

Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

## Asian Pacific Journal of Tropical Disease

journal homepage: [www.elsevier.com/locate/apjtd](http://www.elsevier.com/locate/apjtd)

Document heading

doi: 10.1016/S2222-1808(14)60758-1

© 2014 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

# Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria

Atikya Farjana, Nagma Zerín, Md. Shahidul Kabir\*

Department of Microbiology, Stamford University, Dhaka, Bangladesh

## ARTICLE INFO

## Article history:

Received 14 Jul 2014

Received in revised form 31 Jul 2014

Accepted 15 Aug 2014

Available online 18 Aug 2014

## Keywords:

Antimicrobial activity

Leaf extract

Pathogenic bacteria

## ABSTRACT

**Objective:** To determine antibacterial activity of water, oil and methanol extracts of guava (*Psidium guajava*), green tea (*Camellia sinensis*), neem (*Azadirachta indica*) and marigold (*Calendula officinalis*) against different species of bacteria, *Pseudomonas* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Klebsiella* spp., *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* (*S. aureus*).

**Methods:** Antibacterial activity of plant extracts was measured by agar well diffusion method.

**Results:** Boiled water extracts of guava leaf showed the largest zone of inhibition (22 mm) against *V. parahaemolyticus*. Water extracts of green tea leaf at boiling and room temperature showed 17.5 mm and 19 mm zone of inhibitions against *V. parahaemolyticus* and *S. aureus*, respectively. Boiled water extract of neem leaf showed moderate zone of inhibition against *Escherichia coli* (10 mm) and *Klebsiella* spp. (11 mm). Water and oil extracts of marigold leaf at both boiling and room temperature did not show any zone of inhibition against any of the tested microorganisms. Methanol extracts of both guava and green tea leaves showed same zone of inhibition against *Pseudomonas* spp. (18 mm). Methanol extract of neem leaf showed antibacterial activity against *Klebsiella* spp. (16 mm) and *Vibrio cholerae* (14 mm) and that of marigold leaf showed antimicrobial activity against *S. aureus* (18 mm) and *Klebsiella* spp. (12 mm).

**Conclusions:** The results from the study suggest that the leaves of guava, green tea, neem and marigold show antibacterial activity against different bacterial species. They could be used as alternatives to common antimicrobial agents for treatment of bacterial infections.

## 1. Introduction

Plant leaves have been used as herbal medicine for their healing properties since ancient times. Some bioactive compounds within these plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloids, tannin, flavonoid and phenolic compounds[1]. Their concentrations may vary in different plants which result in unique medicinal properties for a specific plant. Leaves and bark of the guava plant are well recognized for the treatment of diarrhoea, gastrointestinal

disorders, toothaches, colds, and swelling[2]. Tea consumption (especially green tea) is considered to provide protection against lung, esophagus, pancreas, liver, breast, colon, and skin cancers induced by chemical carcinogens[3]. Neem leaves are capable of preventing hepatitis and controlling diabetes[4], and marigold leaf is known to be highly effective in healing of burns and bruises.

During the last few decades, the global interest in the study of various medicinal plants has increased rapidly due to their antibacterial and antioxidant activities, low toxicity and the potential to be a cheaper alternative to costly synthetic drugs[5]. The determination of antibacterial activities of different medicinal plants is of special interest these days due to the current global issue of increasing antibiotic resistance of microorganisms. It is assumed that the drug resistance in pathogenic

\*Corresponding author: Dr. Md. Shahidul Kabir, Associate Professor, Department of Microbiology, Stamford University, 51 Siddheswari Road, Post Code 1217, Dhaka, Bangladesh.

Tel: +8801949622783

Fax: +88-02-9143531

E-mail: mskabir@yahoo.com

Foundation Project: Supported by the Department of Microbiology of the Stamford University Bangladesh.

microorganisms is developing due to indiscriminate use of commercial antimicrobial drugs. Antimicrobial resistance threatens the prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. Therefore, it is highly imperative to determine compounds which can be used to develop novel medicines with higher antimicrobial properties.

