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Antimicrobial effects of different extracts of medicinally used green leafy vegetables collected from local market of Dhaka, Bangladesh

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Abstract

The present study was carried out to determine the antimicrobial efficacy of green leafy vegetable samples which are traditionally used as medicinal herbs. Therefore, three samples each of Neem (*Azadirachta indica,* leaves), Ivy Gourd (*Coccinia grandis*, leaves), Water Spinach (*Ipomoea aquatica*, leaves) and Skunkvine (*Paederia foetida*, leaves) were collected and subjected to microbiological analysis, and agar well diffusion and microdilution assays to check antimicrobial activity. The samples contained total viable bacteria and fungi up to 10^7 and 10^5 CFU/g, respectively. *Staphylococcus* spp., *Klebsiella* spp. and *Pseudomonas* spp. were recovered in all the samples. All the samples showed potential antibacterial activity against most of the tested bacteria, especially their ethanolic and methanolic extracts. Although, crude and hot water extracts almost had no effect on the bacterial growth. The MIC value of the samples was found in a range of 3 mg/mL to 12 mg/mL and the average MIC value was recorded to be 6 mg/mL. Overall, the findings of the present study justified the therapeutic potential of the tested green leafy vegetable samples.

1. Introduction

Since ancient times, green leafy vegetables have been used as medicine and have a great impact on our diet and nutrition. They are known to be the sources of carbohydrates, fats, important proteins, vitamins, minerals, essential amino acids, and fibers (Bhat and Al-Daihan, 2014). Many widely consumed vegetables, apart from medicinal herbs, may comprise of essential phytochemicals with pharmacological properties (Amagase and Farnsworth, 2011). In addition, certain indigenous vegetables, especially dark colored leafy green vegetables have been reported to contain bioactive compounds such as alkaloids, tannins, flavonoids, and phenolic compounds with antioxidant and antimicrobial activities (Bhojane *et al*., 2014). Vegetables merely have any adverse effects as medicinal plants (Matasyoh *et al*., 2009), and capable to synthesize a variety of secondary metabolites of relatively complex structures with antimicrobial effects (Dhiman *et al*., 2012). Green leafy vegetables also contain a large number of compounds those have anti-diabetic (Kesari *et al*., 2005), anti-histaminic (Yamamura *et al*., 1998), anti-carcinogenic (Rajeshkumar *et al*., 2002) and hypo-lipidemic (Khanna *et al*., 2002) properties.

Due to the increasing rate of antibiotic resistance, there is an urgent need of novel antimicrobial agents against emerging and re-emerging infectious diseases (Chakraborty *et al*., 2020). Therefore, researchers are focusing more on traditional or folk medicine in recent times (Sukanya *et al*., 2009). Plants produce a number of bioactive molecules, rendering them a rich source of various medicines. Several studies reported on the use of many plant-derived products with antimicrobial properties against different pathogenic bacteria and fungi (Sukanya *et al*., 2009; Ahmed *et al*., 2014; Jahan *et al*., 2018; Chakraborty *et al*., 2020). Green leafy vegetables have been a valuable source of natural products for maintaining human health as well as can possibly be applied for prevention and therapeutic purposes (Rattanasena, 2012). Many of them, though, remain unexplored.

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The present study was therefore designed to investigate the antibacterial properties of green leafy vegetables using their crude and solvent extracts against some bacterial strains by agar well diffusion method as well as by MIC assay using microdilution technique. Microbiological quality of the vegetables was also checked.

2. Materials and methods

2.1 Study area, sampling and sample processing

The present study was carried out with three samples each of four types of green leafy vegetables including Neem (*Azadirachta indica,* leaves), Ivy Gourd (*Coccinia grandis*, leaves), Water Spinach (*Ipomoea aquatica*, leaves) and Skunkvine (*Paederia foetida*, leaves) which were collected from different local market of Dhaka, Bangladesh during November 2017 to February 2018. All the samples were processed in aseptic manner followed by homogenizing 10 mL of each sample with 90 mL normal saline and serially diluted up to 10^{-4} for microbiological assay (Ahmed *et al*., 2014; Sharmin *et al*., 2014; Jahan *et al*., 2018; Chakraborty *et al*., 2020).

