, in Ethiopia it is also used for the treatment of “Kuruba, Chiffea, Mageriat geter (meningitis), nessir (epistaxis), wefbeshita leafs also used for’yekusilmerz (worsening external figure injury) and yeahyakintarot (wart) (37). Also, the roots are a remedy for malaria. The Root extract of *C. ficifolius* is recorded to be used in local honey-wine or “Tej” to make the beverage more intoxicating (27).

Natural compounds are a source of numerous therapeutic agents. Recent progress to discover drugs from natural sources has resulted in compounds that are being developed to treat cancer, resistant bacteria and viruses, and immunosuppressive disorders (2). Phytoconstituents are the natural bioactive compounds found in plants that could prevent diseases and inhibit pathogenic microorganisms (22, 35). Phytoconstituents are divided into two groups, i.e. primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins, and chlorophyll while secondary constituents consist of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, and tannins. Phytoconstituents could prevent diseases and inhibit pathogenic microorganisms (21).

Phytochemical analysis of the plants belonging to the Cucurbitaceae family confirms the presence of various phytochemicals like tannins, cardiac glycosides, terpenoides, saponins, carotenoids, and phytosterols. Phytochemical screening of the leaves revealed the presence of tannins, phlorotannins, flavonoids, steroids, terpenoids, saponins, Terpinoid, Quinone, phenol, and cardiac glycosides, which are the most important bioactive constituents of medicinal plants (3).

Scientists are in search of new phytochemicals that could develop as useful anti-microbial for the treatment of infectious diseases (47). Currently out of 80% of pharmaceuticals derived from plants, very few of them are used as anti-microbial. But, Plants are rich in a wide variety of secondary metabolites that have found anti-microbial properties (34). The current demand for herbal remedies in both developed and developing countries is increasing (7). Screening active compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer (37, 21).

Since *Cucumis ficifolium* belongs to the Cucurbitaceae family it has different phytochemical constituents. Therefore the presence of tannins, terpenoides, Quinone, saponins, flavonoids, and steroids in *Cucumis ficifolium* had a different role in the treatments of the pathogen. Despite people using *Cucumis ficifolium* as traditional medicinal value for wound healing, treatment of fowl disease, treat syphilis, fumigation, etc. However, the antibiotic activity and phytochemicals composition of this plant is not known thus the study was providing the phytoconstituents and antibiotic activity of *Cucumis ficifolium* plant on the selected pathogen.

The specific objective of this study were

To assess the antibacterial activity of the aqueous-methanol extracts of the *Cucumis ficifolius* seed, leaf, and fruit parts.

To perform the preliminary phytochemical screening of crude extracts from fruit, seed, and leave parts of *Cucumis ficifolius*.

### 2 Materials And Methods

#### 2.1 Collection and authentication of the plant material

Fresh whole parts of *Cucumis ficifolius* used for this experiment were collected from Arema natural frosts in Delgi which were found in North Gondar during October 2017. The identification of the plant sample was authenticated at the University of Gondar, College of Computational and Natural Sciences, Department of Biology. Finally, the voucher specimen had been deposited in the University of Gondar, College of Veterinary medicine and animal sciences.

#### 2.2 Preparation of plant for extraction
The collected plant material was dried at room temperature without exposing it to direct sunlight in a shaded area to bring down the initial large moisture content for about 4 weeks. The fruit and seed part of the plant material was cut by scalpel blade with the help of a hand and the dried material was then ground using a mechanical grinder to form fine powder material of the plant. About 126gm, 94gm, and 162gm powder of leaf, fruit, and seed plant material respectively were gained after the plant materials were ground. Then the powdered material was extracted with methanol and water combination (90%: 10%) within three days’ interval three times and shaken by hand for mixing purposes. The extracted result solution had been filtered using clean double gauze into a clean ask and stored at +4°C. Then the filtrated solution was evaporated by rotary evaporator with 70°C temperature and stored at +4°C with sterilized flusk until used for experiments (17).

2.3 Phytochemical screening

The aqueous-methanol extract of C. ficifolius leaf, fruit, and seed parts was used for phytochemical screening. To identify the chemical constituents of the plant extract, standard procedures were followed. The plant was screened for alkaloids, saponins, flavonoids, cardiac glycosides, polyphenols, quinines, and terpenoids as the following procedures proceed.

Test for terpenoids (Salkowski test): To 0.25 g of each of the crude extract of Cucumis ficifolius leaves, fruit, and seed parts by methanol, 2 ml of chloroform was added. Then 3 ml concentrated sulfuric acid was carefully added to form a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids (4).