This study was conducted to determine the antimicrobial properties in some common medicinal plants *e.g.* guava (*Psidium guajava*), green tea (*Camellia sinensis*), neem (*Azadirachta indica*) and marigold (*Calendula officinalis*) leaves available in Bangladesh. The primary objective of the study was to aid to the progressive research works related to the antimicrobial activity of plants.

## 2. Materials and methods

### 2.1. Sample collection

Green tea (*Camellia sinensis*) dust (Kazi & Kazi Tea Estate Ltd., Bangladesh) was collected from a super market in Dhaka City. Guava and marigold leaves were collected from Siddheswari campus area of Stamford University Bangladesh in Dhaka. Neem (*Azadirachta indica*) leaves were collected from a nursery near Kamalapur Railway Station, Dhaka, Bangladesh.

### 2.2. Preparation of plant extract

Each plant leaf sample was air dried at room temperature (25 °C). Commercial green dust tea and other plant leaves were grounded into fine powder using mortar and pestle.

#### 2.2.1. Distilled water and methanol extraction

All the extractions were done by following the methods mentioned elsewhere with slight modification[6]. For water extraction, 20 g sample was mixed with 80 mL distilled water into two sterile bottles. One was placed in a shaker water bath at 130 r/min at 37 °C overnight. Another bottle of sample was allowed to boil at 100 °C for 5 min. The liquid samples were then filtered with Whatman No. 2 filter paper. Methanol extraction was done similarly where sample was mixed with methanol at a ratio of 2:10 and was placed in a shaker water bath following similar conditions as mentioned above. The extracted samples were stored in universal bottles and refrigerated at 4 °C prior to use.

#### 2.2.2. Oil extraction

Oil extraction was done by adding sample in the mustard

oil at a ratio of 2:10 into two sterile containers. One was allowed to boil at 100 °C for 30 min and another one was placed in a shaker water bath at 130 r/min at 37 °C overnight. Both the liquid samples were then filtered using Whatman No. 2 filter paper. Extracts were refrigerated at 4 °C for subsequent analysis.

### 2.3. Microorganisms

The test organisms include five Gram negative bacteria: *Pseudomonas* spp., *Vibrio cholerae* (*V. cholerae*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Klebsiella* spp., *Escherichia coli* (*E. coli*), *Salmonella* spp. and one Gram positive bacteria *Staphylococcus aureus* (*S. aureus*). They were previously isolated, identified and stored in the Department of Microbiology of Stamford University Bangladesh.

### 2.4. Agar well diffusion assay

The antimicrobial activity of the leaf extracts was evaluated by agar well diffusion method. Bacteria were grown in Muller Hinton broth (HiMedia Laboratories Ltd., India) to match the turbidity of 0.5 McFarland standards to be inoculated on Muller–Hinton agar (HiMedia Laboratories Ltd., India)[7]. After inoculation, plates were dried for 15 min, and the wells were punched using sterile cork borers. Once wells were formed, they were filled with 100 µL of plant extracts and blanks (water, methanol and soybean oil). Commercially available gentamycin (10 µg) discs were used as a positive control in this study. Plates were incubated for 24 h at 37 °C to allow leaf extracts to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different leaf extracts against different bacteria were measured in millimetre for further analysis. An agar well (6 mm) showing no zone of inhibition was considered as no antimicrobial activity. All experiments were done in triplicate and the average values were used for drawing bar diagrams.

