Antibacterial evaluation of the chosen medical plants on pathogenic bacteria commonly related to the upper respiratory system infections

Ghaidaa J Mohammed and Ali A Alsudani

Department of Biology, College of Science, University of Al-Qadisiyah, Iraq

Email: ghaidaa.mohammed@qu.edu.iq

Abstract. Some curative plants have been picked to screen their inhibitory activity against seven bacterial isolates commonly associated with the upper respiratory tract infections. The plant's extractors, in the concentration of 100 µg/ml have been examined. Acacia nilotica ssp. nilotica, Psidium guajava, and Eucalyptus camaldulensis were the most effective plants. Maximum activity of plant extracts was observed on Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. In contrast to the reference antibiotics, Acacia nilotica ssp. nilotica showed a vast spectrum of antibacterial effectiveness and it was present distinctly excellent. These findings should stimulate the search for novel, natural products as antibacterial agents.

Keywords: Antibacterial activities, Medicinal plants, Respiratory tract infections, Pathogenic bacteria

Introduction:

Pathogenic bacteria possess expanded resistance toward numerous antibiotics because of the random user of the antimicrobial medications [1-2], that generated issues in the curing of the treatment of contagious illnesses [3-4]. Moreover, antibiotics are occasionally correlated with the side impacts, while there are several benefits of utilizing the antibacterial components from a plant source, like little side-influences, superior patient bearing, comparatively least costly, admission belong to the lengthy history of using and having the renewable characteristics in nature [5-6]. While this information highlights the necessity for modern alternate medication manners.

Sharp infections of the respiratory system are a major reason of the infancy morbidity and death rate worldwide but those at the elevated hazard in the evolving countries, possessing restricted arrival to clinical assistance [7].

The main purpose of the recent work is to confirm the supposed efficacy of elect plant species as antibacterial agents.

Materials and Procedures:

Plant part: Acacia nilotica ssp. nilotica “Sunt”, Citrus paradise “Grape Fruit”, Combretum hartmanianum “Habeel”, Croton zambesicus “Um Glegla”, Eucalyptus camaldulensis “Kafor Ahmer”, Magnifera indica “Manga”, Psidium guajava “Guava”, Syzygium cumini “Colate”, Terminalia brownii “Shaf”, Ziziphus spina-christi “Sidr”. It has
been insurance the plant samples in the plant laboratory, Biology department, Sciences College, University of Al-Qadisiyah.

**Plant extractors elaboration:**

100 grams from the dried organ of each plant were separately macerated in ethanol (80%) for 24 hours at room temperature (30°C). The crude ethanolic extractor was purified out and the dehydrated remaining of plant material was repeatedly overdoing with petroleum ether to 24 h, filtrated and was vaporized to desiccation at room temperature (petroleum ether extract). The remaining of the plant material gained like the up step, macerated with ethyl acetate to 24 h, filtrated and vaporized to filtered and evaporated to get ethyl acetate extractor. The last residual plant material macerated with methanol for 24h, then filtered and evaporated at room temperature, while plant material residue was discarded [8].

**Bioassays:**

**a-Bacterial isolates:**

A collecting of seven bacterial isolates commonly related to the infections of the upper respiratory system, was chosen for this work; involving Gram-positive and Gram-negative bacteria: (1) Gram-positive bacteria represented by Streptococcus pyogenes, Streptococcus pneumonia, and Staphylococcus aureus. (2) Gram-negative bacteria included Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Haemophilus influenzae. The bacterial isolates were stimulated and repeated three consecutive times on nutrient agar and stocked on the nutrient agar slants at 4 C. Following cultivation and tests were cultivation and tests were achieved on the nutrient agar medium.

**b- Antibacterial testing:**

The inhibitory effect of plant extracts against bacteria was screened by the well-agar diffusion procedure [9]. 250 ml of disinfected nutrient agar was utilized to do the test. The inoculum size of every tested bacterial species was checked to a hanging of 10^6 cells. 2ml from bacterial culture(24 hours) was added up to 250 ml of melted down and cooled down nutrient agar and then mixing up, nearly 20 ml of the cultivated agar were teemed in the pre-sterilized plates and let to settle. Three wells with a diameter (10mm) were done in the nutrient agar by a sterilized cork pricket and the well disks were taken away. 0.1 ml aliquot of the prepared extract was put in the well with a pipette and then left the plates for 2 hours at room temperature to allow the diffusion of the extractor in the agar. Thereafter, the plates incubated at 37°C for 24 hours. After nursery, the inhibition zones' diameters were measured with mm [9]. The antibiotics; gentamycin, tetracycline, ampicillin, penicillin, and cephalosporin with concentrations ranged from 40µg/ml to 5 µg/ml were used as a positive control and Dimethylsulphoxide (DMSO) used as a negative control.

