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Microbiological analysis and detection of anti-bacterial activity of *Centella asiatica* and *Aloe vera* samples collected from different areas of Dhaka city, Bangladesh

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The present study was performed to detect the presence of contaminating microorganisms in two commonly available herbal samples (*Centella asiatica* and *Aloe vera*) collected from different areas of Dhaka city, Bangladesh and to assess their antibacterial activity. Out of twenty samples (having ten samples of each categories) studied; the range of total viable bacterial count was approximately 10^3 to 10^8 cfu/g. Presence of *Staphylococcus aureus* was found in all the samples, followed by *Klebsiella* spp. in 15 samples, *Pseudomonas* spp. in 14 samples, *Bacillus* spp. in 12 samples, *Escherichia coli* in 9 samples and *Vibrio* spp. in 7 samples. *Salmonella* spp. was detected in neither of the sample. 17 samples showed a high fungal load up to 10^7 cfu/g. Antibacterial activity of *C. asiatica* samples was demonstrated against eight laboratory isolates. Only four *C. asiatica* samples showed activity against *Klebsiella spp*. On the contrary, *Aloe vera* samples (12-14) showed antibacterial activity only against *Staphylococcus* spp.

Key words: *Centella asiatica*; *Aloe vera*; Contamination; Antimicrobial activity

Besides a mass practice of antibiotics to fight diseases, most of the medicines have been derived from natural resources around the world until now (1-3). Many antibiotics have become out-of-date due to the appearance of resistant or even the multidrug-resistant strains, or reported to be associated with undesirable side effects (4-6). Researchers have found that, bacterial resistance to tetracycline; penicillin and erythromycin are encoded by over 100 different genetic mechanisms, a number of which are readily transferable to other bacteria via conjugal elements, transposons, plasmids etc (7). Therefore, inauguration of herbal medication could be an alternate to treat diseases caused by multi-drug resistant bacteria (8, 9). Some other reasons for the practice of herbal medicines include the minor side effects with small or no toxicity, cheap in price, better ease of access etc. (10, 11). Therefore, more researches are going on focused onto developing drugs from herbal sources with slightest or no side effects having better outcome (12).

C. asiatica is a small stonoliferous, perennial, frost-tender creeping aromatic plant belonging to the family Apiaceae (Umbelliferae) and subfamily Mackinlaya (13), which was formerly included in hydrocotyle (14). In ancient times, *Centella asiatica* and its extracts was used for the treatment of various skin disease such as leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhea, fever, and diseases related to the female genitourinary tract (15). Various reports have claimed that *Centella asiatica* have been used conventionally in decreasing high blood pressure, treating a range of deficiencies, enhancing memory and brainpower, easing nervousness and wound repairing (16).

Aloe vera is a member of liliaceae family. *Aloe vera* (Synonym *A. brobadensis*) is a cactus like plant with green, dagger- shaped leaves that are fleshy, tapering. *Aloe vera* gel is extensively used in gastrointestinal disorders including peptic ulcer (17). *A. vera* has been used to treat numerous skin conditions such as cuts, burns and eczema. There are suggestions on the properties of *A. vera* juice on wound healing (18). There are several studies on *A. vera* which are considerable. Ethanolic extracts of *A. vera* showed advanced antibacterial activity than aqueous extract (19).

Medicinal plants horde a wide spectrum of microorganisms with various individual properties and with considerable differences regarding qualitative & quantitative aspects. In principle, the microbial load of plants is the result of a series of influences caused by animate & inanimate sources and microbial contaminates are easily transferred via air and soil borne vectors (20-23). Furthermore, original microflora in plants, presence of microorganisms within processing plant, dust, using contaminated water and animal human

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excreta, before and after harvest procedure including processing, storage and distribution may be the origins of microbial contamination (20, 24). In addition, the collection and handling of medicinal plants does not usually done in sanitary conditions and unhygienic cultivation conditions can increase pollution, influence the maintenance period and destruct the aspect and the potential advantage of medicinal plants (9, 25).

