Jahangirnagar University J. Biol. Sci. **10**(1 & 2): 1-8, 2021 (June & December)

Occurrence of Enterobacteriaceae in medicinal plants sold in local markets of Savar, Dhaka

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Abstract

Medicinal plants can be contaminated with a large diversity of potentially pathogenic bacteria. Ten medicinal plant samples *Plantago psyllium*, *Terminalia bellirica*, *Terminalia chebula*, *Phyllanthus emblica*, *Ocimum basilicum*, *Glycyrrhiza glabra*, *Withania somnifera*, *Andrographis panicula*, *Bombax ceiba* were collected from Amin Bazar and Savar of Savar area to study the presence and abundance of Enterobacteriaceae family bacteria in the herbal plant samples. A total of fifty five (55) bacterial isolates have been isolated from different medicinal plant samples using spread plate method on nutrient Agar media. Culture of bacterial isolate on a number of selective and differential media and biochemical tests (Catalase activity, hemolytic property, fermentation test, Starch hydrolysis, VP test, MR test, MIU test, Indole and casein test etc.) revealed the presence of different Enterobacteriaceae family bacteria in the plant samples. Out of 55 isolates 6 isolates were *Salmonella* sp. (11%), 2 isolates were *Klebsiella* sp. (3.63%) and 2 isolates were *Enterobacter* sp. (3.63%). Antibiotic sensitivity test of bacterial isolates showed that all the identified Enterobacteriaceae family bacteria were sensitive to Kanamycin but resistant to Amoxycilin, Aztreonam and Penicillin-G. Therefore, this study highlighted the necessity for constant quality assessment of herbal medicines to facilitate and ensure safe production and supply of medicinal products suitable for human consumption.

Key words: Occurrence, Enterobacteriaceae, medicinal plants, local market, Savar area.

INTRODUCTION

Plant is a key source of medicines and performs as a major role in health treatment (Sandberg and Corrigan, 2001). Various uses of plants are constantly expanding worldwide day by day. The diverse uses of medicinal plants are not only confined to the treatment of diseases but also act as a potential source for maintaining good health and conditions. Herbal medicines are natural and rare to cause serious side effect as compared to conventional drugs. The emergence of different diseases also increases the demand for medicinal plants. But without proper scientific knowledge and proper treatment of the herbal medicine it cannot be an appropriate treatment alternative. Therefore, the safety and quality of medicinal plants are also of major concern (Abba *et al*., 2009).

Herbal plants can be contaminated with a large diversity of potentially pathogenic bacteria. Microbiological contamination refers to the contamination of herbal plant by different types of microorganisms like viruses, bacteria, insects (larvae and eggs),

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protozoa, fungi (moulds) and other different organisms. The presence of these pathogenic microorganisms in medicinal plants influences the quality of the final products as well as might cause a hazard to public health (Banerjee *et al*., 2003). Different pathogenic microorganisms present in the herbal plants include; *Listeria monocytogenes, Escherichia coli*, *Salmonella* sp. and spore-forming different microorganisms like *Clostridium perfringens* and *Bacillus cereus* (Witkowska, 2011).

Enterobacteriaceae sp. is a bacterial family commonly found on the harvested part of plants and raises the problems of deterioration and damage of the quality of foods. These are rod shaped, Gram-negative, facultative anaerobic and non-spore-forming bacteria. As Enterobacteriaceae is ubiquitous in nature, many species exist in diverse environment like both aquatic and terrestrial habitats and as free living in distinct ecological niches. Some species may be associated with plants, animals or insects only (Octavia, 2014). Though Enterobacteriaceae are found in nature but they possess some indicative values towards fecal contamination. Hence, their presence could be considered as an index of the degrees of contamination which indicates possible presence of disease causing or harmful organisms (APHA, 1992; Pelczer, 1993 and Jay, 2012).The occurrence of *E. coli* and Enterobacteria indicate the situations relating to faecal contamination (Czech, 2001). Together with the group of coliform, it could be used as an indicator of undesirable hygiene condition after considering the magnitude of total viable counts measured (Kneifel, 2002).

