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Original Article

Phytochemical Screening and Antibacterial Activities of Aqueous and Alcoholic Extracts of *Averrhoa bilimbi* Leaf against Bacteria Isolated from Oral Cavity

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Abstract

Medicinal herbs have been used as traditional treatments for many pathogens and extracted bioactive compounds from medicinal plants with a suitable therapeutic index for the production of new drugs. Moreover, they are utilized to evaluate different concentrations of aqueous and alcoholic extracts of *Averrhoa bilimbi* leaves and antibiotics against bacteria isolated from the oral cavity. This study was conducted simultaneously at the Departments of Botany and Biology, Shatrah Hospital, Thi-Qar, Iraq, during March and August 2021. *A. bilimbi* leaf extracts were utilized in the plant component examination and the assessment of the antibacterial activity. The bacterial strain of *Escherichia coli* and *Klebsiella pneumoniae* was isolated from the oral cavity. To test the antibacterial impact of the extracts on bacteria, the agar well diffusion method was used. The phytochemical screening indicated the presence of Alkaloids, Flavonoids, Sapiens, Steroids, Tannins, Glycosides, and Carbohydrates, followed by the absence of Tannins in aqueous extract. Due to the *A. bilimbi* leaf aqueous and methanol extract against *E. coli*, areas of inhibition were found (0.20 cm and 0.19 cm) at the concentration of 100 mg/ml, respectively. However, there were no regions of inhibition of the *K. pneumoniae* trend for both extracts. The sensitivity of bacterial isolates of *E. coli* and *K. pneumonia* to antibiotics was also tested through Gentamicin, Amoxycillin, Azithromycin, Ciprofloxacin, Penicillin, and Polymyxin B, and the regions of inhibition appeared against *E. coli* (0.5cm, 0 cm, 0.34 cm, 0.45 cm, 0 cm, and 0.12 cm, respectively). Furthermore, the regions of inhibition appeared against *K. pneumoniae* (3 cm, 0.3 cm, 0.4 cm, 0.55 cm, 0 cm, 0.66 cm, respectively). The antibiotics showed a higher inhibition zone, compared to the aqueous and alcoholic extracts; however, further studies are required to be conducted to validate its reliability.

Keywords: Antibacterial activity, *Averrhoa bilimbi*, *Escherichia coli*, *Klebsiella pneumoniae*

1. Introduction

The human oral cavity commonly contains certain temperatures and moisture, as well as several nutritional compounds, such as carbohydrates, lipids, and proteins, which shelter the growth of bacteria, and occasionally, play an incubator role in some pathogenic microorganisms (1). Furthermore, the oral cavity provides two types of surfaces for bacteria colonization (e.g., soft tissue and hard tooth enamel exposed root surfaces) allowing various bacterial populations to grow. Pathogenic bacteria are the most common infections in humans, including *Escherichia coli* which is found in the human intestine. It usually colonizes in the digestive tract and attaches to the mucus of the large intestine through food or water (2). Infections of bacteria are usually treated with antibiotics; however, *E. Coli* is immune to many effective antibiotics. Serious food poisoning, urinary tract infections,

diarrhea, extreme illness, or death may be induced by this specific bacteria (3). Other bacteria, such as *Klebsiella pneumoniae* can also be pathogenic in humans and are found in the mouth, skin, and intestines; moreover, they can cause urinary tract infections and pneumonia in critically ill and immunocompromised hospitalized patients (4). For thousands of years, medicinal plants are being utilized as conventional remedies for various pathogens. Researchers now pay special concern to alternate phytomedicines and biologically active compounds extracted from plant species being utilized in herbal drugs with an appropriate therapeutic index for the production of new medications (5, 6). Many species of plants have reported drug properties and are known to have different secondary metabolites, such as Glycosides, Sapiens, Flavonoids, Steroids, Tannins, Alkaloids, and Terpenes (7). According to the World Health Organization survey, about 80% of the world's inhabitants are using medicinal plants for their primary health care (8). This study examined the leaves of the *A. bilimbi* for its phytochemical screening and antibacterial activity in the continuation of the development of new drugs. *A. bilimbi* belongs to the family *Oxalidaceae*, a tropical plant, native from South-East Asia, the least resistant to cold, and growing best in rich and well-drained soils. The *A. bilimbi* tree is long-lived and reaches up to 5-10 m in height; in addition, its leaves are about 3-6 cm, long arranged in the alternate, imparipinnate, and cluster at branch extremities (Figure 1). It is utilized as an antibacterial and antiscorbutic; moreover, a leaf infusion is efficient or can be used as an after-birth tonic. Previously, the Philippines used the *A. bilimbi* leaf as dough on itches, tumefaction, rheumatism, mumps or skin rash, inflammation of the rectum and diabetes, hypertension, stomach pain, aphthous sore, and as a cooling drink. Malaysians utilized fresh *A. bilimbi* leaves to treat genital illness (9). The fruit is very sour due to its high oxalic acid (10). Amino acids, citric acid, cyanidin-3-O-h-D-glucoside, phenolics, potassium ions, vitamin A, and sugar are among the chemical components of *A. bilimbi* (11, 12). Former phytochemical investigations showed that chloroform extracts of the *A. bilimbi* leaves have antibacterial action against different bacterial strains (13, 14). In the Ayurvedic method of medicine, different medicinal plants express anti-cancer activity. *A. bilimbi* extracts have proven anti-cancer performance and different cell lines (8, 15). Therefore, this study aimed to assess the ability of aqueous and alcoholic extracts of *A. bilimbi* leaf parts against *E. coli* and *K. pneumonia* strains of bacterial isolations from the oral cavity.

