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Microbiological profiling and the demonstration of *in vitro* anti-bacterial traits of the major oral herbal medicines used in Dhaka Metropolis

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Abstract

Present study attempted to assess the level of microbiological contamination in oral herbal medicines, frequently used for medications, through conventional cultural and biochemical tests along with the antibiogram of the isolates. Moreover, the anti-bacterial potential of the herbal medicines was also aimed to be checked by the agar well diffusion method and minimum inhibitory concentration (MIC) assay. Out of 10 categories of liquid oral herbal medicine samples ($n = 50$) studied, all were found to be contaminated with bacteria (10^3 - 10^5 cfu/mL), specifically with *Staphylococcus* spp. in 8 samples; while 2 samples harbored *Klebsiella* spp. Fungal presence was observed only in one sample. Study of antibiogram revealed *Klebsiella* spp. to be strongly resistant against penicillin G and erythromycin, whereas *S. aureus* possessed 80% sensitivity. The *in vitro* anti-bacterial activity was observed in 7 samples. Of them, one sample was found to exhibit the activity against almost all the test bacteria and another was found effective against 5 out of 8 test bacteria. Five samples showed the activity within a minor range while 3 samples were devoid of such trait. Samples 2 and 4 were found to stall the bacterial growth below 10 mg/mL of concentration in MIC test. Overall, the prevalence of specific pathogens was not so significant in the samples studied as well as only one drug-resistant isolate was identified. Besides, the anti-bacterial trait of 5 samples indicated that most of herbal medicines might be considered effective for medication.

Keywords: Drug resistance; *In vitro* anti-bacterial activity; Oral herbal medicines; Pathogens; Public health; Spoilage microorganisms

Background

Natural products have long been used as precious sources for the formulation of useful drugs (Cragg and Newman 2013; World Health Organization 2013; Veeresham 2012; Gyasi et al. 2011; Izzo and Ernst 2009; Kraft 2009). Besides combating diseases with the use of antibiotics, herbal medicines are also widely known for their implications in mitigating a number of clinical complications (Behera et al. 2013; Noor et al. 2013a; Tabassum and Ahmad 2011; Damery et al. 2011; Ernst 2011; Mendes et al. 2010). According to WHO estimation, 21,000 plants have been found globally to be used for medicinal purposes (Umashanker and Shruti 2011). Along with the progressive growth in synthetic medicines sourced from pharmaceuticals and biotech companies, medicinal plants

have long been indeed readily available, reasonable and culturally suitable source of primary health care for the majority of Asia's population (Srivastava et al. 1996).

An important shortfall of using antibiotics in recent days has appeared through the emergence of drug-resistant bacteria which in turn, resulted in the ineffectiveness of antibiotics (Dutta et al. 2013; Molton et al. 2013; Tenover 2006; Khan et al. 2013). Furthermore, a huge number of reports revealed the adverse effects and drug interactions of antibiotics resulting in fatal toxicity (Somasundaram and Manivannan 2013; Eric et al. 2008; Aster and Bougie 2007; Granowitz and Brown 2007; Apter et al. 2006; Navarro and Senior 2006; Baddour et al. 2005; Chow et al. 2005; Faulkner et al. 2005; Micek et al. 2005). Besides, when first-line and then second-line antibiotic treatment options get limited by the surfacing of bacterial drug-resistance traits or due to the drug-unavailability, physicians employing antibiotics appear to be eventually

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more toxic to the users. Patients with drug-resistant infections are often much more likely to expire, and even the survivors often need a longer hospital stays with prolonged healing. Earlier notation of anti-bacterial traits of natural products thus made an array of herbs to be the suitable alternative medication means of antibiotics (Barbour et al. 2004). Afterwards a number of reports revealed the antagonistic feature of herbs against a wide range of microorganisms as well as proved the lesser side effects with small or no toxicity of the herbal medicines compared to that of antibiotics (Djeussi et al. 2013; Kucekova et al. 2013; Oreagba et al. 2011; Kala et al. 2006).

In addition to the advantages over antibiotics, an important aspect is to be considered that herbs are more likely to harbor a huge number of bacteria including *Escherichia coli*, *Salmonella* spp., *Shigella* spp., aerobic spores, and the fungal population, usually originating in plantation soil or may be disseminated from organic fertilizer (Duraisankar and Ravindran 2013; Steven et al. 2013; World Health Organization 2007; Nakajima et al. 2005). Besides the method of harvesting herbs or herbal products, the production process, unhygienic handling, transportation and inappropriate storage may also cause microbial contamination as well as the chemical toxicity of the herbal medicines (World Health Organization 2007; Ting et al. 2013; Abba et al. 2009; Chan 2003).

In Bangladesh, a huge pharmacological evaluation and ethnomedicinal survey of medicinal plants have been conducted; however, the microbiological survey of herbal medicines have not been accomplished well (Das et al. 2012; Rahmatullah et al. 2010). While the research on microbiological assessment of the pharmaceutical products is abundant in this country, such work on the herbal products is quite petite (Das et al. 2013; Khanom et al. 2013; Noor et al. 2013a; Moniruzzaman et al. 2012; Hossain 2009). Along these lines, present study endeavored to assess microbiological contamination level within the commonly available herbal medicines; and to further demonstrate the anti-bacterial activity of the tested medicinal samples.