Although there are several reports based on the determination of antibacterial activity of *C. asiatica & A. vera*, little is known about the harboring microorganism associated with these samples. Based on these facts and considerations, the recent study not only deals with the evaluation of antibacterial efficacy of the crude extracts of these herbal plants, but also to measure their level of bacterial contamination when collected from the market places from various areas of Dhaka city.

MATERIALS AND METHODS

Collection of samples & processing. 10 *Centella asiatica* samples and 10 *Aloe vera* samples were collected randomly for the detection of microbiological analysis and antibacterial activity from different locations of Dhaka city within a period of October 2015 to November 2015. Samples were collected early in the morning and transported quickly to the laboratory according to the standard scheme. For preparing the sample suspension for microbiological examinations, 10g of each sample was weighed and homogenized in 90 ml normal saline to prepare 100ml sample suspension. Then, it was subjected to serial dilution (made up to 10^{-5}) for microbiological analysis (9, 26-29).

Total viable bacterial count (TVBC), total bacilli count and total fungal count. 0.1 ml from 10^{-2} & 10^{-5} dilution of each sample was spread over nutrient agar (NA) for TVBC & 0.1 ml from 10^{-2} dilution was spread over Starch agar and SDA plate for the detection of *Bacillus* spp. and total fungal load respectively (26, 27, 30).

Determination of *Pseudomonas* spp., *Staphylococcus* spp., *Escherichia coli,* & *Klebsiella* spp. 0.1 ml sample from 10^{-2} & 10^{-5} dilution was spread on Cetrimide agar, MSA (Mannitol Salt Agar), MAC (MacConkey Agar) for the determination of *Pseudomonas* spp., *Staphylococcus* spp. and coliform, respectively and incubated overnight at 37 °C (27, 28, 31).

Detection of *Vibrio* spp., *Salmonella* spp., *and Shigella* spp. As *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. remain in the environment as viable but non-culturable (VBNC) state, they do not appear readily during microbiological test procedures. Before conducting serial dilutions, 1 ml of 10^{-2} diluted samples were enriched with alkaline peptone water (APW) for *Vibrio* spp. and selenite cystein broth (SCB) for *Salmonella* spp. & *Shigella* spp. at 37 °C for 2-4 hrs. The samples were then spread on the SS agar and TCBS agar for detecting *Salmonella* spp. (with black precipitates), *Shigella* spp. (reddish or pink color*)* & *Vibrio* spp. (small, yellow colored colony) (27, 28, 31).

Determination of antibacterial activity of the herbal plants. The antibacterial activity of the samples was carried out by using agar well diffusion method. At first, the suspension (with standard turbidity compared to that of McFarland standard of 0.5) of each of the test bacteria (*Escherichia coli, Bacillus spp., Staphylococcus* spp., *Vibrio* spp., *Salmonella* spp., *Pseudomonas* spp., *Klebsiella* spp., & *Listeria* spp.) were spread evenly over the MHA using cotton swab which in turn resulted into uniform lawns. Wells made in the MHA was generated by cork-borer. Each of the samples then introduced separately in the specified well along with a positive control Gentamicin (GEN-10µg) and a negative control (normal saline). Existence of clear zone surrounding the sample solution (if any) was indicative of the presence of antibacterial activity of the samples tested (26, 27, 32, 33).

RESULTS AND DISCUSSIONS

In the ancient times, plants have been a source of medicine and today scientists and the public recognize their value as a source of new or complimentary medicinal products (34). The traditional system of medicine in particular herbal medicine is in great demand in developed as well as in developing countries because of their lesser side effect, wide biological activities and lesser cost than synthetic drugs (9, 35).