Figure 1. *Averrhoa bilimbi* leaves; Ken and Robert (10)

2. Materials and Methods

2.1. Collection and Identification of the Plant Materials

A. bilimbi leaves were obtained from trees planted in North Iraq and were collected from three different levels of the tree during March and August 2021. The plant had to be clean and free of infection.

2.2. Preparation of the Plant Extracts

The fresh leaves of *A. bilimbi* were washed completely three times with running and sterile distilled water to remove the dust particles and then dried under shade for seven days. The dried leaves were then ground into fine powders utilizing a laboratory grinding mill. Samples were extracted from 100 grams of powdered *A. bilimbi* leaf, soaked in 300 mL of ethanol and distilled water for 24 h at room temperature, and protected from light with periodic shaking. The solvent was changed until the solution became pure, and the supernatant was filtered through Whatman filter paper. Maceration solutions were concentrated under reduced pressure utilizing a rotary evaporator at 500˚C. The crude extracts were collected and dried at room temperature (9).

2.3. Phytochemical Screening

The Steroids, Glycosides, Flavonoids, Tannins, Saponins, Carbohydrates, and Alkaloids were detected as described in a study by Ghani (16) and Sravanthi, Ramana (17) (Table 1).

Table 1. Phytochemical examination of aqueous and alcoholic leaf extracts of *A. bilimbi*

No	Phytochemicals	Ethanol	Aqueous
1	Alkaloid	+	+
2	Saponin	+	+
3	Tannin	+	-
4	Flavonoid	+	+
5	Steroid	+	+
6	Carbohydrate	+	+
7	Glycoside	+	+

2.4. Isolation and Identification of Pathogens

This study collected swabs from the nose and mouth of patients, and the identification of bacterial pathogens of *E. coli* and *K. pneumonia*, as well as isolation, were conducted on culture media (MacConkey agar and blood agar) and incubated at 37°C for 24 h. After incubation, the individual colonies with different morphologies were picked using sterile incisors, grown in nutrient broth, and incubated at 37°C for 24 h. Consecutively, using the standard culture techniques, for antimicrobial assays, these stock cultures were maintained at 4°C (18).

2.5. Antibacterial Activity Test

The antibacterial activity of methanol and aqueous extracts of *A. bilimbi* was evaluated using an agar well diffusion test (19). In this method, 0.1 ml of test organism was inoculated on the Muller Hilton Agar. It was then spread upon the surface of the agar using a sterilized glass spreader. After 10 min of inoculation, the wells were prepared by utilizing a sterilized steel cork borer (6 cm diameter). The wells were made in each plate and loaded with each aqueous and alcoholic *A. bilimbi* leaves at the concentration of 100 ug/ml. All the plates were then incubated at 35±2°C for 24 h. The antibacterial activity of the extracts was determined by measuring the inhibition zone in cm against strain bacterial isolated. Results were compared with standard antibiotics, such as Gentamicin, Amoxycillin, Azithromycin, Ciprofloxacin, Penicillin, and Polymyxin B.

2.6. Analytical Statistics

Each experiment was carried out in triplicates, with the means from absolute data being reported. The statistical analysis of the acquired data was conducted in SPSS software (version 21) through ANOVA.