Methods

Study area, sampling and sample processing

The study was carried out with ten categories of oral herbal drugs in liquid formulations (details of the samples have been provided in the Additional file 1). Five (5) samples from each of the 10 categories were randomly collected from different drug's store with appropriate dates of manufacturing and expiry within the city of Dhaka during the time frame of December 2013 to January 2014 according to the standard sampling method suggested by American Public Health Association (1998). All the samples were aseptically processed followed by homogenizing 10 ml of each samples with 90 ml normal saline and

diluted up to 10^{-6} for the isolation and quantification of pathogenic bacteria and fungi.

Microbiological analysis and confirmative biochemical tests

Estimation of total viable bacteria and fungi

For the enumeration of total viable bacteria (TVB) and the total fungal load, 0.1 mL of each sample was introduced onto the nutrient agar (NA) and Sabouraud's dextrose agar (SDA) plates, respectively, by means of spread plate technique (Cappuccino and Sherman 1996). Plates were incubated at 37°C for 24 hours and at 25°C for 48 hours for total viable bacteria and fungi, respectively.

Estimation of fecal coliform, *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp. and actinomycetes

From the dilutions 10^{-3} and 10^{-5} , 0.1 mL of each sample was spread onto the membrane fecal coliform (MFC) agar and MacConkey agar for the enumeration of total fecal coliform (TFC), and coliforms (especially, *Escherichia coli* and *Klebsiella* spp.), respectively. Plates were incubated for 24 hours at 44.5°C and 37°C for fecal coliform and coliforms, correspondingly. Likewise, *Staphylococcus* spp. and actinomycetes were isolated onto Mannitol Salt Agar (MSA) and actinomycetes agar, respectively by adding 0.1 mL of diluted sample each, and all the plates were then incubated at 37°C for 24 hours.

Isolation of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp.

Ten (10) mL of sample was transferred into 90 ml of selenite cysteine broth (SCB) and alkaline peptone water (APW) for the enrichment of *Salmonella*, *Shigella*, and *vibrio* spp., respectively and incubated at 37°C for 6 hours. After incubation, the samples were diluted up to 10^{-6} and then 0.1 mL of samples from each of the 10^{-3} and 10^{-5} dilutions were spread onto *Salmonella-Shigella* (SS) agar and thiosulfate citrate bile salt sucrose (TCBS) agar for the isolation of *Salmonella* spp. and *Shigella* spp., and *Vibrio* spp., consecutively. Plates were incubated at 37°C for 48 hours for the detection of typical colonies. Finally, all the isolates were biochemically examined following standard procedures as described earlier (Cappuccino and Sherman 1996; Alfrad 2007).

Antibiotic susceptibility test

Antibiotic susceptibility traits of the pathogenic isolates were examined (either drug resistant or sensitive) by the disc diffusion assay on Mueller-Hinton agar (MHA, Difco, Detroit, MI) against the commonly used antibiotics following the standard protocol (Munshi et al. 2012; Ferraro et al. 2001; Bauer et al. 1968). Commercially available laboratory grade antibiotic discs of Penicillin G (10 µg), Gentamicin (10 µg), Oxacillin (1 µg) Amoxicillin (30 µg), Imipenem (30 µg), Erythromycin (15 µg), Tetracycline

(30 µg), Ciprofloxacin (5 µg), Trimethoprim-sulfamethoxazole (25 µg), Azithromycin (15 µg), Nalidixic acid (30 µg) and Ampicillin (10 µg) were aseptically placed over the surface of Mueller-Hinton agar plates at spatial distance of 5 mm which had been previously inoculated (with the afterward formation of bacterial lawn over the agar surface) with the pathogenic test bacterial suspensions with prescribed turbidity (compared to that of the McFarland standard of 0.5).

Determination of anti-bacterial activity of herbal medicine

The anti-bacterial activity of the samples was performed by using agar well diffusion method (Ahmed et al. 2014; Jagessar et al. 2008; Hussain et al. 2010). At first, the suspensions (with standard turbidity compared to that of the McFarland standard of 0.5) of each of the test bacteria; i.e., *Pseudomonas* spp., *Listeria* spp., *Bacillus* spp., *Vibrio* spp., *Salmonella* spp., *Klebsiella* spp., *Staphylococcus* spp. and *E. coli* was spread evenly over the MHA using cotton swab which in turn resulted in the uniform lawns. Wells were then made spanning the MHA by means of sterile cork-borer. Each of the samples was then introduced separately in a concentration of 100 µL with a residual herbal medicine concentration of 2.4 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 1 mg/mL, 3.5 mg/mL, 2.5 mg/mL, 3.1 mg/mL, 6.4 mg/mL, 0.9 mg/mL and 1.5 mg/mL for samples 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, consecutively in the specified well along with a positive control (Gentamicin, 10 µg) and a negative control (normal saline). Presence of clear zone around the sample solution (if any) was analytical for the existence of the antibacterial activity of the samples tested.