However, Medicinal plants could be subjected to a wide range of microbial contamination during pre and post harvest stages and they can harbor high microbial loads. Researchers investigated the microbial load of medicinal plants & spices and found the presence of different contamination including pathogenic bacteria such as *Staphylococcus aureus*, *Shigella* spp., *Salmonella*, *Escherichia coli*, *Clostridium perfringens*, fungi, molds, mesophilic aerobic bacteria (total count) and Enterobacteriaceae (36, 38, 39). In this study, all herbal samples (*C. asiatica* & *Aloe* vera) were found to exhibit huge load of total viable bacteria within the range of 10^3 to 10^8 cfu/g as shown in Table 1. Here, *Staphylococcus aureus* was present in all the samples, followed by *Klebsiella* spp. (in 15 samples), *Pseudomonas* spp. (in 14 samples), *Bacillus* spp. (in 12 samples), *E. coli* (in 11 samples) and *Vibrio* spp. (in 10 samples). *Salmonella* spp. was absent in all samples (Table 1). This result was quite similar with the study conducted by Le et al. (39). Here, 19 samples showed a high fungal load up to 10^7 cfu/g, which can be indicative to the presence of various mycotoxins in the food samples that may pose severe threat to the human health (39). Also, Presences of the high load of other contaminating pathogenic bacteria are also a matter of great concern.

Anti-bacterial activity of the herbal sample. Chemically synthesized medicines may have numerous undesirable side effects on our body but natural antimicrobial agents from the herbal plant such as *Aloe vera* could be used safely in near future as anti-infective. It has several uses as laxative, anti-helminthic, hemorrhoid remedy, and uterine stimulant. It is used often together with licorice root, to treat eczema or psoriasis (42). On the contrary, *Centella asiatica* and its extracts was documented to be used for the treatment of various skin diseases like leprosy, lupus, varicose ulcers, psoriasis, diarrhea, fever, amenorrhea, and diseases of the female genitourinary tract (27). Some other reports have also claimed that *Centella asiatica* have been used traditionally in decreasing of high blood pressure, treating a range of deficiencies, enhancing memory and brainpower, easing nervousness and wound repairing (28).

In this present study antibacterial activity of herbal samples (*C. asiatica* & *Aloe vera*) was demonstrated against eight laboratory isolates (Table 2). Only four *C. asiatica* samples (1, 2, 5 and 6) showed activity against *Klebsiella spp*. On the contrary, *Aloe vera* samples (12, 13, 14) showed antibacterial activity against *Staphylococcus* spp. This result was not similar with the results found by Lalitha et al. (34), Udoh et al. (16),

TABLE 1. Microbiological analysis of the herbal samples

Sample	Microbial load (cfu/g)								
	TVB	***Bacillus* spp.**	**Fungi**	***E. coli***	***Klebsiella* spp.**	***Staphylococcus* spp**	***Pseudomonas* spp.**	***Vibrio* spp.**	***Salmonella* spp.**
S-1	1.0×10^{7}	8×10^{3}	1.0×10^{7}	1×10^{4}	0	2.6×10^{5}	1.5×10^{4}	2.2×10^{5}	0
S-2	1.0×10^{7}	1×10^{4}	1.0×10^{7}	1.6×10^{5}	9×10^{4}	4.3×10^{5}	1.9×10^{4}	3.8×10^{5}	0
S-3	1.0×10^{7}	7×10^{3}	1.0×10^{7}	0	0	7.8×10^{2}	0	3.2×10^{5}	0
S-4	2.1×10^{7}	0	2.4×10^{5}	3.8×10^{5}	4.1×10^{5}	4.5×10^{5}	1.3×10^{5}	2.5×10^{5}	0
S-5	3.7×10^{8}	0	3.4×10^{5}	3.4×10^{5}	3.7×10^{5}	1.5×10^{4}	1.92×10^{5}	0	0
S-6	2.3×10^{8}	0	1.65×10^{5}	3.3×10^{5}	3.6×10^{5}	3×10^{4}	1.38×10^{5}	1.0×10^{4}	0
S-7	2.4×10^{5}	0	1.0×10^{7}	2.2×10^{5}	2.7×10^{5}	1.8×10^{4}	6×10^{3}	0	0
S-8	1.6×10^{5}	0	1.0×10^{7}	1.6×10^{5}	2.9×10^{5}	2.5×10^{4}	6.9×10^{4}	0	0
S-9	1.7×10^{7}	0	1.0×10^{7}	1.8×10^{5}	2.0×10^{5}	3.8×10^{4}	1.0×10^{5}	0	0
S-10	1.7×10^{5}	0	1.0×10^{7}	1.2×10^{5}	1.8×10^{5}	7.0×10^{3}	8.5×10^{4}	0	0
S-11	2×10^{6}	0	2×10^{3}	0	0	1×10^{6}	0	0	0
S-12	1.72×10^{6}	4×10^{3}	5×10^{3}	0	3.7×10^{4}	1.5×10^{4}	8×10^{3}	3.5×10^{5}	0
S-13	2×10^{4}	1.60×10^{4}	0	0	2×10^{3}	2×10^{4}	2×10^{3}	3×10^{5}	0
S-14	2.88×10^{4}	1×10^{4}	1.32×10^{5}	1×10^{3}	5×10^{3}	2.2×10^{5}	0	2.5×10^{5}	0
S-15	6.20×10^{4}	7×10^{3}	4.20×10^{5}	7.90×10^{4}	3.99×10^{5}	3.70×10^{4}	1.29×10^{5}	0	0
S-16	5.59×10^{4}	1.12×10^{5}	4.48×10^{5}	0	9×10^{3}	3.5×10^{7}	1.10×10^{5}	1.0×10^{4}	0
S-17	2.70×10^{7}	1.15×10^{5}	3×10^{5}	0	7.3×10^{4}	3.7×10^{4}	4.1×10^{4}	0	0
S-18	3.52×10^{6}	1.10×10^{5}	1.20×10^{5}	0	1.10×10^{5}	4×10^{4}	0	1.5×10^{5}	0
S-19	3.10×10^{7}	1×10^{6}	0	0	0	1.51×10^{3}	0	0	0
S-20	2.61×10^{7}	1.308×10^{5}	0	0	0	3.20×10^{7}	0	0	0