3. Results and Discussion

The results reveal that the current extracts were more efficient against bacteria, which is consistent with the results of earlier publications (13). In this study, *E. coli* and *K. pneumonia* were isolated from different clinical samples of the oral cavity and were grown on culture media. This media confirmed the growth of *E. coli* and *K. pneumonia* isolates, and after 24 h of incubation, a colony of most *E. coli* and *K. pneumoniae* isolates on blood agar started developing characteristic colors. *K.pneumoniae* is a negative bacterial, non-motile, encapsulated, lactose-fermenting, facultatively anaerobic, and penis-shaped bacterium. In the blood agar medium, *K. pneumoniae* colonies are non-hemolytic which show Gamma Hemolysis (γ-hemolysis). Furthermore, in MacConkey agar medium, the colonies of *K. pneumoniae* are pink colored due to the lactose fermentation, which became more eminent after 48 h. Incubated at 37°C, *E. coli* colonies appeared on sheep blood agar after 24 h.

Most strains of *E.coli* produce smooth, circular, and low-convex colonies with an entire edge that is about 3-4 mm in diameter. They are greyish, butyrous, and readily emulsified. Partial digestion of erythrocytes may cause profound discoloration of agar under colonies that are quite often beta-hemolytic. Colony colors displayed *E. coli* and *K. pneumoniae* isolates on blood Agar that are given in figure 2.

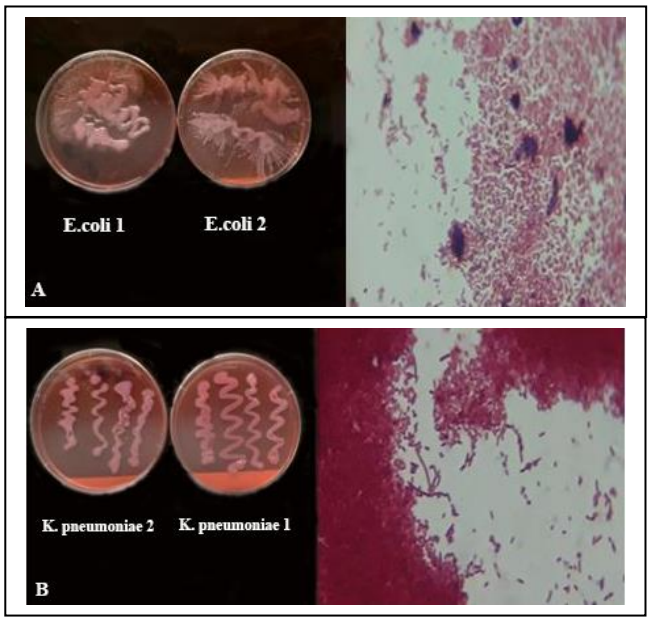


Figure 2. A. *E.coli* and **B.** *K. pneumonia* growing on blood agar and diagnosis

3.2. Preliminary Phytochemical Screening

The phytochemical analysis detected the existence of Alkaloids, Saponins, Tannins, Flavonoids, Steroids, Carbohydrates, and Glycosides, which are present in aqueous and alcoholic leaf extracts of *A. bilimbi* only (Table 1), while Tannins are absent in aqueous extract.

The antibacterial activity of the plant can be imputed to its phytochemical compounds, which work as armor against bacterial pathogens. Alkaloids, Saponin, Tannin, Flavonoid, Steroid, and Carbohydrate Glycosides are the most significant phytochemical substances (20), and according to Abuga, Sulaiman (21), they can limit bacterial development by destroying the bacterial cell wall. Alkaloids naturally exist as organic molecules that contain large nitrogen atoms. They can have pharmacological effects to be used as medical medicines. Flavonoids improve vitamin C's effects and act as antioxidants. Liver toxins, cancers, viruses, and other bacteria are biologically active (22). Terpenoids from plants are used largely because of their aromatic properties. They are playing a part in classic medicinal herbal products and are being investigated for anti-bacterial, anti-neoplastic, and other pharmaceutical properties (6). Tannins can have antiviral, antibacterial, and antiparasitic properties. Red blood cells are hemolyzed by saponins (6). The saponins contain the capacity to cause the infiltration of proteins and specific enzymes in the cell.