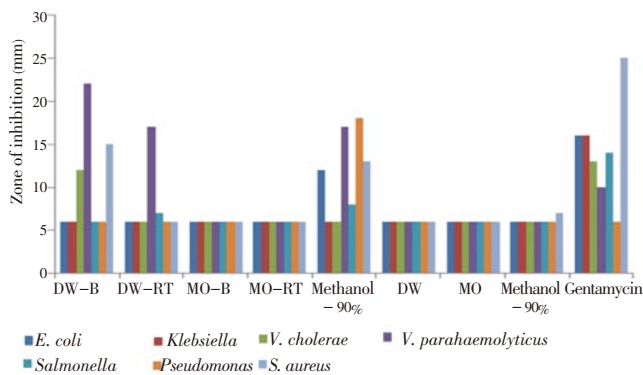
## 3. Results

Guava, green tea, neem and marigold leaf extracts showed some antibacterial activity against the selected pathogens, but they varied in different extraction process.

### 3.1. Antimicrobial activity of leaf extracts in water

Leaf extracts were prepared at two different temperatures (boiled and room temperature) in water for determination of

antibacterial activity. No zone of inhibition was produced by water extracts of guava and green tea leaves against *E. coli*, *Klebsiella* spp., *Salmonella* spp. and *Pseudomonas* spp. at both temperatures. The strongest antimicrobial activity was found to be 22 mm (Figure 1) in guava leaf extract (boiled water) against *V. parahaemolyticus*. Guava leaf extract (boiled) also showed antibacterial activity against *S. aureus* (15 mm). Boiled water extracts of green tea showed 17.5 mm and 17 mm zone of inhibitions against *V. parahaemolyticus* and *S. aureus*, respectively (Figure 2). However, for room temperature water extracts of green tea, the zones of inhibitions were slightly different for *V. parahaemolyticus* (17 mm) and *S. aureus* (19 mm) (Figure 2). Boiled water extract of neem leaf showed moderate zone of inhibition against *E. coli* (10 mm) and *Klebsiella* spp. (11 mm) (Figure 3). No antibacterial activity was shown by water extracts of marigold leaf against the tested isolates (Figure 4).

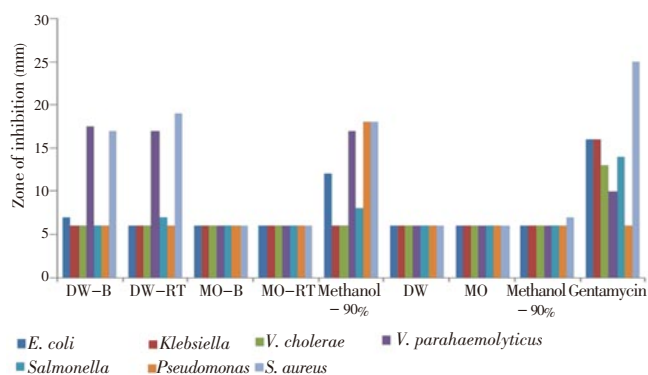


**Figure 1.** Antimicrobial activity of guava leaf extracts.

DW: Distilled water; MO: Mustard oil; DW-B: Boil extraction in DW; DW-RT: DW extraction at room temperature; MO-B: Boil extraction in MO; MO-RT: MO extraction at room temperature.

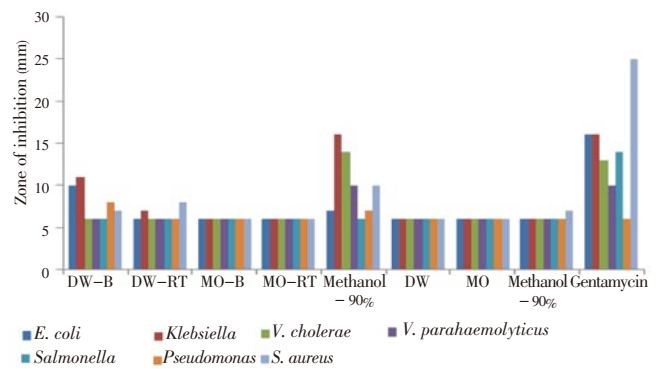
### 3.2. Antimicrobial activity of leaf extracts in oil

No antimicrobial activity was found in mustard oil extracts for any leaf sample at two different temperatures, boiling and room temperatures (Figures 1–4).