2.2 Isolation and enumeration of microorganisms

From the dilutions 10^{-2} and 10^{-4}, 0.1 mL of each sample was introduced by means of spread plate technique onto the Nutrient agar (NA) and Sabouraud's dextrose agar (SDA) plates for the enumeration of total viable bacteria (TVB) and fungi, respectively. In order to isolate coliforms (especially, *Escherichia coli* and *Klebsiella* spp.), fecal coliform, *Staphylococcus* spp. and *Pseudomonas* spp.*,* 0.1 mL of each sample from dilutions 10^{-2} and 10^{-3} was spread out onto MacConkey (MAC) agar, Membrane Fecal Coliform (mFC) agar, Manitol Salt Agar (MSA) and *Pseudomonas* agar (PA) plates, consecutively. NA, MAC, MSA and PA Plates were incubated for 24 hrs at 37°C. While SDA plates were incubated at 25°C for 48 hrs and the incubation of mFC agar plates was carried out at 44.5°C for 24 hrs (Jahan *et al*., 2018; Munshi *et al*., 2018; Chakraborty *et al*., 2020).

2.3 Preparation of the samples for solvent extraction and conduction of antimicrobial assay

For the preparation of methanolic and ethanolic extracts, the powdered form of each sample was prepared through grinding and 15 g of powder was added in 85 mL of ethanol and methanol in Durham's bottle. Then the bottles were kept in shaking water bath at 24°C for 24 hrs at 130 rpm. Afterward, the pellets of the samples were collected following filtration of the extract solutions (Jahan *et al*., 2018; Chakraborty *et al*., 2020).

To observe the anti-bacterial properties of crude, hot water and solvent extracts of the green leafy vegetable samples against different previously isolated laboratory strains such as *Escherichia coli*, *Pseudomonas* spp., *Vibrio* spp., *Klebsiella* spp., *Staphylococcus* spp., *Listeria* spp., *Salmonella* spp. and *Bacillus* spp., agar well diffusion method was employed (Jahan *et al*., 2018; Chakraborty *et al*., 2020). At first, the lawn from each bacterial suspension (10^5 CFU/mL or 0.5 OD measured by spectrophotometer) onto Mueller-Hinton agar (MHA) were prepared and 100 µl (at a concentration of ~11.1mg/mL) each of the crude, hot water, ethanolic and methanolic extracts were introduced into the wells formed on MHA (Jahan *et al*., 2018; Chakraborty *et al*., 2020). Buffer peptone water, absolute ethanol and methanol were used as negative controls while the antibiotic discs of gentamicin (10 µg) were used as the positive control (Chakraborty *et al*., 2020). Plates were incubated at 37°C for 12-18 hrs and examined for formation of the zone of inhibitions (mm).

2.4 Assessment of the minimal inhibitory concentration (MIC) through broth micro-dilution method

The antibacterial activity of natural products was studied by employing a microdilution method, using Mueller-Hinton (MH) broth culture media (Sharmin *et al*., 2014). A total of 100 µL of the overnight (~12 hrs) culture of each of the test bacteria was inoculated into the appropriately labelled sterile tubes containing MH broth (Oxoid Ltd, England) at the turbidity adjusted with 0.5 McFarland standards. Green leafy vegetable samples at a concentration of 0.4, 0.8, 1.5, 3, 6, 12 and 24 mg/mL were introduced onward in the suspension. All the tubes were incubated at 37°C for 24 hrs. The least concentration (mg/mL) of each sample which visibly could retard the multiplication of the tested bacteria was considered as the MIC value (Sharmin *et al*., 2014).

3. Results and discussion

3.1 Recovery of microorganisms from green leafy vegetable samples

A vast array of pathogenic bacteria, fungi, viruses an d parasites may ruin vegetables (Beuchat, 2002). Raw vegetables can be bruised during processing and distribution, leading to the release of plant nutrients that can act as potential growth factors for microorganisms (Ahmed *et al*., 2014). To verify the fact, the microbiological analysis was carried out in the present study and microbial contaminations were evident. All the samples harbored total viable bacteria in an average of 10^7 CFU/g, while fungi were present in a range of 10^4 - 10^5 CFU/g (Table 1). All the samples conferred the presence of specific bacterial isolates to some extent.