**Results:**

Results of the antibacterial activity of the screened plant extracts are shown in table 1.

**1- Acacia nilotica ssp. nilotica extracts:**

Ethanol extracts of flowers exhibited elevated activity against all the examined Gram-negative bacteria and only S. aureus among Gram-positive bacteria. Ethyl acetate and methanol extracts of flowers showed promising results, on the whole, examined Gram-negative and Gram-positive bacteria except S. pneumonia. Aqueous extract displayed significant inhibitory effects on Gram-negative bacteria; E. coli and P. aeruginosa, and against S. aureus and S. pneumoniae among Gram-negative bacteria.

**2- Citrus paradise extracts:**

Ethanol extract of internal peel was found effective against H. influenzae Gram-negative bacteria whereas methanol extract showed activity against S. pyogenes Gram-positive bacteria.
3- **Combretum hartmanianum** extracts:

All bacterial isolates were found to be resistant against ethanol extract of stem bark except *S. pyogenes*. Ethanol and aqueous extracts were found moderately active against *H. influenzae* and *S. aureus*.

4- **Croton zambesicus** extracts:

Ethanol extract of fruits was found effective on *S. aureus*, *H. influenzae*, *K. pneumonia*, *P. aeruginosa*, and *E. coli*. Methanol extract appeared efficacy on *S. aureus*, *S. pyogenes*, and *E. coli*. Aquatic extractor inhibits the growth of *E. coli* and *P. aeruginosa*.

5- **Eucalyptus camaldulensis** extracts:

Ethanol and methanol extracts of leaves were found effective against *S. aureus* within Gram-positive bacteria and *E. coli*, *P. aeruginosa* and *K. pneumonia* of Gram-negative bacteria, in addition to *H. influenzae* (Gram-negative bacteria) was also sensitive to methanol extract. Aqueous extract inhibits the growth of *S. aureus*, *P. aeruginosa*, and *E. coli*.

6- **Mangifera indica** extracts:

Ethanol extract of stem bark did not exhibit any efficiency on Gram-negative bacteria, also on *S. aureus* of Gram-positive bacteria. It was found active against *Streptococcus spp.* Methanol and aqueous extracts showed no antibacterial effect on the all examined species of Gram-positive and Gram-negative bacteria (except methanol extractor against *K. pneumoniae*).

7- **Psidium guajava** extracts:

Ethanol and methanol extracts of leaves exhibited a broad spectrum of antibacterial activity contra the whole examined bacterial isolates exclude *E. coli* and *S. pyogenes* as well as *E. coli* respectively. Aqueous extract showed antibacterial properties against three bacterial species (*E. coli*, *P. aeruginosa*, and *H. influenzae*).

8- **Syzygium cumini** extracts:

Ethanol extract of stem bark was found effective on *P. aeruginosa* and *K. pneumoniae* within Gram-negative bacteria. Methanol extractor appeared rise antibacterial effectiveness on the whole isolates of Gram-negative bacteria (except *H. influenzae* and *S. pneumoniae* Gram-positive bacteria).

9- **Terminalia brownii** extracts:

Ethanol extract of stem bast was existed ineffective on the Gram-negative and Gram-positive bacteria except for *S. pyogenes*. The methanol extract was found ineffective against *E. coli* and *P. aeruginosa* of Gram-negative bacteria and *S. pneumoniae* within Gram-positive bacteria. Aqueous extracts were effective against most of the tested bacterial species but no activity was observed against *P. aeruginosa*, *S. pyogenes*, and *S. pneumoniae*.

10- **Ziziphus spina-Christi** extracts:

*Klebsiella pneumoniae* and *E. coli* of Gram-negative bacteria were sensitive to methanol and aqueous extracts respectively.
Table (1): The inhibition zones (mm) of the inspected plant extractors on the selected bacterial isolates.