**Figure 2.** Antimicrobial activity of green tea leaf extracts.

DW: Distilled water; MO: Mustard oil; DW-B: Boil extraction in DW; DW-RT: DW extraction at room temperature; MO-B: Boil extraction in MO; MO-RT: MO extraction at room temperature.

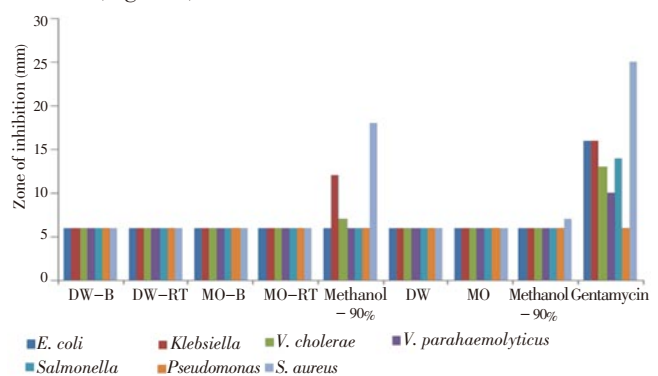


**Figure 3.** Antimicrobial activity of neem leaf extracts.

DW: Distilled water; MO: Mustard oil; DW-B: Boil extraction in DW; DW-RT: DW extraction at room temperature; MO-B: Boil extraction in MO; MO-RT: MO extraction at room temperature.

### 3.3. Antimicrobial activity of leaf extracts in methanol (90%, w/v)

The highest zone of inhibition of methanol extracts of guava (18 mm) was demonstrated against *Pseudomonas* spp. (Figure 1). *Pseudomonas* spp. and *S. aureus* showed the same zone of inhibition (18 mm) against green tea extracts in methanol (Figure 2). Methanol extract of neem leaf showed inhibition zones of 16 mm and 14 mm against *Klebsiella* spp. and *V. cholerae*, respectively (Figure 3). Both *S. aureus* and *V. parahaemolyticus* demonstrated the same zone of inhibitions of 10 mm for methanol extract of neem (Figure 3). Interestingly, the methanol extract of marigold leaf showed antimicrobial activity against *S. aureus* (18 mm) and *Klebsiella* spp. (12 mm), which was not found in water and oil extracts (Figure 4).



**Figure 4.** Antimicrobial activity of marigold leaf extracts.

DW: Distilled water; MO: Mustard oil; DW-B: Boil extraction in DW; DW-RT: DW extraction at room temperature; MO-B: Boil extraction in MO; MO-RT: MO extraction at room temperature.

## 4. Discussion

Natural antimicrobial agents have been more popular due to their efficacy against antibiotic resistant microorganisms and campaign for consumption of natural products.

According to previous reports, various solvent extracts of guava, green tea, neem and marigold leaves demonstrated antimicrobial activity against different microorganisms. Ethanol extracts of guava leaf showed antibacterial activity against *S. aureus* and *Staphylococcus epidermidis* with zone of inhibitions being 21.6 mm and 24.9 mm, respectively for 25 µL of extract solution[2]. Comparatively, in this study *S. aureus* exhibited lower zone of inhibition (15 mm) for boiled water extract of guava for 25 µL of extract solution.

Previously ethanol extracts of green tea exhibited inhibition against various bacteria: *S. aureus* (8–12 mm), *Pseudomonas aeruginosa* (7–10 mm) and *Streptococcus* spp. (9–13 mm) with volume of extract solutions ranging from 10–30 µL[8]. In this study, *S. aureus* showed the highest zone of inhibition (19 mm) for water extract (room temperature) of green tea. Methanol extract of green tea showed slightly lower inhibition (18 mm) against *Pseudomonas* spp. and *S. aureus*. In both cases, the extract volumes were 100 µL.