Test for saponins: To 0.5 ml of the crude extract of methanol plant materials in a test tube, 5 ml of distilled water was added and the mixture was vigorously shaken. Formation of a froth Persistent for 30 min confirms the presence of saponins (13)

Test for tannins: About 0.25 g of crude extract was boiled in 10 ml of water in a test tube and then filtered. The addition of a few drops of 0.1% ferric chloride to the filtrate resulting in blue, blue-black, green or blue-green coloration or precipitation was taken as evidence for the presence of tannins (4).

Test for flavonoids: About 10ml of ethyl acetate was added to 0.25 g of the crude extract and heated on a water bath for 3 min. The mixture was cooled and filtered. Then, about 4 ml of the filtrate was taken and shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow color in the ammonia layer indicated the presence of flavonoids (4).

Test for cardiac glycosides (Keller-Killiani test)

To 0.25 g of the crude extract diluted to 5 ml in water, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was under lied with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (4).

Test for Quinones

To test the quinone phytochemical presence, in a test tube 1ml of crude extract and 1ml of concentrated sulphuric acid (H2SO4) were added. Formation of red color (37).

Test for phenols

To 5 ml of the crude extract, 1 ml of FeCl3 (1%) and 1 ml of K3(Fe(CN)6) (1%) were added. The appearance of fresh reddish blue color indicated the presence of polyphenols (14).

2.4 ANTIBACTERIAL ACTIVITY ASSAY

Inoculum Preparation and Preparation of Test Solutions: The tested microorganisms were separately cultured on sterilized Muller-Hinton Agar (MHA) at 37°C for 24 hours by using the streak plate method. Then, well-isolated overnight cultured colonies of the same morphological type were selected from the cultured media. Each colony was touched with a flamed wire loop and the growth was transferred into a sterilized test tube containing 5 ml sterile normal saline solution. The test tubes that contain the bacterial suspension were vortexed to be mixed well uniformly. Then, the bacterial suspension was adjusted with 0.5 McFarland turbidity standards. The adjustment and comparison of turbidity of Inoculum tubes were performed by visually observing them with a naked eye against a 0.5 McFarland turbidity equivalence standard with white background and contrasting blue lines in the presence of...
adequate light. The adjusted bacterial suspensions should be used as inocula within 15 minutes; otherwise, they are not used for testing purposes.

**Tested Microorganisms**

A total of 7 bacterial microorganisms were used in this experiment for the zone of inhibition and MIC and MBC assays. The bacteria were purchased from National Veterinary Institute (NVI), Bishoftu, Ethiopia, and gained from National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia. The bacterial strains under the study include *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 6539), *Citrobacter freundii* (ATCC 43464), *Klebsiella pneumonia* (ATCC 700603), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 29213).

### 2.4.1 Determination of Zone of Inhibition (Zone of Inhibition Test)

For the determination of the zone of inhibition, oxytetracycline (30 μg/disc) was used as a standard antibiotic for comparison of the results. The antibacterial activities of the crude aqueous-methanol extract of the seed fruit and leaf parts of *Cucumis ficifolius* were tested against six Gram-negative bacterial and one Gram-positive bacterium (40). Pathogenic test bacteria were streaked to MHA plate and incubated at 37°C for 24 hrs. Before this, suspensions of the bacterial isolates were made in sterile normal saline and adjusted to 0.5 McFarland's standard solution. Small volumes of bacterial suspensions were swabbed to each MHA plate and then evenly seeded and streaked utilizing a sterile cotton swab on the agar plate surface. This procedure was repeated by streaking two or more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums, and, finally, the rim of the agar was swabbed. Then the agar wells were prepared by using a sterilized cork borer with 6mm diameter (40). By using a micropipette, four different concentrations of the plant extract solutions (500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml of plant extracts were mixed with 5% of DMSO) and 5% DMSO were carefully added to the respective wells in the plate media and performed in triplicate. The antibiotic disc (oxytetracycline 30 μg/disc) was dispensed with a dispensing apparatus (sterile pair of forceps) onto the surface of the inoculated agar plate and pressed down to ensure complete contact with the agar surface. The plant extracts and antibiotic disc were allowed to diffuse for about one hour before incubation and then the plates were incubated in an upright position at 37°C for 24hrs. After overnight incubation, the diameters of inhibition zones were measured in mm using Caliber and the results were recorded separately. Oxytetetracycline (30 μg/ml) was used as positive control separately, while 5% DMSO was used as a negative control.

### 2.4.2 Determination of the Minimum Inhibition Concentration (MIC)- by 96-Well Microtiter Plate Using Tetrazolium Chloride.