Therefore, it is essential to examine the bacterial contamination and Enterobacterial presence in medicinal plant products (Zin *et al*., 2013) locally available in different markets. The presence of the microbial contaminants in different non-sterile pharmaceutical products can reduce or even inactivate the therapeutic activities of the products. It also has the potential to adversely affect the patients taking these medicines. As different herbal medicinal products are complex mixtures originating from biological sources, great efforts are needed to guarantee an adequate and constant quality. Therefore, this study was aimed at determining the existence of Enterobacteriaceae family bacteria in medicinal plant samples collected from different locations of Savar city, Dhaka.

MATERIALS AND METHODS

Sample collection: A total number of ten medicinal plant samples were taken from different local markets of Savar and nearby areas (Amin Bazar, Savar) of Dhaka, Bangladesh from November to December, 2018. All samples were gathered in sterile polythene bags and after collection, they were handled carefully. For further detailed study, they were kept at 4ºC.

Table 1. List of medicinal plants, plant parts used in this study collected from Savar and Amin bazer, Dhaka, Bangladesh

Medicinal plants		Used parts
Local name	Scientific name	
1. Isupguler vushi	*Plantago psyllium*	Seed husk
2. Tokma	*Ocimum basilicum*	Seed
3. Bohera	*Terminalia bellirica*	Seed
4. Horitoki	*Terminalia chebula*	Seed
5. Amloki	*Phyllanthus emblica*	Fruit
6. Yasthimadhu	*Glycyrrhiza glabra*	Root
7. Aswagandha	*Withania somnifera*	Root and Fruit
8. Kalomegh	*Andrographis paniculata*	Whole plant
9. shimul mul	*Bombax ceiba*	Root
10. Trifola		Fruit

Isolation of bacteria: Bacterial isolation and enumeration were done in Nutrient Agar media (NA) plate by spread plate method (Afrin *et al*., 2019). The microbial hygiene and safety conditions were then assessed using the methods recommended by International Commission on Microbiological Specifications for Foods (ICMSF, 2005). Each sample was diluted upto 10^{-10} and 0.1ml of each dilution was equally spread on the NA media plate in petridish. Then they were incubated for 24 hours at 37°C. After incubation, plates were observed carefully. Bacterial plates having well discrete colony were used for counting. Further selection was made on the basis of definite colony morphology, and the isolates were purified by repeated streaking. Finally, they were stored at 4°C in NA slant as a glycerol stalk for further analysis.

Microbial load determination: After incubation, colony forming unit (CFU/g) was calculated using the following equation - CFU/ g = Number of colonies on the agar plates/(Total dilution of tube × amount plated) (Afrin *et al*., 2019).

Identification of the isolates by biochemical analysis: The isolates were examined to monitor various morphological characters *viz.* form, color, surface, margin, elevation; optical characters etc. as stated by Eklund & Lankford (1967). Bacterial isolates were then grown on different selective and differential media like: MacConkey, EMB, SSA, BGA etc. A number of biochemical tests (Indole test, Casein test, Starch hydrolysis test, Fermentation test, Catalase test, MR-VP test, Motility test etc.) were also performed. Results of the biochemical and physiological tests of selected bacterial colonies were analyzed following Bergey's Manual of Determinative Bacteriology ((Buchanan & Gibbons, 1974).

Antibiotic Sensitivity test: The sensitivity of identified bacteria was studied using four antibiotics namely Kanamycin, Amoxycilin, Aztreonam and Penicillin-G following Standard Kirby-Bauer disk diffusion method (Yu *et al*., 2019). All results were recorded appropriately and interpreted using the National Committee for Clinical Laboratory Standards interpretation chart (CLSI, 2008). The isolates were classified as susceptible

(S), resistant (R) or intermediate (I) according to the guidelines of CLSI and interpreted according to the zone diameter interpretation criteria in Table 3.

