Isolation of potential bacterial pathogens from the phylloplane of some selected medicinal plants

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Abstract

Microorganisms are ubiquitous and their impact could be appreciated directly or indirectly. This study was aimed at determining the occurrence of pathogenic bacteria on the phylloplane of some medicinal plants commonly used to treat diseases by oral administration. Leaves samples were collected aseptically from Cassia fistula (Cassia) Mangifera indica (Mango) and Psidium guajavum (Guava) and their bacterial contents were assessed. Higher bacterial counts were observed in raw samples followed by those washed with sterile water. Samples washed with sterile warm water (blanched) were shown to have lower counts although without statistical significance (p ≤ 0.05). Guava leaves had higher counts (3.4 × 10⁶ cfu/g) in the raw samples and cassia in blanched (6.4 × 10⁵ cfu/g). However, mango leaves had the lowest (8.4 × 10⁵ cfu/g and 2.0 × 10⁵ cfu/g) in both the treatments respectively. Twenty bacterial species were isolated comprising Gram negative and Gram positive species. Bacterial identification revealed that Proteus mirabilis (35%), Escherichia coli (15%), Klebsiella pneumoniae (5%), Morganella morganii (5%), Salmonella typhi (10%), Enterobacter sp. (5%), Staphylococcus aureus (5%) and Vibrio cholerae (5%) were the prevalent species. These organisms could be potential pathogens and proper washing with hot water may serve a better means of reducing the microbial contents and thus, it is recommended.

Keywords: Phylloplane; Medicinal; Plants; Bacteria; Washing.
3. Results and discussion

Results for bacterial enumerations showed the occurrence of large bacterial cells on the phylloplanes of the leaves samples. In G1 treatment, bacterial counts were more prominent compared to G2 and G3. M. indica had the lowest counts in all the treatments whereas P. guajavum had the highest. C. fistula was shown to contain 1.36×10⁶ cfu/g, 1.28×10⁶ cfu/g and 6.4×10⁵ cfu/g in the unwashed (G1), washed (G2) and blanched (G2) samples respectively (Fig. 1). Similarly, there was steady decline in the bacterial load observed in the phylloplane of M. indica where highest number (8.4×10⁵ cfu/g) was observed in the unwashed samples. However, there was drastic decline in the bacterial count in P. guajavum in which 3.40×10⁶ cfu/g and 4.4×10⁵ cfu/g were recorded as the highest and lowest values respectively (Fig. 1).

Statistical analysis indicated a significant difference between treatments in a particular leaves sample (p ≤ 0.05) but insignificantly among leaves type. The occurrence of large number of bacteria on the plants’ phylloplane was never unprecedented due to the fact that bacteria are ubiquitous and many are known to be epiphytic. This agreed with the findings of [4] and [11] who observed the presence of microorganisms on phylloplane of different plants using culture and microscopic techniques respectively. Their presence also suggests the availability of basic carbon and energy requirements for microbial life and possible symbiotic relationship [12]. Washing of leaves with warm water (G3) was shown to considerably reduce the bacterial load on the leaves when compared with the unwashed. This indicated the ability of warm water to displace more bacterial cells than cool water; and might be a result of having more de-waxing ability of leaves cuticles by the warm water. Alternatively, the warm water facilitated the dissolution of exopolysaccharides synthesized by the microbes for improving adhesion, aggregate formation or protection from desiccation. This is supported by the work of [13] who observed the presence of exopolysaccharides as major contributor to colonization and survival of bacterial cells on phylloplane.

Lowest bacterial counts (Fig. 1) were observed in M. indica as against C. fistula and P. guajavum. This might be attributed to the fact that C. fistula and P. guajavum have rough phylloplanes that enabled easy attachment of large number of microorganisms on the surfaces. The work of [14] have suggested many factors that influence the abundance and diversity of phylosphere microbes in which plant type was a major determinant. However, despite the reduction in bacterial load by seasonal influence (lower bacteria in summer than in winter [4]) and washing with warm water, the bacterial count recorded was still of great health concern. This is due to the fact that the values observed in G3 are above the limits outlined by some international organizations like World Health Organization (WHO), American National Standard for Dietary Supplements (ANSI), United State Pharmacopeia (USP) and American Herbal Products Association (AHPA) [15]. The standards set by the above organizations ranged between 10⁶ cfu/g and 10⁵ cfu/g in raw materials, powdered extracts or powdered ingredients of botanical origin intended for oral administration.

Bacterial characterization and identification revealed that most of the isolates were members of the family enterobacteriaceae. Many studies have revealed the presence of enterobacteriaceae as members of phylloplane communities [6], [16], [17], [18]. Proteus mirabilis was shown to be more abundant (35%, n = 20), followed by P. vulgaris (15%) and Escherichia coli (15%). Salmonella typhi had 10% occurrence while Klebsiella pneumoniae, Morganella morganii, Vibrio cholerae, Enterobacter sp and Staphylococcus aureus had 5% occurrence each. Figure 2 provides a diagram showing percentage occurrence of the isolates. The presence of enterobacteriaceae in general and some of these isolates in particular on plants’ phylloplane have been stated severally by some investigators, thus supporting our findings. For instance, the occurrence of K. pneumoniae [17], Enterobacter sp [4], [6], E. coli [16] and Staphylococcus sp [4] have been reported. All of the isolates could be pathogenic due to the fact that the pathogenicity of strains of these species is well-known documented. Of particular interest, demonstration by [16] that some of phylloplane bacteria contain different antibiotic resistance genes could increase the health risks associated with these bacteria.
In addition to the bacterial load, the identity of the bacteria strongly suggests the risk associated with consuming such preparation as medicaments. Some international organizations have strict limits for the presence of potential pathogens in herbal medicines. The Association of South East Asian Nations (ASEAN) for example, have limited the number of E. coli to 10^3 cfu in 1g or 1ml and total absence of Salmonella in 25g or 25ml preparations for oral administration [19]. Similarly, WHO have completely ruled out the presence of the two organisms in some herbal preparations [20].

4. Conclusion

In the present study, large number of bacteria were observed to be occupying the phylloplanes of C. fistula, M. indica and P. guajavum. All of the bacterial species present were members of the family enterobacteriaceae, with the exception of Staphylococcus aureus - the only Gram positive species. The number of the organisms and their identities clearly demonstrated their health associated risks due to their pathogenic potentials. Washing with warm water reduced the bacterial load insignificant associated risks due to their pathogenic potentials. Therefore, a better method for eliminating potential pathogens (e.g. using boiling water) is required for proper medication and to avoid further infection leading to complication. Hence the need for enlightenment campaign especially in rural communities where sole dependence on plant-derived medicine is common.

References