The minimum inhibitory concentration (MIC) for plant extract was evaluated according to the method described by (11) with minor modification employing 96-well microplates. For each plate, 50 μl of MHB was placed to each well followed by 100 μl of plant extract (which contains 500mg/ml of plant extract) added to the first column of the microplates. This made each well of the first column have a total volume of 200 μl. Starting from the first column serial dilution was conducted up to the 10th column with double folding; the final volume (50μl) of the plant extract and the broth were drawn from the 10th column. One milliliter of MHB was mixed with 100 μl of bacterial suspension from which 50 μl was filled into the wells up to the 10th column aseptically. Subsequently, the plates were incubated for 24 hrs at a 37°C incubator. The minimum inhibitory concentration (MIC) was determined by adding 30 μl (2mg/ml) of 0.02% p-iodonitrotetrazolium chloride (INT) and incubated at 37°C for 30minutes. INT was used as an indicator for bacteria growth; bacteria metabolize it and changed into pink color. The wells that had no change in color after the addition of INT indicated no growth of the microorganisms and they were taken as MIC values (11).

### 2.4.3 Determination of Minimum Bactericidal Concentration (MBC)

The MBC is defined as the lowest concentration where no bacterial growth is observed. This was determined by aseptically subculturing the contents of wells from the MIC results for an individual bacterium to antimicrobial free agar as described in different studies (32). In this technique, the contents of all wells containing a concentration of test material above the MIC value from every four wells, in the MIC determination test, was dropped using a micropipette of about 3μl on colony counting agar plates aseptically and incubated at 37°C for 24h. The lowest concentration of the extract which showed no bacterial growth after incubation was observed for every four wells and noted as the MBC. The average value was taken for the MBC of test material against each bacterium.

### 2.5 Statistical Analysis
The experimental data are expressed as mean ± Standard Error of the Mean (SEM). Data are analyzed using the Statistical Package for the Social Sciences (SPSS), version 20.0 software. The statistical differences of the mean zone of inhibition of crude extract by methanol for individual bacterium were carried out by employing a one-way analysis of variance (ANOVA) followed by Tukey Post Hoc at a significance level of \( P < 0.05 \). The MIC and MBC are analyzed using one-way analysis of variance (ANOVA) using SPSS software. Moreover, the concentration-dependent antibacterial activities of the crude extract by methanol for each bacterium were determined by linear regression analysis using the SPSS software.

3 Results

3.1.1 Phytochemical Screening

The present study revealed that methanol extract of *Cucumis ficifolius* fruit contained flavonoids, tannins, phenols, terpenoid, saponins, and quinone. Methanol extracts *Cucumis ficifolius* leaf contained saponins, flavonoids, phenol, tannins, and terpenoids. *Cucumis ficifolius* seed aqueous-methanol extract contained saponin, flavonoids, phenols, and terpenoids. Moreover, terpenoid, flavonoids, and saponins compounds were found in almost all three parts as shown in Table 1.

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Fruit</th>
<th>Leaf</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terpinoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinone</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: The preliminary phytochemical screening of methanol (99.5%) extracts from fruit, leaf, and seed parts of *Cucumis ficifolius* 2018.

3.1.2 Antibacterial Activity Test

The results from the present study showed that the crude extracts (methanol) from fruit, leaf, and seed parts of *Cucumis ficifolius* displayed antimicrobial activities against all seven strains of tested bacteria. The zones inhibition of the mean diameter of the plant extract against the tested bacteria is tabulated in Tables 2, 3, and 4). The extracts exhibited a broad spectrum of activity.
Table 2
Zone of inhibition (in mm) of the different concentrations of methanolic crude extract of *Cucumis ficifolius* fruit part against gram-positive and gram-negative bacteria.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Bacteria</th>
<th>E. coli stand</th>
<th>K. pneumonia stand</th>
<th>Citrobacter freundii stand</th>
<th>E. faecalis stand</th>
<th>P. aeruginosa stand</th>
<th>S. Typhi stand</th>
<th>S. aureus stand</th>
</tr>
</thead>
<tbody>
<tr>
<td>500mg/ml</td>
<td>11.00 ± 0.16a1b1c2</td>
<td>14.16 ± 0.14a1b1c1d1</td>
<td>10.41 ± 0.31a1b1c2</td>
<td>10.29 ± 0.35a1b1c1d2</td>
<td>10.23 ± 0.14a1b1c1</td>
<td>7.77 ± 0.18a1b1c2</td>
<td>7.89 ± 0.16a1b1c1</td>
<td></td>
</tr>
<tr>
<td>250mg/ml</td>
<td>9.50 ± 0.26a1b2</td>
<td>11.47 ± 0.21a1b1c2</td>
<td>9.26 ± 0.01a1b1c2</td>
<td>9.14 ± 0.01a1b1c2</td>
<td>8.48 ± 0.24a1b1c2</td>
<td>6.26 ± 0.06c2</td>
<td>7.37 ± 0.19a1b1c1</td>
<td></td>
</tr>
<tr>
<td>125mg/ml</td>
<td>7.50 ± 0.30a1b1</td>
<td>9.61 ± 0.11a1b1</td>
<td>8.67 ± 0.09a1b1</td>
<td>7.29 ± 0.14a1b1</td>
<td>8.19 ± 0.19a1b2</td>
<td>Non</td>
<td>6.82 ± 0.04a2b2</td>
<td></td>
</tr>
<tr>
<td>62.5mg/ml</td>
<td>Non</td>
<td>7.18 ± 0.11</td>
<td>Non</td>
<td>Non</td>
<td>Non</td>
<td>Non</td>
<td>Non</td>
<td></td>
</tr>
<tr>
<td>Oxy 30µg/disc</td>
<td>13.60 ± 0.20</td>
<td>10.58 ± 0.26</td>
<td>14.28 ± 0.15</td>
<td>14.25 ± 0.07</td>
<td>10.77 ± 0.17</td>
<td>8.15 ± 0.17</td>
<td>8.65 ± 0.41</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M (n = 3), analysis was performed with One-Way ANOVA followed by Tukey test; a compared to the positive control, b to crude 62.5mg/ml, c to crude 125mg/ml, d to crude 250mg/ml, e to crude 500mg/ml, 1 p < 0.05, 2 p ≤ 0.01. The negative control has shown no antibacterial activity, Non = no inhibition zone, Stand = standard (ATCC). Oxy = oxytetracycline it continues to next page...

