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Research paper

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 ($p \leq 0.05$). 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%), *Proteus vulgaris* (15%), *Escherichia coli* (15%), *Klebsiella pneumoniae* (5%), *Morganella morganii* (5%), *Salmonella typhi* (10%), *Enterobacter* sp. (5%), *Staphylococcus aureus* (5%) and *Vibrio cholarae* (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.*

1. Introduction

Medicinal plants have for long, been remedies against human diseases because they contain components with therapeutic value [1]. According to the world health organization, over 80% of the world′s population or 4.3 billion people, rely upon such traditional plant-based systems of medicine to provide them with primary health care [2]. The use of plants for treatment of various ailments is considered one of the important means of health care system. Two years back, there were many claims by scientists and herbalists that, use of some plant extracts could cure the deadly Ebola infection that caused epidemics in some West African countries including Nigeria.

However, the use of plants as medications could lead to health hazards instead of cure, especially in rural communities where exclusive dependence on these medications is not a mere speculation but reality. Plant leaves are consumed without proper treatment or washing that can help eliminate or reduce the microbial load. This type of treatment can rather cause more havoc to the consumer or might lead to emergence of new infection(s) [3].

With the current situation of health care systems in most rural communities, which involve consumption of different parts of plants materials; determining the occurrence of pathogenic bacteria is highly essential. This is due to the fact that bacteria are known to cause infections and their occurrence is ubiquitous. Many studies have reported the occurrence of microbes on the phylloplane of plants among which are members of enterobacteriaceae, Bacilli and Pseudomonads [4], [5] and could be potentially pathogenic. Investigating the possibility of having pathogenic bacteria on some of medicinal plants' phylloplane is crucial in order to prevent complications instead of recovery from infections. This will enable us create awareness among public on the better way of leave treatment before being used for medication to prevent the impending health risks.

2. Materials and methods

This study was carried out in the Microbiology laboratory, Department of Microbiology, University of Maiduguri, Borno State (coordinates: 11.83° N, 13.15° E), Nigeria; between July and September, 2014. Leaves samples were collected from three different types of plants in the University Garden. The plants include *Cassia fistula, Mangifera indica* and *Psidium guajavum*. Triplicate samples were aseptically collected from each of the plants at random and conveyed to the laboratory immediately.

Samples collected were divided into three (3) groups and analyzed as described by [4], [6] with modifications. In the first group (G1), the samples were vigorously shaken in order to remove dust and suspended particles. Three pieces of each of the leaves samples were aseptically homogenized into a single paste and serially diluted by measuring one gram (1g) and suspending it into 9ml sterile distilled water. After the dilution, 0.1ml aliquots of 10^4 dilutions were inoculated into Nutrient and MacConkey agar and incubated at 37^0C for 24 hours. For the second (G2) and third groups (G3), the samples were treated the same way except that, sterile and sterile warm water were used to wash the samples respectively before homogenization. The colonies that emerged were enumerated and expressed as colony forming units per gram. Isolation of pure colonies was achieved by repeated subculture on nutrient agar and characterized later according to the methods of

[7] and [8]. The isolates were identified by their colonial, morphological and biochemical characteristics as outlined by [9] and [10].

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 1.36×10^6 cfu/g, 1.28×10^6 cfu/g and 6.4×10^5 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 (8.4×10^5 cfu/g) was observed in the unwashed samples. However, there was drastic decline in the bacterial count in *P. guajavum* in which 3.40×10^6 cfu/g and 4.4×10^5 cfu/g were recorded as the highest and lowest values respectively (Fig. 1).

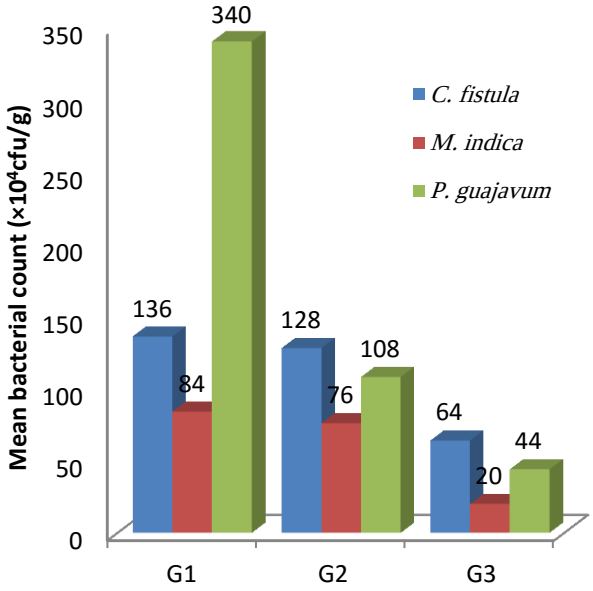

Fig. 1: Bacterial Counts.

Statistical analysis indicated a significant difference between treatments in a particular leaves sample ($p \leq 0.05$) but insignificant 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 10^4cfu/g and 10^5cfu/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 cholarae*, *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.

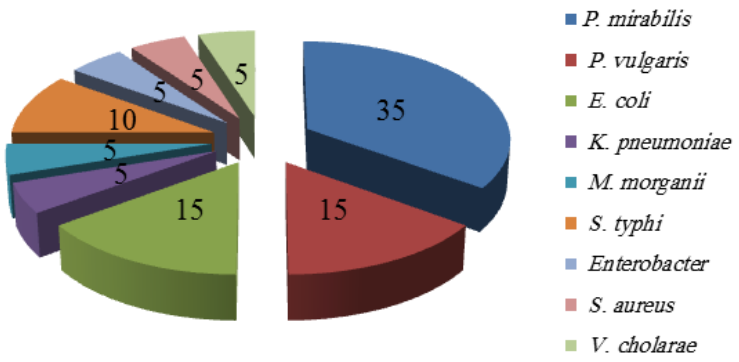

Fig. 2: Percentage Occurrence of Phylloplane Bacteria.

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 insignificantly and also above the limits stipulated by regulatory bodies. 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.

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