TVB= Total viable bacteria
The average load has been shown
Microbial limit (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)

Irshad et al. (42). The difference in the results of these studies might be due to the employment of the different solvent extraction method (such as methanol or ethanol) for scavenging the bioactive components from the *A. vera* samples & minimum inhibitory concentration (MIC) procedure which was proved to be worthwhile.

CONCLUSION

In this study, a variety of bacterial load was observed in *Centella asiatica* and *Aloe vera*. Therefore, hygienic conditions must be enhanced in different stages of cultivation, produce, transfer, processing and packaging. Considering the importance of medicinal plants in the human health and the large practice of the medicinal herbs in various forms for disease prevention and cure; culturing, harvesting, conveying and processing of these crops should be done in sanitary conditions. Thus, it is important to control environmental conditions and improvements in hygiene procedures during production and processing of herbs. A suitable sanitization method for disinfection before and when packaging medicinal plants would be recommended. In addition, a further investigation is necessary to assess antimicrobial activity by performing various solvent (ethanol extraction, methanol extraction, hot and cold-water extraction) extraction method.

TABLE 2. *In vitro* anti-bacterial activity of herbal samples

Sample	Zone of inhibition (mm)							
	E. coli	***Klebsiella* spp.**	***Pseudomonas* spp.**	***Vibrio* spp.**	***Staphylococcus* spp.**	***Listeria* spp.**	***Bacillus* spp.**	***Salmonella* spp.**
S-01	**0**	11	**0**	**0**	**0**	**0**	**0**	**0**
S-02	**0**	11	**0**	**0**	**0**	**0**	**0**	**0**
S-03	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-04	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-05	**0**	11	**0**	**0**	**0**	**0**	**0**	**0**
S-06	**0**	11	**0**	**0**	**0**	**0**	**0**	**0**
S-07	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-08	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-09	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-10	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-12	**0**	**0**	**0**	**0**	18	**0**	**0**	**0**
S-13	**0**	**0**	**0**	**0**	14	**0**	**0**	**0**
S-14	**0**	**0**	**0**	**0**	14	**0**	**0**	**0**
S-15	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-16	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-17	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-18	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-19	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
S-20	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Positive Control (GEN-10µg)	19	18	20	21	19	16	17	18
Negative Control (Normal Saline)	0	0	0	0	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.

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