Table 3
Zone of inhibition (in mm) of the different concentrations of methanolic crude extract of *Cucumis ficifolius* leave part against gram-positive and gram-negative bacteria.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Bacteria</th>
<th>E. coli stand</th>
<th>K. pneumonia stand</th>
<th>Citrobacter freundii stand</th>
<th>E. faecalis stand</th>
<th>P. aeruginosa stand</th>
<th>S. Typhi stand</th>
<th>S. aureus stand</th>
</tr>
</thead>
<tbody>
<tr>
<td>500mg/ml</td>
<td>10.64 ± 0.26a1b1c2</td>
<td>12.65 ± 0.41a2b1c2d2</td>
<td>10.67 ± 0.51a1b1c2</td>
<td>11.84 ± 0.16a1b1c1d2</td>
<td>12.19 ± 0.32a1b1</td>
<td>9.02 ± 0.28b1</td>
<td>9.08 ± 0.22a1b1c2</td>
<td></td>
</tr>
<tr>
<td>250mg/ml</td>
<td>9.68 ± 0.23a2b1</td>
<td>9.11 ± 0.25a1b1c2</td>
<td>8.90 ± 0.35a1b2c2</td>
<td>8.75 ± 0.41a1b2c2</td>
<td>9.48 ± 0.32a2b1c2</td>
<td>8.53 ± 0.24b2</td>
<td>8.32 ± 0.13a1b1</td>
<td></td>
</tr>
<tr>
<td>125mg/ml</td>
<td>8.46 ± 0.36a1b2</td>
<td>8.09 ± 0.28a1b2</td>
<td>7.59 ± 0.32a1b1</td>
<td>7.91 ± 0.39a1b1</td>
<td>7.75 ± 0.33a1b3</td>
<td>7.95 ± 0.49b2</td>
<td>7.36 ± 0.19a1b1</td>
<td></td>
</tr>
<tr>
<td>62.5mg/l</td>
<td>Non</td>
<td>7.66 ± 0.24a1</td>
<td>7.02 ± 0.27a1</td>
<td>Non</td>
<td>Non</td>
<td>Non</td>
<td>Non</td>
<td></td>
</tr>
<tr>
<td>Oxy 30µl/disc</td>
<td>13.85 ± 0.27</td>
<td>10.68 ± 0.31</td>
<td>14.05 ± 0.23</td>
<td>14.48 ± 0.10</td>
<td>10.56 ± 0.9</td>
<td>8.93 ± 0.4</td>
<td>9.39 ± 0.34</td>
<td></td>
</tr>
</tbody>
</table>
Among the tested bacteria, *K. pneumonia* (14.16mm) was highly susceptible as compared to the other tested bacteria within the concentration of 500mg/ml of plant extract of aqueous methanol of *Cucumis ficifolius* fruit part in this study. As depicted in Table 2, the highly susceptible bacterium at 500mg/ml was *K. pneumonia* (14.16mm) followed by *E. coli*, *Citrobacter freundii*, *E. faecalis* stand, *Pseudomonas aeruginosa* stand, and *S. aureus*, and with a mean of a zone of inhibition diameters (11mm, 10.41mm, 10.29mm, 10.23mm and 7.89mm), respectively. From the tested bacteria, *S. Typhi* is slightly resistant to the plant extract at the concentration of 500mg/ml with 7.77mm mean of inhibition zone diameter; even in the lower plant extract, concentrations showed a lower inhibition zone of diameter. Based on the mean value zone of inhibition, the plant extracts antibacterial activity ability was depending on the concentrations of the plant extracts used. At the concentration of 62.5mg/ml of plant extract, most of the tested bacteria had no inhibition zone, whereas *K. pneumonia* has shown a better mean zone of inhibition diameter (7.81mm) in the same concentration (62.5mg/ml).