3.3. Antibacterial Activity

The *A. bilimbi* leaf medicinal plant showed strong antibacterial effects against bacterial isolates of *E. coli* and *K. pneumonia* from the oral cavity. The higher concentration of the *A. bilimbi* leaf extract leads to the greater diameter of the inhibition zone against bacterial isolates of *E. coli* and *K. pneumonia*. Due to the *A. bilimbi* leaf aqueous and methanol extract against *E. coli*, an area of inhibition was found (0.20 cm, 0.19 cm) at the concentration of 100 mg/ml, respectively (Figure 3, Graph 1). However, there were no regions of inhibition of the *K. pneumonia* trend for both extracts (Figure 4, Graph 1). The sensitivity of bacterial isolates of *E. coli* and *K. pneumonia* bacteria to antibiotics was also tested (Gentamicin, Amoxycillin, Azithromycin, Ciprofloxacin, Penicillin, and Polymyxin B), and the region of inhibition appeared against *E. coli* (0.5 cm, 0 cm, 0.34 cm, 0.45 cm, 0 cm, and 0.12 cm), respectively (Figure 3, Graph 1). Furthermore, the region of inhibition appeared against *K. pneumonia* (3 cm, 0.3 cm, 0.4 cm, 0.55 cm, 0 cm, and 0.66 cm), respectively (Figure 4, Graph 1).

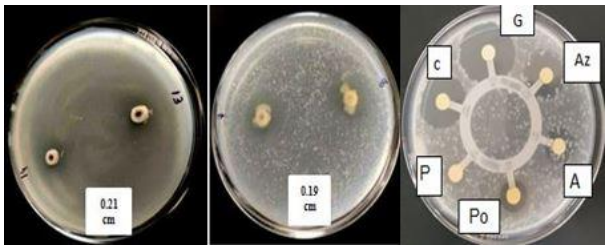
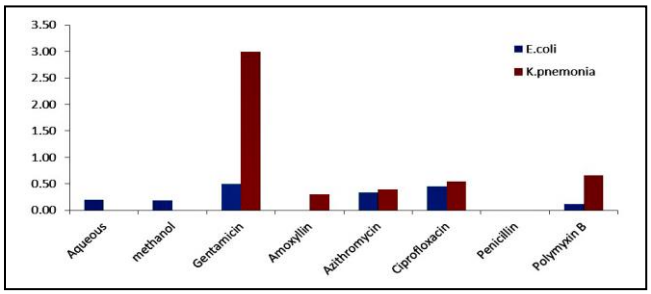


Figure 3. Impact of *A. bilimbi* leaf extracts and antibiotics against bacterial isolates of *E. coli*
G=Gentamicin 0.5, A=Amoxyllin 0, AZ=Azithromycin -0.34, C=Ciprofloxacin -0.45, P=Penicillin 0, P= Polymyxin B -0.12



Graph 1. Impact of *A. Bilimbi* leaf extracts and antibiotics against bacterial isolates of *E. coli* and *K. pneumonia*

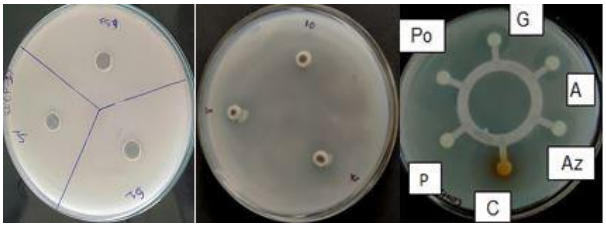


Figure 4. Impact of *A. bilimbi* leaf extracts and antibiotics against bacterial isolates of *K. pneumonia*
G=Gentamicin 3, A=Amoxyllin 3, Az=Azithromycin 4, C=Ciprofloxacin 5, P=Penicillin 0, Po=Polymyxin B 6.

The results of this study revealed that the extracts of the *A. bilimbi* leaf had strong antibacterial actions. The findings also indicated that the extracts from the *A. bilimbi* leaves and antibiotics showed high inhibition zones against strains of *E. coli* and *K. pneumonia* isolations from the oral cavity. The isolation of potent antibacterial chemicals from the leaves of *A. bilimbi* might be of great importance in the development of novel medicines.

Authors' Contribution

Study concept and design: M. M. A.
Acquisition of data: H. M. A.
Analysis and interpretation of data: H. S. J.
Drafting of the manuscript: M. M. A.
Critical revision of the manuscript for important intellectual content: H. M. A.
Statistical analysis: H. S. J.
Administrative, technical, and material support: M. M. A.

Conflict of Interest

The authors declare that they have no conflict of interest.

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