Determination of Minimal Inhibitory Concentration (MIC)

Besides the agar well diffusion method, additionally the minimal inhibitory concentration (MIC) or broth microdilution assay was performed to determine the lowest concentration of each of the exoerimental herbal medicines capable of trimming down the extent of viability of the test bacteria (Carson et al. 1995). According to the suggested method by Clinical and Standard Laboratory Institute: two fold serial broth dilution method was used to determine the MIC (Clinical and Laboratory Standards Institute 2006). An aliquot of 100 µL of the overnight (~12 hours) culture of each of the test bacteria was inoculated into the appropriately labeled sterile tubes containing Mueller Hinton (MH) broth (Oxoid Ltd, England) at the turbidity adjusted with 0.5 McFarland standard and the different volumes (32 µL, 64 µL, 128 µL, 256 µL, 512 µL, 1024 µL and 2048 µL) of herbal medicine samples were introduced onward to make a total volume of 3 ml. Then the residual or extract concentrations of the herbal medicines for each of the above mentioned volume of all the aqueous samples used in the MIC assay was

determined (Additional file 2). All the tubes were incubated at 37°C for 24 hours. The least concentration (mg/mL) of each sample extract which could retard the multiplication of the tested bacteria, as judged visually by lack of turbidity in the tube comparable to the McFarland standard, was recorded and considered as the MIC value.

Results and discussion

The practice and prospect of herbal medicines in Bangladesh is to its brim since the country has a number of operational traditional herbal medicinal systems including homeopathy, Ayurveda, Unani, folk medicine and home remedies (Akter et al. 2012; Das et al. 2012; Rahmatullah et al. 2010). The routine monitoring of the hygiene as well as the anti-bacterial potency of these drugs are thus required to ensure a sustainable health management system. To our knowledge, such examination of herbal medicinal samples is not that frequent in our country, which in turn, may pose the probable unidentified adverse toxicity upon usage of the medicines.

Microbial prevalence within the herbal medicine samples

The microbial spoilage of herbal medicines is quite evident from our study where all the samples have been detected to harbour contaminant bacteria; nevertheless, all were indeed within the specified range of 10^3 - 10^5 cfu/mL (Table 1). However, contamination with the specific pathogenic bacterium *Staphylococcus aureus* (10^3 - 10^5 cfu/mL) in 8 samples were indicative of improper handling during harvesting, processing, manufacturing, distribution or storage of the medicinal plant samples (Duraisankar and Ravindran 2013; Steven et al. 2013; Duraisankar and Ravindran 2013, 2007; Ting et al. 2013; Abba et al. 2009; Chan 2003). Two of the samples tested were found to be contaminated with *Klebsiella* spp., which possibly might take place during the plant harvesting or even from the farming manure; and certainly due to the unhygienic handling (Duraisankar and Ravindran 2013; Steven et al. 2013; World Health Organization 2007). Interestingly the fungal species were quite infrequent and only one sample exhibited the fungal proliferation (Table 1).

Drug-resistance trait of the pathogens isolated from herbal medicine samples

The surfacing of drug-resistant bacteria within an array of products (including water, food and pharmaceuticals) resulted in the incompetence of the synthetic drugs or antibiotics (Acharjee et al. 2013a, b; Ahmed et al. 2013; Dutta et al. 2013; Molton et al. 2013; Tenover 2006). Among the 2 pathogens found in our samples, *Klebsiella* spp. exhibited up to 60% resistance, and the pathogen was specifically found to be highly resistant against penicillin G and erythromycin; while conversely *S. aureus* was found to be sensitive towards most of the antibiotics

Table 1 Microbiological conditions of the samples (cfu/mL)

Samples	TVB	Fungi	<i>Klebsiella</i> spp.	FCC	<i>Staphylococcus</i> spp.	Actinomycetes	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Vibrio</i> spp.
Sample 1 (N = 5)	6.5×10^5	0	1.0×10^6	0	4.0×10^4	0	0	0	0
Sample 2 (N = 5)	7.0×10^5	0	0	0	3.7×10^5	0	0	0	0
Sample 3 (N = 5)	2.4×10^4	0	0	0	2.0×10^3	0	0	0	0
Sample 4 (N = 5)	1.14×10^5	0	0	0	4.6×10^4	0	0	0	0
Sample 5 (N = 5)	9.0×10^4	0	0	0	3.0×10^3	0	0	0	0
Sample 6 (N = 5)	4.12×10^5	0	0	0	2.1×10^4	0	0	0	0
Sample 7 (N = 5)	1.06×10^5	1.8×10^4	4.5×10^4	0	2.0×10^3	0	0	0	0
Sample 8 (N = 5)	2.0×10^3	0	0	0	0	0	0	0	0
Sample 9 (N = 5)	3.5×10^4	0	0	0	6.0×10^2	0	0	0	0
Sample 10 (N = 5)	7.7×10^3	0	0	0	0	0	0	0	0

TVB: Total viable bacteria; FCC: fecal coliform count.
 The average load has been shown.
 Microbial limits (World Health Organization 2007).
 Total aerobic bacteria 10^5 cfu/ml.
Escherichia coli 10^1 cfu/ml.
Salmonella spp. absent.
 Enterobacteria 10^3 cfu/mL.

used in this study (Figure 1). Nevertheless, existence of drug-resistant pathogenic isolate in 2 of the samples studied may pose further threat to the overall public health remedies through the application of these medicines.