Among the tested bacteria, *K. pneumonia* (12.65mm) was moderately susceptible as compared to the other tested bacteria within the concentration of 500mg/ml of plant extract of aqueous methanol of *Cucumis ficifolius* leaf part in this study. As depicted in Table 3, the moderate susceptible bacterium at 500mg/ml was *K. pneumonia* (12.65mm) followed by *Pseudomonas* stand, *E. faecalis* stand, *Citrobacter freundii*, *E. coli*, and *S. aureus*, and with a mean of a zone of inhibition diameters (12.19mm, 11.84mm, 10.67mm, 10.64mm and 9.22mm), respectively. From the tested bacteria, *S. Typhi* is slightly resistant to the plant extract at the concentration of 500mg/ml with 9.02mm mean of inhibition zone diameter; even in the lower plant extract, concentrations showed lower inhibition zone of diameter.

Among the tested bacteria, *K. pneumonia* (6.13mm) was slightly susceptible as compared to the other tested bacteria within the concentration of 500mg/ml of plant extract of aqueous methanol of *Cucumis ficifolius* seed part in this study. As depicted in Table 4, the slightly susceptible bacterium at 500mg/ml was *K. pneumonia* (6.13mm) followed by *Pseudomonas* stand with a mean of a zone of inhibition diameters (5mm). From the tested bacteria, *Citrobacter freundii*, *E. coli*, *S. aureus* *E. faecalis* stand, and *S. Typhi* were highly resistant to the plant extract at the concentration of 500mg/ml with a mean of inhibition zone diameter (4.76mm, 3.81mm, 3.56, 3.48mm, and 2.44mm) respectively; even in the lower plant extract, concentrations showed lower inhibition zone of diameter.

When the three crude extracts were compared with each other and with that of positive control. Some of the extract parts were seen to have greater potential compared to that of oxytetracycline. Crude extracts from fruit, leaf, and seed parts of *C. ficifolius* results showed that the diameter of inhibition zones ranging from 7.02 to 14.16 mm, with the highest inhibition zone observed against *K. pneumonia* (14.16mm), followed by *P. aeruginosa* (12.19 mm), *E. faecalis* (11.84 mm) and *Citrobacter freundii* (10.79). The least inhibition zone was observed against *S. Typhi* (7.77 mm) followed by *S. aureus* (7.89mm) at 500mg/ml. Despite the inhibition zone observed against *S. aureus* was only 7.89 mm, it is noteworthy when compared with that of positive control. Out of the 7 pathogenic strains tested, the fruit and leaf parts showed inhibition zones comparable with that of the positive control (oxytetracycline) used.
Crude extract of *C. ficifolius* fruit exhibited the highest inhibition zone against the standard strain of *K. pneumonia* followed by the standard strain of *E. faecalis* whereas the standard strain of *S. typhi* and *S. aureus* showed the least inhibition. Crude extract of *C. ficifolius* leave and seed parts exhibited the highest inhibition zone against *K. pneumonia* followed by *P. aeruginosa* while *S. typhi* and *S. aureus* were the least inhibited.

The present study also revealed that inhibition zone of crude extract from a fruit, leaves, and seed parts *Cucumis ficifolius* concentration were statistically different when compared with each other and that of their respective positive control (p < 0.05) against most of the tested bacteria as showed on the tables (2,3, 4). Moreover, the inhibition zone of crude extract *C. ficifolius* fruit at 500 mg/ml was significantly different (p < 0.05) when compared to that of its zone of inhibition at 250 mg/ml against the growth of each test bacterium except for the standard strain of *E. coli*, *S. Typhi* and clinically isolated strain of *S. aureus*.

The zone of inhibition at 250mg/ml was significantly different (p < 0.05) as compared with that of the inhibition zone at 125mg/ml against the growth of each tested bacterium except that of the standard strain of *E. coli*. The zone of inhibition at 125mg/ml was significantly different (p < 0.05) as compared with that of a zone of inhibition at 30µg/ml against the growth of each tested bacterium except the standard strain of *S. typhi*. Generally, among the tested standard strain *K. Pneumonia, E.faecalis, Citrobacter freundii, P aeruginosa*, and *E. coli* were more susceptible than that of the *S. typhi* and *S. aureus* at the corresponding tested concentrations of crude extract from a fruit, leaf, and seed parts of *Cucumis ficifolius*, especially at 500 mg/ml and 250mg/ml mg/ml. As depicted in tables (2, 3, 4).