In earlier studies, ethanol extracts of neem leaf showed antimicrobial activity against *Streptococcus salivarius* (8.33–15.66 mm) for different neem concentrations (100–500 µg) and *S. aureus* (8.33–14.33 mm) for neem concentrations ranging from 50%–100%[4,9]. In comparison to these results, in this study the *S. aureus* showed lower zone of inhibition (10 mm) for methanol extract of neem with extract concentration of 0.2 g/mL.

Methanol extracts of marigold petals exhibited antimicrobial activity against *S. aureus* (18 mm) and *Klebsiella aerogenes* NCTC 9528 (19 mm) in an earlier research work[10]. According to the results of this study, *S. aureus* showed similar zone of inhibition (18 mm) and *Klebsiella* spp. showed lower zone of inhibition (12 mm) in methanol extract of marigold leaf.

As no significant activity was observed for mustard oil extraction of any test plant against pathogenic microorganisms, it is quite evident that mustard oil is not an effective solvent to test for antimicrobial activity. Other edible oils may be used in the future study to determine the antibacterial activity against common pathogenic bacteria.

In conclusion, significant inhibitory activity of water and methanol extracts of guava, tea, neem and marigold was noted against different pathogenic microorganisms. These four plant extracts could be studied further as future alternatives to control contamination in foods and diseases associated with common pathogenic bacteria. The toxicity study of the plant extracts need to be performed in order to determine the risk and benefits of potential applications in humans. Also, the antioxidant property of these plant extracts could be evaluated. Phytochemical analysis could be carried out to isolate the bioactive compounds of these plant species, which act as antioxidant and antimicrobial agents. These separated compounds then could be used

to produce new drugs, which could prove to be effective against antimicrobial resistance as well as against cancer.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

This research was supported by the Department of Microbiology of the Stamford University Bangladesh.

### References

- [1] Shihabudeen MH, Priscilla DH, Thirumurugan K. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. *Int J Pharma Sci Res* 2010; **1**: 430–434.
- [2] Richard FT, Joshua AT, Phillips AJ. Effect of aqueous extract of leaf and bark of guava (*Psidium guajava*) on fungi *Microsporum gypsum* and *Trichophyton mentagrophytes*, and bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Adv Med Plant Res* 2013; **1**(2): 45–48.
- [3] Mukhtar H, Ahmed N. Tea polyphenols: prevention of cancer and optimizing health. *Am J Clin Nutr* 2000; **71**: 1698S–1702S.
- [4] Sarmiento WC, Maramba CC, Gonzales MLM. An *in-vitro* study on the antibacterial effect of neem (*Azadirachta indica*) leaf extract on methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. *PIDSP J* 2011; **12**(1): 40–45.
- [5] Chew AL, Jessica JJ, Sasidharan S. Antioxidant and antibacterial activity of different parts of *Leucas aspera*. *Asian Pac J Trop Biomed* 2012; **2**: 176–180.
- [6] Archana S, Abraham J. Comparative analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens. *J Appl Pharm Sci* 2011; **1**(8): 149–152.
- [7] Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement, M100S21. New York: Clinical and Laboratory Standard Institute; 2011. [Online] Available from: <http://www.techstreet.com/products/1760826> [Accessed on 10th July, 2014]
- [8] Kumar A, Kumar A, Thakur P, Patil S, Payal C, Kumar A, et al. Antibacterial activity of green tea (*Camellia sinensis*) extracts against various bacteria isolated from environmental sources. *Recent Res Sci Technol* 2012; **4**(1): 19–23.
- [9] Lekshmi NCJ, Sowmia N, Viveka S, Brindha JR, Jeeva S. The inhibiting effect of *Azadirachta indica* against dental pathogens. *Asian J Plant Sci Res* 2012; **2**(1): 6–10.
- [10] Efstratiou E, Hussain AI, Nigam PS, Moore JE, Ayub MA, Rao JR. Antimicrobial activity of *Calendula officinalis* petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens. *Complementary Ther Clin Pract* 2012; **18**: 173–176.