Antimicrobial resistance is one of the most serious health threats. Clinical complications and infections from drug-resistant resistant bacteria are now frequently commencing, and surprisingly some pathogens have been identified to be resistant to multiple types or classes of antibiotics; i.e., multi-drug resistant (Dutta et al. 2013; Noor et al. 2013b). The thrashing of effectual antibiotics emasculates the capacity to fight against infectious diseases and gets along the infectious complications frequent in susceptible patients. Routine examination of herbal medicines for the presence of drug-resistant bacteria is thus obligatory.

In vitro anti-bacterial activity of the herbal medicine samples

A number of reports showed the anti-bacterial features of natural herbs suggesting them appropriate substitute of synthetic medicines (Djeussi et al. 2013; Kucekova et al. 2013; Oreagba et al. 2011; Kala et al. 2006). However, the experimental demonstration of the existence of such anti-bacterial activity of the common and popular herbal medicines used by the Bangladeshi community is still lacking. Our study, for the first time, revealed such trait within our common herbal medicines whereby 70% of the tested samples were found to exhibit the in vitro anti-bacterial activity (Table 2). One of the samples (Sample no. 2) studied possessed the activity against almost all the test bacteria employed, one (Sample no. 5) was found to be effective against 5 test bacteria, one (Sample no. 6) against 3 test bacteria, 2 samples (Sample

3 and 9) against 2 test bacteria, while another 2 samples (Samples 1 and 10) exhibited the activity against only one bacterium. The anti-bacterial activity was found to be completely absent in 3 samples (Samples 4, 7 and 8).

In addition, the in vitro anti-bacterial activity of the samples was further supported by observing the result of MIC (Table 3). In this parameter all the samples were found to show their anti-bacterial activity against different tested bacteria; i.e., *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Bacillus* spp., *Salmonella* spp., *Listeria* spp., *Staphylococcus* spp., and *Vibrio* spp. The highest MIC was scored at 66 mg/mL while the lowest value was noted to be 1 mg/mL (Table 3). For sample 1, the ideal MIC was found to be 25 mg/mL against all the tested pathogenic bacteria except *Pseudomonas* spp. which was found to be inhibited at a concentration of 50 mg/mL. Sample 2 revealed the MIC at a maximal level of 7 mg/mL to stall the bacterial growth. For sample 3, the MIC was recorded to be not more than 6 mg/mL to inhibit any of the tested bacterial growth. Sample 4 showed the MIC potential at 10 mg/mL against most of the tested pathogenic bacteria while 5 mg/mL and 20 mg/mL were found to be effective against *E. coli* and *Listeria* spp., respectively. Sample 5 exhibited the MIC range between 18 mg/mL to 36 mg/mL to inhibit the bacterial growth. In case of sample 6, 13 mg/mL was effectual to hinder most of the test bacterial growth excluding *Listeria* spp. and *Vibrio* spp. In case of sample 7, the MIC was found at 16 mg/mL against *E. coli*, *Klebsiella* spp. *Bacillus* spp., 50 mg/mL against *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp., *Vibrio* spp., and 66 mg/mL against *Listeria* spp. For sample 8, the MIC was recorded at 66 mg/mL against most of the bacterial species except *E. coli* and *Staphylococcus* spp. (with an MIC of 33 mg/mL). For samples 9 and 10, the