### 3.1.3 Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of crude extract from fruit, leaf, and seed parts of *C. ficifolius* were evaluated against six standard strains of gram-negative bacteria and one clinically isolated gram-positive bacteria. The results are summarized in Tables 5 and 6. The result revealed significant differences in MIC values among fruit, leaf, and seed extracts and tested bacterial strains with MIC values ranging from 0.52 mg/l to 20.83 mg/l. The antibacterial activities of this plant were found to vary between the leaf, fruit, and seed parts of *C. ficifolius*.

**Table 5**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Crude extract of fruits of <em>C. ficifolius</em></th>
<th>Crude extracts of leaf of <em>C. ficifolius</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td><em>E.coli</em> stand</td>
<td>4.17 ± 0.33</td>
<td>5.20 ± 0.33</td>
</tr>
<tr>
<td><em>K.pneumonia</em> stand</td>
<td>0.52 ± 0.13</td>
<td>0.65 ± 0.13</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em> stand</td>
<td>4.17 ± 0.33</td>
<td>4.17 ± 0.33</td>
</tr>
<tr>
<td><em>E.faecalis</em></td>
<td>2.62 ± 0.33</td>
<td>4.17 ± 0.33</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> stand</td>
<td>8.33 ± 0.33</td>
<td>12.5 ± 0.33</td>
</tr>
<tr>
<td><em>S.typhi</em> stand</td>
<td>20.83 ± 0.33</td>
<td>25.00 ± 0.00</td>
</tr>
<tr>
<td><em>S.aureus</em> stand</td>
<td>12.50 ± .33</td>
<td>12.5 ± 0.33</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M (n = 3), analysis was performed with One-Way ANOVA followed by Tukey test, Stand = standard. Continue...
Table 6
The MIC (in mg/ml) crude extract of *Cucumis ficifolius* seed part against gram-positive and gram-negative bacteria.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Crude extract of <em>C. ficifolius</em> seed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
</tr>
<tr>
<td><em>E. coli</em> stand</td>
<td>8.33 ± 0.33</td>
</tr>
<tr>
<td><em>K. pneumonia</em> stand</td>
<td>4.17 ± 0.33</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em> stand</td>
<td>6.25 ± 0.00</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>4.17 ± 0.33</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> stand</td>
<td>8.33 ± 0.33</td>
</tr>
<tr>
<td><em>S. typhi</em> stand</td>
<td>20.83 ± 0.33</td>
</tr>
<tr>
<td><em>S. aureus</em> stand</td>
<td>16.67 ± 0.33</td>
</tr>
</tbody>
</table>

Table 7
Material and Equipment

<table>
<thead>
<tr>
<th>Personal protective equipment</th>
<th>Gown, glove, shoes, face mask</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample preparation materials</td>
<td>Cotton swab, Plastic shed, conical flask, beaker, scalpel blade</td>
</tr>
<tr>
<td>Laboratory material</td>
<td>Petri dish, tree refrigerator, autoclave, scalpel blade, tree, electro balance, borer, test tube, aluminum foil, Rota vaporizer machine, pork, Petri dish, wire loop, Incubator, Bunsen burner, waste disposal, etc.</td>
</tr>
<tr>
<td>Laboratory chemicals and reagents</td>
<td>Alcohol, sulfuric acid, HCl, ethyl acetate, ferric chloride, DMSO, salt (sodium chloride), chloroform, barium chloride, distilled water</td>
</tr>
<tr>
<td>Different media and standards</td>
<td>McFarland, media (Muller-Hinton agar), plate count agar, Muller-Hinton broth</td>
</tr>
</tbody>
</table>

The MIC range against different indicator bacteria was 0.52–20.83 mg/mL for *C. ficifolius* fruit part, 1.82 to 20.83 mg/mL for *C. ficifolius* leaf part, and 4.17 to 20.83 mg/mL for the seed part of *C. ficifolius*. Thus the order of antibacterial activities of *C. ficifolius* parts was in the order of fruit > leaf > seed. The MBC range against different indicator bacteria was 0.65–25 mg/mL for fruits of *C. ficifolius*, 3.64 to 25 mg/ml for the left part of *C. ficifolius*, and 5.21– 25 mg/mL for seeds of *C. ficifolius*. Thus the order of antibacterial activities of plant species was in the order of fruits of *C. ficifolius* > leaf of *C. ficifolius* > seeds of *C. ficifolius*. The overall result showed that methanol extract of crude *C. ficifolius* fruit exhibited the highest antibacterial activity against *K. pneumonia* (MIC values of 0.52 mg/mL) as compared to that of seed and leaf extracts as depicted in tables (5, 6) while crude extract of *Cucumis ficifolius* seed part exhibited the least antibacterial activity against the tested bacteria as compared to the other parts as depicted in Table 6.