| Plant (Part)                  | Extract      | Tested bacteria | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|-------------------------------|--------------|-----------------|----|----|----|----|----|----|----|
| *Acacia nilotica ssp. nilotica* (Flowers) | Ethanol      |                | 38 | 37 | 25 | 24 | 37 | -  | -  |
|                              | Ethyl acetate|                | 37 | 38 | 24 | 19 | 31 | 22 | -  |
|                              | Methanol     |                | 36 | 43 | 25 | 30 | 40 | 17 | -  |
|                              | Aqueous      |                | 16 | 17 | -  | -  | 18 | 11 | -  |
| *Citrus paradisi* (internal peel of fruits) | Ethanol      |                | -  | -  | 27 | -  | -  | -  | -  |
|                              | Methanol     |                | -  | -  | -  | -  | 25 | -  | -  |
| *Combretum hartmanianum* (stem bark) | Ethanol      |                | -  | -  | -  | -  | 14 | -  | -  |
|                              | Methanol     |                | -  | -  | 15 | 16 | -  | -  | -  |
|                              | Aqueous      |                | -  | -  | 17 | 14 | -  | -  | -  |
| *Croton zambesicus* (Fruits) | Ethanol      |                | 16 | 22 | 12 | 11 | 30 | -  | -  |
|                              | Methanol     |                | 11 | -  | -  | -  | 13 | 11 | -  |
|                              | Aqueous      |                | 18 | 18 | -  | -  | -  | -  | -  |
| *Eucalyptus camaldulensis* (Leaves) | Ethanol      |                | 23 | 25 | 16 | -  | 36 | -  | -  |
|                              | Methanol     |                | 29 | 34 | 20 | 15 | 39 | -  | -  |
|                              | Aqueous      |                | 16 | 17 | -  | -  | 18 | -  | -  |
| *Mogifera indica* (stem bark) | Ethanol      |                | -  | -  | -  | -  | 15 | 15 | -  |
|                              | Methanol     |                | -  | -  | 15 | -  | -  | -  | -  |
| *Psidium guajava* (Leaves)   | Ethanol      |                | -  | 26 | 15 | 15 | 25 | 17 | 14 |
|                              | Methanol     |                | -  | 23 | 16 | 19 | 24 | -  | 18 |
|                              | Aqueous      |                | 23 | 18 | -  | 21 | -  | -  | -  |
| *Syzgium cumini* (stem bark) | Ethanol      |                | -  | 15 | 14 | -  | -  | -  | -  |
|                              | Methanol     |                | 25 | 24 | 15 | -  | 19 | 18 | -  |
| *Terminalia brownie* (stem bark) | Ethanol      |                | -  | -  | -  | -  | 14 | -  | -  |
|                              | Methanol     |                | -  | -  | 15 | 14 | 23 | 21 | -  |
|                              | Aqueous      |                | 22 | 20 | 19 | 22 | -  | -  | -  |
| *Ziziphus spina-Christi* (Fruits) | Methanol      |                | -  | -  | 18 | -  | -  | -  | -  |
|                              | Aqueous      |                | 16 | -  | -  | -  | -  | -  | -  |

1- *Escherichia coli*, 2- *Pseduomonas aeruginosa*, 3- *Klebsiella pneumonia*, 4- *Haemophilus influenzae*, 5- *Staphylococcus aureus*, 6- *Streptococcus pyogenes*, and 7- *Streptococcus pneumonia*. The values illustrate the suppression area diameters (mm) which are the average of three repeats; (-): means no suppression. DMSO did not appear any repressed effect.

Table 2: the Antibacterial potential of antibiotics against the tested bacteria

| Antibiotics | Gentamycin (µg/ml) | Tetracycline (µg/ml) | Ampicillin (µg/ml) | Penicillin (µg/ml) | Cephalosporin (µg/ml) |
|-------------|--------------------|----------------------|---------------------|--------------------|-----------------------|
| Bacterial Isolates | 4 | 2 | 1 | 5 | 4 | 2 | 1 | 5 | 4 | 2 | 1 | 5 | 4 | 2 | 1 | 5 |
| *E. coli* | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | - | - | - | - | 1 | 1 | 1 | - |
| *P. aeruginosa* | 0 | 8 | 5 | 2 | 9 | 6 | 4 | 1 | 5 | - | - | - | - | 1 | - | - | 8 | 5 | 3 | - |
| *K. pneumonia* | 2 | 1 | 1 | - | 2 | 1 | 1 | 1 | 2 | 1 | 1 | - | - | - | - | - | - | 2 | 1 | 1 | - |
| *H. influenzae* | 0 | 8 | 2 | - | 0 | 8 | 6 | 3 | 2 | 0 | 8 | 5 | 5 | - | - | - | 8 | 4 | 7 | 5 |
| *S. aureus* | 1 | 1 | - | 1 | 1 | - | - | - | - | - | - | - | - | - | - | 1 | - | 1 | - | 7 | 2 | 5 |
| *S. pyogenes* | 2 | - | - | 1 | 1 | 1 | - | 1 | 1 | - | - | - | - | 2 | 1 | 1 | - | 2 | 9 | 5 | - |
| *S. pneumonia* | 9 | 7 | 6 | 2 | - | - | - | - | - | - | - | - | - | 1 | 1 | 1 | - | 2 | - | 9 | 7 | 4 |