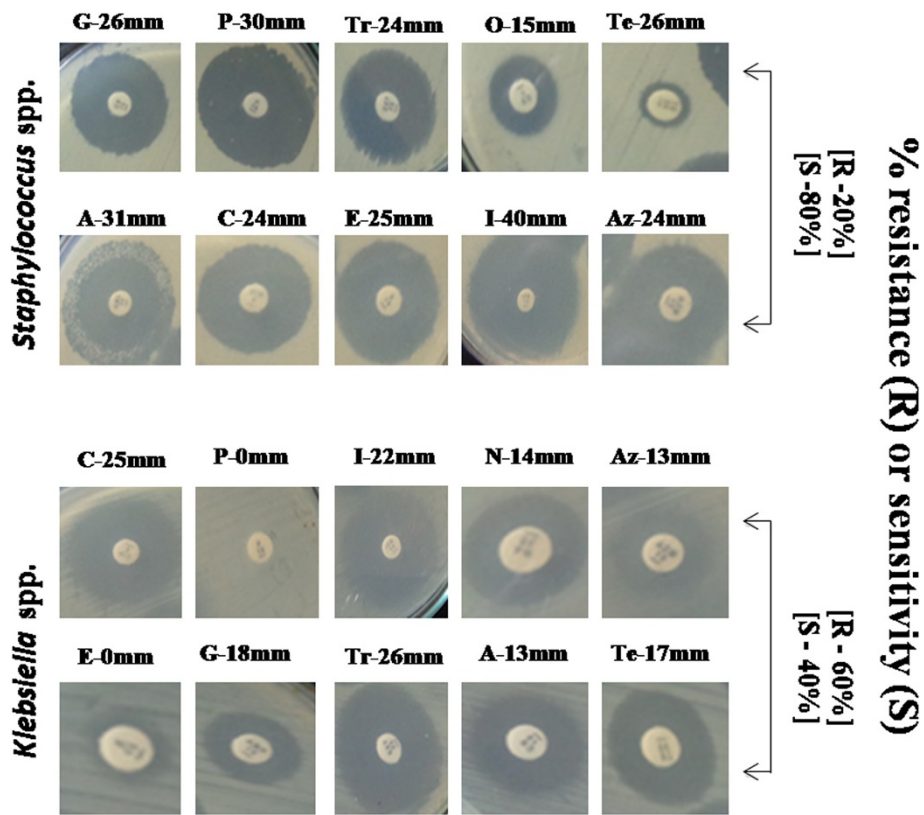


Figure 1 Determination of resistance or susceptibility of *Staphylococcus* spp. and *Klebsiella* spp. towards commonly used antibiotics. The arrows indicate the frequency of resistance/sensitivity of the isolates. *Klebsiella* spp. exhibited elevated resistance (60%) than *Staphylococcus* spp. (20%). P = Penicillin G (10 µg), G = Gentamicin (10 µg), O = Oxacillin (1 µg), A = Amoxicillin (30 µg), I = Imipeneme (30 µg), E = Erythromycin (15 µg), Te = Tetracycline (30 µg), C = Ciprofloxacin (5 µg), Tr = Trimethoprim-sulfamethoxazole (25 µg), Az = Azithromycin (15 µg), N = Nalidixic acid (30 µg), A = Ampicillin (10 µg), R: resistant, S: sensitive.

Table 2 Antimicrobial activity of the herbal medicine samples tested

Herbal medicine samples (concentration in mg/mL)	Zone of inhibition (mm) against test bacteria							<i>Listeria</i> spp.
	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>Salmonella</i> spp.	<i>Vibrio</i> spp.	<i>Pseudomonas</i> spp.	<i>Bacillus</i> spp.	<i>Staphylococcus</i> spp.	
Sample 1 (2.4)	0	0	0	0	0	0	0	11 mm
Sample 2 (0.4)	11 mm	15 mm	20 mm	20 mm	17 mm	20 mm	18 mm	20 mm
Sample 3 (0.6)	0	11 mm	0	0	0	0	0	7 mm
Sample 4 (1.0)	0	0	0	0	0	0	0	0
Sample 5 (3.5)	0	18 mm	0	10 mm	11 mm	10 mm	0	10 mm
Sample 6 (2.5)	0	16 mm	0	0	0	8 mm	0	9 mm
Sample 7 (3.1)	0	0	0	0	0	0	0	0
Sample 8 (6.4)	0	0	0	0	0	0	0	0
Sample 9 (0.9)	0	11 mm	0	13.9 mm	0	0	0	0
Sample 10 (1.5)	0	0	0	12 mm	0	0	0	0

The experiments were conducted three times independently, and the results were found to be reproducible. One representative data has been shown.

Table 3 Minimum Inhibitory Concentration (MIC) of the samples

Sample	Organisms							
	<i>E. coli</i>	<i>Klebsiella spp.</i>	<i>Salmonella spp.</i>	<i>Vibrio spp.</i>	<i>Pseudomonas spp.</i>	<i>Bacillus spp.</i>	<i>Staphylococcus spp.</i>	<i>Listeria spp</i>
Sample 1	25 mg/mL	25 mg/mL	25 mg/mL	25 mg/mL	51 mg/mL	25 mg/mL	25 mg/mL	25 mg/mL
Sample 2	1 mg/mL	3 mg/mL	3 mg/mL	3 mg/mL	7 mg/mL	7 mg/mL	3 mg/mL	7 mg/mL
Sample 3	3 mg/mL	1 mg/mL	3 mg/mL	3 mg/mL	6 mg/mL	3 mg/mL	6 mg/mL	6 mg/mL
Sample 4	5 mg/mL	10 mg/mL	10 mg/mL	10 mg/mL	10 mg/mL	10 mg/mL	10 mg/mL	20 mg/mL
Sample 5	18 mg/mL	36 mg/mL	36 mg/mL	36 mg/mL	36 mg/mL	18 mg/mL	18 mg/mL	36 mg/mL
Sample 6	13 mg/mL	13 mg/mL	13 mg/mL	26 mg/mL	13 mg/mL	13 mg/mL	13 mg/mL	26 mg/mL
Sample 7	16 mg/mL	16 mg/mL	50 mg/mL	50 mg/mL	50 mg/mL	16 mg/mL	50 mg/mL	66 mg/mL
Sample 8	33 mg/mL	66 mg/mL	66 mg/mL	66 mg/mL	66 mg/mL	66 mg/mL	33 mg/mL	66 mg/mL
Sample 9	9 mg/mL	18 mg/mL	9 mg/mL	9 mg/mL	9 mg/mL	18 mg/mL	9 mg/mL	18 mg/mL
Sample 10	15 mg/mL	15 mg/mL	15 mg/mL	15 mg/mL	30 mg/mL	7 mg/mL	30 mg/mL	30 mg/mL

Residual concentrations of the herbal medicines have been provided. The experiments were conducted three times independently, and the results were found to be reproducible. One representative data has been shown.

maximal MICs were determined to 18 mg/mL and 30 mg/mL, respectively (Table 3).