4 Discussions

Infectious diseases are one of the major problems in developing as well as developed countries.

Traditional medicinal plants are widely used to treat microbial diseases due to their rich source of antimicrobial activity and less cost (16). The present study permitted the evaluation of some biological properties of *C. ficifolius*, including the antibacterial activity of some selected bacterial species. Groups of phytochemical compounds commonly associated with combating microbial resistance and having antimicrobial activity in medicinal plants are flavonoids, alkaloids, tannins, triterpenoids, essential oils, saponins, glycosides, and phenols (30). Despite this time it is difficult to judge the mechanism of actions of the bioactivity of the extract of the study plant of the *C. ficifolius* fruit, leaf, and seed parts. It is possible to say that the plant has antibacterial activity based on the chemical detection methods in the phytochemical screening of aqueous-methanol extract.
The methanol extracts of C. cifolius. In the present study methanol crude extract from the fruit part of Cucumis cifolius was subjected to preliminary tests that revealed the presence of various constituents like quinones, phenols, saponins, tannins, flavonoids, and terpenoids. On another hand, the methanol crude extract of the leaf part contained saponin, tannins, flavonoids, phenols, and terpenoids and the seed part contained saponin, flavonoids, phenols, and terpenoids. There was a report that the biological efficacy of the plants in turn depends on the presence of the required quantity and nature of the secondary metabolite in the crude extract (42). These secondary metabolites are reported to have many biological and therapeutic properties (43). So that the presence of these phytochemicals could be some extent justifies the observed antimicrobial activities in the current study.

The presence of flavonoids in crude extracts of plant contributes their share for the observed antibacterial activities. The possible mechanism of action for the antibacterial effects of flavonoids includes the damage or disruption of the cell membranes and inhibition of the synthesis of nucleic acids which can lead to the death of the susceptible bacterium (10). Therefore the antibacterial activities found in the fruits, leaves, and seeds parts of Cucumis cifolius might be due to the presence of flavonoids in the crude extract.

Terpenoids are the other class of compounds known to have antimicrobial activities. There was one report that the terpenoid fractions isolated from Luffa cylindrical and Elephantopus scaber (20) were found to have antibacterial activities against various pathogenic microbes including S. aureus, E. coli, and P. aeruginosa with varying selectivity. Therefore the susceptibility of P. aeruginosa to methanolic extracts of C. cifolius leave part might be due to the presence of terpenoids in high concentration in the crude extract.

The antimicrobial activity of the crude extracts from fruit and seed parts of C. cifolius was done for the first time. In the present study, the aqueous methanol extracts from leaf, seed, and fruit parts of C. cifolius showed moderate antibacterial action against most of the tested strains of bacteria used in this study. The crude extract from fruit and leaf parts of C. cifolius was found to have a greater antibacterial effect against most of the gram-negative tested bacteria than that of the seed part of Cucumis cifolius. This might be due to the presence of different compositions and a concentration of the secondary metabolites found in the crude extract of fruit and leaf parts of C. cifolius which acts as selectively or synergistically against the tested bacteria than that of the seeds of C. cifolius.

Fruit part of C. cifolius crude extract showed the highest antibacterial activity against K. pneumonia as calculated as the mean diameter of growth inhibition zone in mm average. On the other hand, the seed part of C. cifolius methanolic extract showed the least antibacterial activity. There was a report that the activity difference among the gram-negative and gram-positive bacteria could be because of the partial penetration of the bioactive phytochemicals through the lipopolysaccharide rich outer cell membrane in the cell wall of gram-negative bacteria (33) unlike in the cell wall of the gram-positive bacteria with less effective permeability barrier (37).

Also, the high inhibitory potential of methanolic extract might be due to the high solubility of the phytoconstituents in the polar organic solvent like methanol (41). Therefore the highest sensitivity of K. pneumonia to aqueous methanol extract of C. cifolius fruit part might be due to the partial penetrations of bioactive phytochemicals through the lipopolysaccharide-rich outer cell membrane in the cell wall and high solubility of the phytoconstituents of fruit parts in the methanol. Whereas, of all the test bacteria in this study S. typhi, was relatively found to be resistant to all extracts of the leaf, seed, and fruits of C. cifolius at the highest concentration tested in the present study. This might be due to the presence of bioactive secondary metabolites found in all extracts having less selective against S. typhi.