(-): no suppression
An antibacterial activity related to known antibiotics (Gentamycin, Tetracycline, Ampicillin, Penicillin, and Cephalosporin) was studied. The results are shown in Table 2. Gentamycin, Ampicillin, and Tetracycline at concentration 40 μg/ml were active against *K. pneumoniae*. All tested bacteria were resistant to penicillin at the different concentrations except against *E. coli*, *K. pneumoniae*, *S. aureus* and *S. pneumoniae* where it exhibited moderate activity at the concentration 40 μg/ml. Cephalosporin appeared the effectiveness contra the whole examined bacterial species in the concentration of 20 μg/ml and 40 μg/ml, except *E. coli*, *P. aeruginosa*, *H. influenzae* and *S. pneumoniae* where it showed moderate activity at concentration 20 μg/ml.

**Discussion:**

Five different fractions (ethanol, petrol ether, ethyl acetate, methanol, and aqueous fractions) from ten medical plants were examined to determine the antibacterial effectiveness on seven economically significant respiratory tract bacterial pathogens. It was found that ethanol and methanol fractions possess an efficient antibacterial effectiveness contra most of the examined bacteria whereas petrol ether and ethyl acetate fractions (except ethyl acetate fraction of *Acacia nilotica* ssp. *nilotica*) were not active (data not being shown). The potentially more interesting species were *Acacia nilotica* ssp. *nilotica*, *Psidium guajava* and *Eucalyptus camaldulensis* for their broad spectrum of activity and *Acacia nilotica* ssp. *nilotica* that produced a large area of suppression contra Gram-negative bacteria as well as *S. aureus* among Gram-positive bacteria. The antibacterial screening showed that out of the 44 extracts examined, 31 (70.5 %) exhibited inhibitory effects against at least one microorganism, 18 (40.9 %) of plant extracts showed activity against *E. coli* and *P. aeruginosa*, 16 (36.4 %) against *K. pneumoniae*, 15 (34.1 %) against *H. influenzae*, 19 (43.2 %) against *S. aureus*, 11 (250 %) against *S. pyogenes* and 4 (9.1 %) against *S. pneumoniae*. Several bacterial species, particularly *S. pyogenes* and *S. pneumoniae* having the ability to resist the ordinarily consumed antibiotics, this project may do deserving to search modern antibacterial drugs efficient on the resistant bacteria[10&11].

Khafagi, 1999 [12] reported that the ethanolic extract of the pods of *Acacia nilotica* ssp. *nilotica* showed effectiveness contra Gram-positive bacteria and *Pseudomonas aeruginosa* from Gram-negative bacteria, these findings coincided to the findings obtained in this study. The methanolic and aqueous extracts from *Psidium guajava* leaves showed antibacterial activity[13&14], the findings of this study come in consent with the reported results. Contrasted to the source antibiotics, the antibacterial effectiveness spectrum for some inspected plants was present to be obviously better in a state of *Acacia nilotica* ssp. *nilotica*. This study may contribute to improving ethno-medical knowledge and would help to discover substances with potential therapeutic use as antibacterial agents.

**Conclusion:**

In vitro antibacterial activities were carried out with the ethanol, petrol ether, ethyl acetate, methanol and water extractors of the chosen medical plants against upper respiratory tract bacterial pathogens. The petrol ether extract of all investigated plant species did not exhibit effectiveness contra the bacterial species beneath realizations. The most antibacterial active plants were *Acacia nilotica* ssp. *nilotica*, *Psidium guajava* and *Eucalyptus camaldulensis* whereas the least active plants were *Mangifera indica* and *Ziziphus spinachristi*. Of all extracts, the methanolic extract of *Acacia nilotica* ssp. *nilotica* was the most active. The revelation of the major spectrum of the antibacterial effectiveness by *Acacia nilotica* ssp. *nilotica*, *Psidium guajava*, and *Eucalyptus camaldulensis* might assist to find a new chemical type of antibiotic materials which could avail as elective factors for contagious sickness chemotherapy and monitoring. The effectiveness of these plants on most of the pathogenic microorganisms, and toxicological realizations and moreover refining. Anywise, require to be achieved.
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