However, agar well diffusion test may not be a suitable one to determine the antibacterial activity of natural compounds. The rate of diffusion of natural antimicrobials can be strongly affected by the polarity, the concentration, the molecular size, etc. of the compounds. This fact was evident from our study findings (Tables 2 and 3). When the results of agar well diffusion method and MIC assay were compared, a clear discrepancy was apparent for some samples. Although a relatively low concentration of sample 3 (up to 6 mg/mL) was found to inhibit the growth of all the tested bacteria as revealed from the MIC assay, only *Klebsiella spp.* and *Listeria spp.* were found to be affected by the concentration of 0.6 mg/mL in agar well diffusion method with considerably small zone of inhibition. Similar divergence was noticed for the samples 4, 9 and 10. Additionally, sample 4 exhibited no anti-bacterial activity in agar well diffusion method, whereas the sample was found to possess the MIC level of 10 mg/mL for most of the test bacteria. Sample 9 featured MIC of 9 mg/mL against *Pseudomonas spp.*, *Salmonella spp.*, *Staphylococcus spp.* and *Vibrio spp.*, but neither of the bacteria except *Vibrio spp.* were found to be inhibited in the agar well diffusion method. Sample 10 exhibited an MIC of 7 mg/mL against *Bacillus spp.* which was conversely found to be unaffected in the agar well diffusion assay.

Samples 1, 2, 7 and 8 on the other hand showed nearly consistent results as noticed both from the agar well diffusion assay and the broth microdilution assay. Sample 2 had minimal MIC as sample 3 and exhibited the antibacterial activity against all the tested bacteria as was also noticed in the agar well diffusion assay. Samples 1, 7 and 8 had almost no antibacterial activity and consistently featured moderate to high MIC. Exceptionally,

Sample 5 imparting a relatively moderate MIC showed anti-bacterial activity against most of the bacteria. Considering this exception it can be concluded that agar well diffusion method could be used as screening test but however, more reliable results could be achieved by the minimal inhibitory concentration (MIC); i.e., the broth microdilution assay.

Conclusion

The revelation of the drug-resistant microbial pathogenic contaminants in the herbal medicines tested in our study is suggestive of implementing more hygienic manufacturing and processing operations. Nevertheless, the total viable bacteria were more or less with the specification limits which providentially ensured the overall product quality. The anti-bacterial traits of the samples tested revealed the potency of the medicines against pathogenic bacteria as well as the biological mechanism of action of such natural medicines. However, as revealed from the agar well diffusion tests, in most of the cases, the absence of such activity further urges for a careful formulation of the medicinal products to achieve the treatment efficiency against microbial pathogens. Besides, exploration of the motivating factors for the farmers cultivating medicinal plants as well as expanding the market of herbal medicinal plants in Bangladesh and in other developing countries would further aid in a better management of the overall public health.

Additional files

Additional file 1: Names, composition and indications of the herbal medicine samples studied.

Additional file 2: Residual or extract concentrations of herbal medicines for each volume of aqueous samples used in the minimum inhibitory concentration (MIC) assay.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MS and ITN conducted the experiments and participated in acquisition, analysis, and interpretation of data. MA participated in analysis and interpretation of data, and drafted the manuscript. SK helped in analyzing the data and in drafting the manuscript. RN drew the study conception and design, participated in revising the manuscript critically for important intellectual content and has given the final approval of the version to be submitted. All authors read and approved the final manuscript.

Authors' information

All authors are from Department of Microbiology, Stamford University Bangladesh. MS and ITN is the thesis students of MS program of the department. MA and SK are working as Lecturer. RN, corresponding author of the manuscript, is the Departmental Head.