RESULTS AND DISCUSSION

Microbiological load of different medicinal plants samples were calculated and shown in the Table 2. Among the ten samples, mean heterotrophic bacterial load ranged from 3.37×10^4 to 8.63×10^{10} CFU/g on Nutrient Agar media plate. Maximum heterotrophic bacterial count was observed in the root of *Bombax ceiba* (Shimul root). While minimum bacterial count was observed in the *Phyllanthus emblica* (Amloki). The limit of microbial contamination for herbal medicinal products is determined by European Pharmacopoeia (2007) on the basis of two conditions. Conditin1. total aerobic bacteria should be $<10^7$ CFU/g to those medicinal products where boiling water is added before consumption. Condition 2. total aerobic bacteria should be $<10^5$ CFU/g for those herbal products where boiling water is not added before consumption. Enterobacteria and other Gram-negative organisms should be $<10^3$ CFU/g where *Salmonella* sp. and *E. coli* should be totally absent. Almost all the samples exceeded the standard microbiological limit according to these guidelines except Amloki collected from Amin Bazar. In comparison between two different locations of Savar city, samples collected from Savar Bazar are more contaminated with bacteria than Amin Bazar samples. Among the ten medicinal plant samples 60% samples (*Plantago psyllium*, *Ocimum basilicum*, *Terminalia bellirica*, *Terminalia chebula*, *Phyllanthus emblica* and Trifola) have higher CFU value than standard limit collected from Savar Bazar. Previous studies have also confirmed the presence of potential contaminants in herbal preparation (Okunlola *et al*., 2007). In a study conducted by Idu *et al*. (2015) the results showed that all the polyherbal samples were highly contaminated with microbes with total bacterial count ranging from $2.5x10^3$ to $6.4x10^9$ which is lower than our study. Another study (de Sousa Lima *et al*., 2020) reported that a totality of 31.8% herbal medicine samples exceeded the safety limits of CFU/g $\leq 10^5$, as indicated by WHO guidelines (WHO, 2007) where 16.7% were homemade herbal medicines and 15.1% were commercial herbal medicines. Abba *et al.* (2009) reported that the herbal medicine samples were contaminated with pathogenic bacteria at different levels. Among 150 samples, nineteen (12.67%) were free from bacterial contamination, thirty five (23.33%) had bacterial counts in the range of 1.0×10^7 to 4.5×10^7 CFU/g; while forty six (30.07%) had bacterial count between 5×10^7 to 8.5×10^7 CFU/g. So, the bacterial counts ranged between 1.0×10^7 to 1.8×10^8 CFU/g respectively in that study which is also lower than our present study.

In this study, a total number of 55 isolates have been obtained from different medicinal plant samples. *Enterobacter* sp., *Salmonella* sp. and *Klebsiella* sp. were identified presumptively through their distinct colony characteristics on selective culture media. Out of 55 isolates, 6 isolates were *Salmonella* sp. (11%), 2 were *Klebsiella* sp. (3.63%) and 2 were *Enterobacter* sp. (3.63%). Antibiotic sensitivity test of different isolates showed that all the identified bacteria were sensitive to the antibiotic Kanamycin and resistant to the antibiotic Amoxycilin, Aztreonam and Penicillin-G.

Table 2. Total viable count of samples on NA media (CFU/g)

Medicinal plant Samples	CFU /g (mean ± SD)		Standard limit (CFU/g) according to WHO guidelines
	Savar	Amin Bazar	
1. *Plantago psyllium* (Isupguler vushi)	$9.67x10^9 \pm 0.58$	$2.17x10^6 \pm 0.58$	10^5
2. *Ocimum basilicum* (Tokma)	$7.8x10^7 \pm 1$	$1.8x10^6 \pm 1$	
3. *Terminalia bellirica* (Bohera)	$6.27x10^6 \pm 0.58$	$6.03x10^6 \pm 1.15$	
4. *Terminalia chebula* (Horitoki)	$5.74x10^5 \pm 0.58$	$4.7x10^4 \pm 1.15$	
5. *Phyllanthus emblica* (Amloki)	$3.8x10^5 \pm 1$	$3.37x10^4 \pm 0.58$	
6. *Glycyrrhiza glabra* (Yasthimadhu)	$4.7x10^8 \pm 1$	$8.03x10^8 \pm 0.58$	
7. *Withania somnifera* (Aswagandha)	$7.86x10^6 \pm 0.58$	$6.73x10^7 \pm 0.58$	
8. *Andrographis paniculata* (Kalomegh)	$3.43x10^8 \pm 1.15$	$5.2x10^8 \pm 1$	
9. *Bombax ceiba* (root shimul mul)	$4x10^8 \pm 1.53$	$8.63x10^{10} \pm 1.15$	
10. Trifola	$6.13x10^8 \pm 1.15$	$4.6x10^6 \pm 0.58$	