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Table 1. Microbiological load in the tested samples.

Green Leafy vegetable Samples (n)	TVB (CFU/g)	Fungi (CFU/g)	*Staphylococcus* spp. (CFU/g)	*Klebsiella* spp. (CFU/g)	*E. coli* (CFU/g)	*Pseudomonas* spp. (CFU/g)
Neem (3)	1.9×10^7	5.5×10^5	2.8×10^4	3.2×10^4	0	3.1×10^5
Ivy Gourd (3)	2.6×10^7	6.1×10^4	2.6×10^4	3.3×10^3	0	1.2×10^4
Water Spinach (3)	1.1×10^6	1.9×10^4	2.2×10^5	4.0×10^4	0	1.1×10^4
Skunkvine (3)	1.9×10^7	2.1×10^5	3.4×10^4	3.0×10^5	2.3×10^4	2.8×10^4

N– Number of samples, TVB- Total viable bacteria. Average count (CFU/g) from all samples have been shown here. Fecal coliform was absent in all samples.

Staphylococcus spp., *Klebsiella* spp. and *Pseudomonas* spp. were encountered in all the samples in an average of 10^4 CFU/g. *E. coli* contamination was found only in Skunkvine leaves samples. Fecal coliform was totally absent in all the tested samples (Table 1). The presence of microbial contaminants in green leafy vegetables was in cohort with the previous studies on similar samples investigated in different parts of the world (Froder *et al*., 2007; Seow *et al*., 2012; Al-Holy *et al*., 2013; Ahmed *et al*., 2014).

3.2 In vitro antibacterial activity of different extracts of green leafy vegetable samples

The present investigation revealed that all the green leafy vegetable samples exhibited antibacterial activity (Tables 2-6). In almost all cases, the crude extract and the hot water extract of the samples showed no antibacterial activity (Tables 2-5). On the other hand, ethanolic and methanolic extracts of the samples were found to be effective in eliminating pathogens, possibly for the better solubility of the active components in organic solvents (Bhojane *et al*., 2014). Exceptionally for Skunkvine leaves samples, the crude extract showed antibacterial potential, while ethanolic and methanolic extracts remained to be ineffective (Table 5). All the samples had antibacterial effects against *E. coli* though results varied for other microorganisms (Tables 2-5). The growth of *Listeria* spp. was only restricted by the solvent extracts of Skunkvine leaves (Table 5). Among all the samples, the extracts of Ivy Gourd leaves failed to inhibit the majority of tested microorganisms (Table 3). Overall, ethanolic extracts were found to be more effective in eliminating pathogens than methanolic extracts in the present study. Although Sukanya *et al*. (2009) reported methanolic extracts to be more effective than ethanolic extracts of plant leaves. Dubey *et al*. (2010), Kim *et al*. (2013) and Bhat and Al-Daihan (2014) found effective and determinative anti-microbial activity in green leafy vegetables against the selected bacterial strains. In addition, the *in vitro* antibacterial activity of the samples in the present study was further supported by observing the results of MIC assay (Table 6). The highest concentration of the samples as MIC value was found at 12 mg/mL which was calculated in four cases. While the lowest concentration as MIC value was determined to be 3 mg/mL. The average MIC value was 6 mg/mL against all tested isolates (Table 6). Sukanya *et al*. (2009) found the highest MIC value of 6 mg/mL and an average of 4 mg/mL MIC in different extracts of plant leaves. Kim *et al*. (2013) reported MIC value of less than 10 mg/mL in the samples they tested.