The antibacterial screening findings in terms of zone of inhibition of the study's plant extract of C. ficifolius against the selected tested bacteria were inversely proportional to their values of MIC and MBC, which means having higher inhibition zones with lower MIC and MBC images, with the exception of S. typhi and S. aureus. The MIC range against the tested bacteria was 0.56-20mg/ml for the plant extract in this study. All the tested bacteria except K. pneumonia and P. aeruginous stand inhibited their growth with the least dilution, which means high concentrations of the plant extract. However, K. pneumonia and P. aeruginous stand inhibited its growth with higher serial dilution or with lower concentration of the plant extract even with 0.52mg/ml.

The crude extract of C. ficifolius fruit part exhibited the highest antibacterial activity against K. pneumonia (MIC values of 0.52 mg/mL) as compared to the seed and leaf parts of C. ficifolius as depicted in the above result (Tables 5, 6). Moreover, aqueous methanol extracts of Cucumis ficifolius seed part had lower antibacterial activity against other tested bacterial strains relative to the
fruit and leaf parts of *C. ficifolius*, especially against the standard strain of *S. typhi* this suggests that the presence of secondary metabolites with relative selectivity against was lowest as compared to the other parts of the extracts.

The highest susceptibility showed by *K. pneumonia* towards fruit extract suggesting that the fruit might contain secondary metabolites selectively against *K. pneumonia*. *P. aeruginosa* has been reported as one of the troublesome gram-negative multiple drugs resistance bacteria. It is known as a common opportunistic pathogen that is responsible for hospital-acquired infections as well as in the community; it causes serious problems such as pneumonia, urinary tract infections, wound or surgical site infections, and bloodstream infections (24). But is highly susceptible to the methanolic extracts of the crude leaf of *C. ficifolius* this might be suggested that the bioactive secondary metabolites found in the leaf part of the plant were selective against *P. aeruginosa*.

Generally, in the present study, there are variable degrees of sensitivities of the tested bacteria, antibacterial activity screening results were still indicative of the potential of the leave, fruit, and seed of *C. ficifolius* as effective medicaments in the treatment of infections caused by the tested bacteria. These effects might be attributed to either the individual class of compounds (bioactive secondary metabolites) present in the crude extract by methanol or to the synergistic effect that each class of compound exerted to give the observed antibacterial activity found.

### 5 Conclusion

Preliminary phytochemical analysis revealed the presence of different phytoconstituent like flavonoids, saponins, and terpenoids in fruit, leaf, and seed parts of *Cucumis ficifolius* that play an important role in antibiotic activities. The present study also revealed that aqueous methanol extract of fruit, leaf, and seed parts of *Cucumis ficifolius* have antibacterial activities against the growth of the selected pathogenic bacteria with varying antibacterial spectrum. Based on the result of the above study it can be concluded that *Cucumis ficifolius* showed higher antibacterial activities against the followed microorganisms like *K. pneumonia*, *E. faecalis*, *Citrobacter freundii*, *P. aeruginosa*, and *E. coli* with having potential for further study to serve as a source of antibacterial agents. Also, it justifies the claimed uses of fruit and leaf parts of the *Cucumis ficifolius* in the traditional system of medicine to treat various infectious diseases caused by the microbes. The tested extracts also exhibited differential MIC and MBC values against the tested bacterial strains. Antimicrobial activities are aggravated by increasing the quantity of this compound, which can be used as an alternative for antibiotics.

### Abbreviations

ANOVA  
One-way Analysis Of Variance  
ATCC  
American Type Culture Collection, *C.ficifolius*: *Cucumis ficifolius*  
CFU/ml  
Colony-Forming Unit  
CSA  
Central Statistical Agency  
DMSO  
Di Methyl Sulf Oxide  
*E. coli*  
*Escherichia coli*  
*E. faecalis*  
*Enterococcus faecalis*  
*K. pneumonia*: MBC: Minimum Bactericidal Concentration  
MHA: Muller Hinton Agar  
MIC  
Minimum Inhibitory Concentration  
NCCLS  
National Committee for Clinical Laboratory Standards
Declarations

Author contribution

Samuel Abebe developed the strategy for the review, screened titles and abstracts, conducted the quality assessment, supported data extraction, contributed to the writing of the manuscript, and performed the descriptive analysis. All things are done by this author.

Funding

The author does not receive any financial support.

Availability of data and material

The data and material used in this publication manuscript will be attached through the supplementary file.

Ethics approval and consent to participate

Not applicable in this publication manuscript

Consent for publication

Not applicable in this publication manuscript.

Conflict of interests

The author declares that he has no conflict of interest in this publication.

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