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References

- Abba D, Inabo HI, Yakubu SE, Olonitola OS (2009) Contamination of herbal medicinal products marketed in Kaduna Metropolis with selected pathogenic bacteria. *Afr J Traditional Complemen Altern Med* 6:70–77
- Acharjee A, Fatema K, Jahan F, Siddique SJ, Uddin MA, Noor R (2013a) Prevalence of *Vibrio cholerae* in different food samples in the city of Dhaka, Bangladesh. *Int Food Res J* 20(2):1017–1022
- Acharjee M, Jahan F, Rahman F, Noor R (2013b) Bacterial proliferation in municipal water supplied in mirpur locality of Dhaka city, Bangladesh. *Clean Soil Air Water* 41:1–8
- Ahmed T, Baidya S, Sharma BC, Malek M, Das KK, Acharjee M, Munshi SK, Noor R (2013) Identification of drug-resistant bacteria among export quality shrimp samples in Bangladesh. *Asian J Microbiol Biotech Env Sci* 15(4):31–36
- Ahmed T, Urmi NJ, Munna MS, Das KK, Acharjee M, Rahman MM, Noor R (2014) Assessment of microbiological proliferation and in vitro demonstration of the antimicrobial activity of the commonly available salad vegetables within Dhaka metropolis, Bangladesh. *Am J Agri Forestr* 2(3):55–60
- Akter S, Das PR, Islam MT, Kabir MH, Haque MM, Khatun Z, Nurunnabi M, Khatun Z, Lee Y, Jahan R, Rahmatullah M (2012) A selection of medicinal plants used as blood purifiers by folk medicinal practitioners of Bangladesh. *American-Eurasian J Sustain Agri* 6(3):188–194
- Alfred EB (2007) *Bensons Microbiological Applications*. Mcgraw-Hill Book Company, New York
- American Public Health Association (1998) *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington, DC
- Apter AJ, Kinman JL, Bilker WB, Herlim M, Margolis DJ, Lautenbach E, Hennessy S, Strom BL (2006) Is there cross-reactivity between penicillins and cephalosporins? *Am J Med* 119(4):354.e11–20
- Aster RH, Bougie DW (2007) Drug-induced immune thrombocytopenia. *N Engl J Med* 357:580–587
- Baddour LM, Wilson WR, Bayer AS, Fowler VG Jr, Bolger AF, Levison ME, Ferrieri P, Gerber MA, Tani LY, Gewitz MH, Tong DC, Steckelberg JM, Baltimore RS, Shulman ST, Burns JC, Falace DA, Newburger JW, Pallasch TJ, Takahashi M, Taubert KA (2005) Infective endocarditis: diagnosis, antimicrobial therapy and management of complications. *Circulation* 111:3167–3184
- Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN (2004) Screening of selected indigenous plants of Lebanon for antimicrobial activity. *J Ethnopharmacol* 93(1):1–7
- Bauer AW, Kirby WMM, Sherris JC, Tierch M (1968) Antibiotic susceptibility testing by a standardized single disc method. *American J Clin Pathol* 45(4):493–496
- Behera B, Purvia R, Yadav D, Mc S (2013) Review on anti-diabetic plants of different nighantus (Ayurvedic Materia Medica). *Ayurpharm Int J Ayurveda Allied Sci* 2(4):92–97
- Cappuccino JG, Sherman N (1996) *Microbiology - A Laboratory Manual*. The Benjamin/Cummings Publishing Co., Inc., Menlo Park, California
- Carson CF, Hammer KA, Riley TV (1995) Broth micro-dilution method for determination of susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Malaleuca alterifolia* (Tea tree oil). *Microbios* 82:181–185
- Chan K (2003) Some aspects of toxic contaminants in herbal medicines. *Chemosphere* 52:1361–1371
- Chow KM, Hui AC, Szeto CC (2005) Neurotoxicity induced by beta-lactam antibiotics: from bench to bedside. *Eur J Clin Microbiol Infect Dis* 24:649–653
- Clinical and Laboratory Standards Institute (2006) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard-Seventh Edition*. CLSI, Wayne, Pennsylvania, USA
- Cragg GM, Newman DJ (2013) Natural products: a continuing source of novel drug leads. *Biochim Biophys Acta* 1830(6):3670–3695
- Damery S, Gratus C, Grieve R, Warmingtons S, Jones J, Routledge P, Greenfield S, Dowsell G, Sherriff J, Wilson S (2011) The use of herbal medicines by people with cancer: a cross-sectional survey. *Br J Cancer* 104(6):927–933
- Das PR, Islam MT, Kabir MH, Haque MM, Khatun Z, Nurunnabi M, Khatun Z, Lee Y, Jahan R, Rahmatullah M (2012) An ethnomedicinal survey conducted among the folk medicinal practitioners of three villages in Kurigram district, Bangladesh. *American-Eurasian J Sustain Agri* 6(2):85–96
- Das KK, Fatema KK, Nur IT, Noor R (2013) Prevalence of microorganisms in commonly used cosmetics samples in Dhaka metropolis. *J Pharma Sci Innovat* 2(6):7–9
- Djeussi DE, Noumedem JAK, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, Nkuete AHL, Kuete V (2013) Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Compl Altern Med* 13:164
- Duraisankar M, Ravindran AD (2013) Microbial quality of herbal medicine in production unit. *Int J Pharm Life Sci* 4(7):2775–2779
- Dutta S, Hassan MR, Rahman F, Jilani MFA, Noor R (2013) Study of antimicrobial susceptibility of clinically significant microorganisms isolated from selected areas of Dhaka, Bangladesh. *Bang J Med Sci* 12(1):34–42
- Eric V, Granowitz MD, Richard B, Brown MD (2008) Antibiotic adverse reactions and drug interactions. *Crit Care Clin* 24:421–442
- Ernst E (2011) Herbal medicine in the treatment of rheumatic diseases. *Rheum Dis Clin North Am* 37(1):95–102
- Faulkner CM, Cox HL, Williamson JC (2005) Unique aspects of antimicrobial use in older adults. *Clin Infect Dis* 40:997–1004
- Ferraro MJ, Craig WA, Dudley MN (2001) *Performance Standards for Antimicrobial Susceptibility Testing*. NCCLS, Pennsylvania, USA
- Granowitz EV, Brown RB (2007) *Adverse Reactions to Antibiotics*. In: Cunha BA (ed) *Infectious Diseases in Critical Care Medicine*, 2nd edn. Informa Healthcare USA, Inc., New York
- Gyasi RM, Charlotte MM, Prince OWA, Seth A (2011) Public perceptions of the role of traditional medicine in the health care delivery system in Ghana. *Global J Health Sci* 3(2):40–49
- Hossain J (2009) Importance of the bioburden test in pharmaceutical quality control. *Pharma Microbiol Forum* 15(1):2–14
- Hussain A, Wahab S, Zarin I, Hussain MDS (2010) Antibacterial activity of the leaves of *Coccinia indica* (W. and A) of India. *Adv Biol Res* 4(5):241–248
- Izzo AA, Ernst E (2009) Interactions between herbal medicines and prescribed drugs: an updated systematic review. *Drugs* 69(13):1777–1798
- Jagessar RC, Mars A, Gones G (2008) Selective antimicrobial properties of leaf extract against various micro-organisms using disc diffusion and agar well diffusion method. *J Nat Sci* 6(2):24–38
- Kala CP, Dhyani PP, Sajwan BS (2006) Developing the medicinal plants sector in northern India: challenges and opportunities. *J Ethnobiol Ethnomed* 2:32
- Khan SA, Feroz F, Noor R (2013) Study of extended spectrum β -lactamase producing bacteria from urinary tract infection in Dhaka city, Bangladesh. *Tzu Chi Med J* 25(1):39–42
- Khanom S, Das KK, Banik S, Noor R (2013) Microbiological analysis of liquid oral drugs available in Bangladesh. *Int J Pharm Pharma Sci* 5(4):479–482
- Kraft K (2009) Complementary/Alternative Medicine in the context of prevention of disease and maintenance of health. *Prev Med* 49(2–3):88–92
- Kucekova Z, Jiri M, Petr H, Otakar R (2013) Edible flowers - antioxidant activity and impact on cell viability. *Central European J Biol* 8(10):1023–1031
- Mendes E, Herdeiro MT, Pimentel F (2010) The use of herbal medicine therapies by cancer patients. *Act Med Port* 23(5):901–908
- Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH (2005) *Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment. *Antimicrob Agents Chemother* 49(4):1306–1311