Table 3. Antibiotic sensitivity pattern of bacterial isolates against following antibiotics

Medicinal plant name	Identified Bacteria	Resistance (%)			
		Penicillin-G 10µg	Amoxycillin 10µg	Aztreonam 30µg	Kanamycin 30µg
Bohera, Horitoki, Isupguler vushi, Yasthimadhu, Ashwagandha, Kalomegh, Shimul mul, Trifola	*Enterobacter* sp.	100	100	100	0
Isupguler vushi, Kalomegh	*Klebsiella* sp.	100	100	100	0
Kalomegh	*Salmonella* sp.	100	100	100	0

According to Adeleye *et al*. (2005) and Okunlola *et al*. (2007) the microorganisms that cause severe health hazards are pathogenic bacteria like *Staphylococcus aureus, Escherichia coli, Shigella*, *Salmonella* and other Gram negative and Gram positive bacteria. The microbiological contamination of powdered medicinal herbal preparations collected from herbal retail outlets at Kaduna of Nigeria found that a number of herbal drugs were contaminated with *Shigella* spp. and *Salmonella typhi* (Abba *et al*., 2009). In

addition, the presence of pathogenic bacteria such as *Shigella* spp., *Enterobacter agglomerans*, *Vibrio fluvialis* , *Klebsiella* spp. was also found by Idu *et al*. (2011) and Alwakeel (2008). All these results are similar to our present study. The contamination of medicinal plants with Enterobacteriaceae family, *Staphylococcus aureus, Salmonella* spp., *Bacillus* spp., *Aspergillus* spp. and *Penicillium* spp. have been also reported by Govender (2006). The elevated level of fungal and bacterial contaminants, like *Escherichia coli*, *Penicillium* spp., yeast, *Fusarium*, and *Aspergillus* were observed in spices and herbs by Kaume (2012); Candlish (2001) and Kneifel (2002). De Sousa Lima *et al.* (2020) showed that the pathogenic bacteria most commonly isolated from the medicinal plants were *S. aureus* (49.2%), followed by *Salmonella* spp. (34.8%), *E. coli* (25.8%), and *P. aeruginosa* (14.4%) which is higher than our study. Pathogenic bacteria, like *Salmonella* spp., *E. coli*, *Shigella*, *P. aeruginosa*, and *S. aureus* were also found in other studies conducted by Trabulsi & Alterthum (2015) and Esimone *et al*. (2007).

The presence of Enterobacteriaceae including *Enterobacter* sp. and *Klebsiella sp.* in medicinal plants is a cause of concern. The occurrence of the bile salt-tolerant Gram-negative bacterial species belonging to Enterobacteriaceae is a key indicator of hygienic precariousness, inadequate processing or post processing contamination. Contamination of herbal plants by these family of bacteria may be due to poor sanitary measures and inadequate hygienic practices during preparation (Zin, 2013) such as unsafe collection, processing, drying, transportation, dispensing or storage processes of herbal medicines (Trabulsi & Alterthum, 2015).The major sources of contamination might be plant or any other raw materials, soil, water and the container used. Besides, these types of bacteria normally adhere to roots, stem, leaves, flowers, seeds, and other parts of plants from which herbal medicines are prepared. Different environmental factors like humidity, temperature and extents of rainfall during post harvesting as well as pre-harvesting periods can also stimulate bacteriological contamination of the medicinal herbs (De Freitas Araujo & Bauab, 2012).

In conclusion, this study elucidated that standard quality is the basis of safety and reproducible efficiency of herbal medicines and to assure the standards of herbal drugs, quality of the plant material or preparations should be maintained precisely.

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