4. Conclusion

Collectively, all the samples exhibited significant antibacterial activity as revealed from the findings of agar well diffusion method as well as of MIC assay. More research is needed to identify specific antibacterial compounds in the green leafy vegetables and their full spectrum of effectiveness. However, the present investigation verified the effectivity of the studied

Table 2. Antimicrobial activity of Neem leaves (*Azadirachta indica*)

Test bacteria	Zone of Inhibition in diameter (mm)							
	Crude fraction	Negative control (BPW)	Hot water extract	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10 µg)
E. coli	0	0	0	0	13 mm	0	12 mm	17 mm
Pseudomonas spp.	0	0	0	0	12 mm	0	17 mm	20 mm
Vibrio spp.	0	0	0	0	12 mm	0	13 mm	18 mm
Bacillus spp.	0	0	0	0	21 mm	0	18	22 mm
Klebsiella spp.	0	0	0	0	13 mm	0	19 mm	22 mm
Staphylococcus spp.	0	0	0	0	0	0	0	20 mm
Listeria spp.	0	0	0	0	0	0	0	24 mm
Salmonella spp.	15 mm	0	0	0	19 mm	0	18 mm	29 mm

Table 3. Antimicrobial activity of Ivy Gourd leaves (*Coccinia grandis*)

Test bacteria	Zone of Inhibition in diameter (mm)							
	Crude fraction	Negative control (BPW)	Hot water extract	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10 µg)
E. coli	0	0	0	0	12 mm	0	11 mm	18 mm
Pseudomonas spp.	0	0	0	0	0	0	0	21 mm
Vibrio spp.	0	0	0	0	0	0	0	19 mm
Bacillus spp.	0	0	0	0	16 mm	0	12 mm	20 mm
Klebsiella spp.	0	0	0	0	0	0	0	20 mm
Staphylococcus spp.	0	0	0	0	15 mm	0	0	20 mm
Listeria spp.	0	0	0	0	0	0	0	16 mm
Salmonella spp.	0	0	0	0	0	0	0	19 mm

Table 4. Antimicrobial activity of Water Spinach (*Ipomoea aquatic*)

Test bacteria	Zone of Inhibition in diameter (mm)							
	Crude fraction	Negative control (BPW)	Hot water extract	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10 µg)
E. coli	0	0	0	0	20 mm	0	20 mm	20 mm
Pseudomonas spp.	0	0	0	0	16 mm	0	13 mm	24 mm
Vibrio spp.	0	0	0	0	0	0	0	20 mm
Bacillus spp.	0	0	0	0	0	0	0	21 mm
Klebsiella spp.	0	0	0	0	15 mm	0	0	19 mm
Staphylococcus spp.	0	0	0	0	15 mm	0	16 mm	22 mm
Listeria spp.	0	0	0	0	0	0	0	25 mm
Salmonella spp.	0	0	0	0	15 mm	0	12 mm	20 mm

Table 5. Antimicrobial activity of Skunkvine leaves (*Paederia foetida*)

Test bacteria	Zone of Inhibition in diameter (mm)							
	Crude fraction	Negative control (BPW)	Hot water extract	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10 µg)
E. coli	0	0	0	0	15 mm	0	0	17 mm
Pseudomonas spp.	12 mm	0	0	0	0	0	0	28.44mm
Vibrio spp.	0	0	0	0	19 mm	0	15 mm	20 mm
Bacillus spp.	0	0	0	0	16 mm	0	14 mm	22 mm
Klebsiella spp.	0	0	0	0	13 mm	0	12 mm	19 mm
Staphylococcus spp.	0	0	0	0	0	0	0	22mm
Listeria spp.	0	0	0	0	17 mm	0	18 mm	24 mm
Salmonella spp.	0	0	0	0	13 mm	0	0	28 mm

Table 6. Minimum Inhibitory Concentration (MIC) of the samples.

Green Leafy Vegetable Sample	Microorganisms							
	Klebsiella spp.	*E. coli*	*Pseudomonas* spp.	*Bacillus* spp.	*Salmonella* spp.	*Listeria* spp.	*Staphylococcus* spp.	*Vibrio* spp.
Neem (mg/mL)	6	6	3	3	3	3	6	6
Ivy Gourd (mg/mL)	6	3	3	3	6	6	12	6
Water Spinach (mg/mL)	6	6	12	12	3	6	6	6
Skunkvine (mg/mL)	3	3	6	12	6	3	3	6

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vegetables in their traditional uses against bacterial infections. The study findings also suggest that the tested green leafy vegetables could potentially be used in developing antimicrobials.

Conflict of Interest

Authors have no potential conflict of interest.

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