- Molton JS, Tambyah PA, Ang BS, Ling ML, Fisher DA (2013) The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. *Clin Infect Dis* 56(9):1310–1318
- Moniruzzaman M, Ashrafi MFF, Mia Z (2012) Qualitative and quantitative microbiological studies of antacid and paracetamol suspensions from different drugstores of Dhaka. *Dhaka Uni J Biolog Sci* 21(1):105–107
- Munshi SK, Rahman MM, Noor R (2012) Detection of virulence potential of diarrheagenic *Escherichia coli* isolated from surface water of rivers surrounding Dhaka City. *J Bang Acad Sci* 36(1):109–122
- Nakajima K, Nonaka K, Yamamoto K, Yamaguchi N, Tani K, Nasu M (2005) Rapid monitoring of microbial contamination on herbal medicines by fluorescent staining method. *Letters Appl Microbiol* 40:128–132
- Navarro VJ, Senior JR (2006) Drug-related hepatotoxicity. *N Engl J Med* 354:731–739
- Noor R, Huda N, Rahman F, Bashar T, Munshi SK (2013a) Microbial contamination in herbal medicines available in Bangladesh. *Bang Med Res Coun Bull* 39(3):124–129
- Noor R, Akhter S, Rahman F, Munshi SK, Kamal SMM, Feroz F (2013b) Frequency of extensively drug resistant tuberculosis (XDR-TB) among re-treatment cases in NIDCH, Dhaka, Bangladesh. *J Infect Chemother* 19(2):243–248
- Oreagba IA, Oshikoya KA, Amachree M (2011) Herbal medicine use among urban residents in Lagos, Nigeria. *BMC Compl Altern Med* 11:117
- Rahmatullah M, Jahan R, Khatun MA, Jahan FI, Azad AK, Bashar ABM, Miajee ZUM, Ahsan S, Nahar N, Ahmad I, Chowdhury MH (2010) A pharmacological evaluation of medicinal plants used by folk medicinal practitioners of Station Purbo Para Village of Jamalpur Sadar Upazila in Jamalpur district, Bangladesh. *American-Eurasian J Sustain Agri* 4:170–195
- Somasundaram S, Manivannan K (2013) An overview of fluoroquinolones. *Annual Rev Res Biol* 3(3):296–313
- Srivastava J, Lambert J, Vietmeyer N (1996) *Medicinal Plants: An Expanding Role in Development*. Agriculture and Forestry Systems, World Bank, Washington, D. C
- Steven GN, Meghan G, Dhivya S, Sathishkumar R, Subramanyam R (2013) DNA barcoding detects contamination and substitution in North American herbal products. *BMC Med* 11:222
- Tabassum N, Ahmad F (2011) Role of natural herbs in the treatment of hypertension. *Pharmacogn Rev* 5(9):30–40
- Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. *Ame J Med* 119(2):3–10
- Ting A, Chow Y, Tan W (2013) Microbial and heavy metal contamination in commonly consumed traditional Chinese herbal medicines. *J Tradit Chin Med* 33(1):119–124
- Umashanker M, Shruti S (2011) Antipyretic, antiulcer, anti-diabetic and anticancer: a review. *Int J Res Pharm Chem* 1(4):1152–1159
- Veeresham C (2012) Natural products derived from plants as a source of drugs. *J Adv Pharm Technol Res* 3(4):200–201
- World Health Organization (2007) *WHO Guidelines for Assessing Quality of Herbal Medicines With Reference to Contaminants and Residues*. WHO Press, Geneva, Switzerland
- World Health Organization (2013) *WHO Traditional Medicine Strategy: 2014–2023*. WHO Press, Geneva, Switzerland

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