

**Research Paper**

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ISSN 0189-6016©2008**MICROBIAL LOAD AND ANTIMICROBIAL PROPERTY OF TWO NIGERIAN HERBAL REMEDIES****Oyetayo, V. O.**

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Abstract

The use of herbal remedies had increased significantly in the last one decade in Nigeria. This has led to the production of herbal products with bogus claims. The microbial quality and antibacterial properties of two Nigerian herbal remedies with such claimed efficacy of curing all manners of microbial diseases were assessed. The herbal remedies were discovered to be contaminated with the following microorganisms: *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus cereus*, *Basidiobotrytis* sp, *Oedocephalum* sp, *Varicosporium* sp and *Articulospora inflata*. Antibacterial analysis using the killing rate revealed that the herbal remedies had bacteriostatic and not bacteriocidal effect. Qualitative phytochemical screening of the herbal remedies revealed the presence of saponin, tannins, alkaloids, anthraquinone and cardiac glycosides which suggest possible antimicrobial effect. However, the presence of microbial contaminants in the herbal remedies suggests that they may serve as source of infection to end users.

Key words: Microbial, load, antibacterial, herbal, remedies

Introduction

The use of herbal medicine has always been part of human culture, as some plants possess important therapeutic properties, which can be used to cure human and other animal diseases. Rios and Receo (2005) reported that the idea that certain plants had healing potentials, indeed, that they contain what would be characterized as antimicrobial principles was well accepted long before mankind discovered the existence of microbes. The healing property of these medicinal plants is usually linked with the presence of secondary metabolites and these differ from one plant to another. It has been reported that a substantial percentage (38%) of prescription contained one or more of the natural products of plant origin as the therapeutic agent (Farnsworth and Bringel, 1977).

The use of plant, plant extract or chemical derived from plants to treat disease is therapeutic modalities which has stood the test of time (Anwannil and Atta, 2005). Suck (1989) had earlier reported that more than 75 pure compounds derived from higher plants are used in herbal medicine but most of those applied in modern medicine are now produced synthetically. In recent studies, extract of various parts of medicinal plants were found to have broad spectrum antimicrobial activities against pathogenic organisms (Sudhakar *et al.*, 2006; Khan *et al.*, 2006; Oyetayo and Oyetayo, 2006).

Herbal medicine is more accessible to most of the population. About 60 to 85% of the populations of every country of the developing world rely on herbal or indigenous forms of medicine. The reasons for the high patronage of herbal medicine are the high cost of very effective antibiotics and the problem of antibiotic resistance which is very common in developing countries (Hack, 2005; Okeke *et al.*, 1999).

A record of medicinal plants in earliest period in Nigeria is virtually not available because there was no documentation for their isolation, selection and preparation. Every fact about potent herbal plant was passed by

word of mouth from generation to generation (Kochhar, 1981). In the last two decades, there has been an upsurge in the circulation of herbal products in Nigeria. Most of the product developers often have bogus claims that their products can cure all forms of ailments. Two of such products are herbal products A and B. According to the producer, herbal product A is a complete natural antimicrobial remedy with claimed efficacy of 99.9% on bacterial, viral and fungal infections, while herbal product B is antibacterial remedy against *Salmonella typhi*. Some of the major shortcomings of herbal medicine are lack of scientific prove, imprecise diagnosis, imprecise dosage and unhygienic condition under which the herbal products are produced. The present study was therefore meant to examine the microbial quality and the antibacterial properties of these two herbal products produced in Nigeria.

Materials and Methods

Source of herbal products

Packets of herbal product A which is made up of capsules and bottles of herbal product B which is liquid were bought from one of the outlets of the herbal producer at Oba Adesida Road, Akure, Nigeria. The herbal products were randomly selected for analysis.

Source of indicator bacteria

The bacteria used as indicator organisms were clinical isolates obtained from the stock culture of Department of Microbiology, University College Hospital, Ibadan, Nigeria. The pure isolates were inoculated on double strength agar slant and stored at 4°C. The organisms are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus cereus* and *Proteus vulgaris*.

Microbial analysis of herbal products

The pour plate method was used to cultivate 1 ml serially diluted portions of herbal products A and B on plate count agar (Oxoid) for the enumeration of bacteria and potato dextrose agar for the enumeration of fungi. The plates were incubated at 37°C for 24 hours for bacteria while fungi plates were incubated at 26±1°C for 72 hours, after which discreet colonies were subcultured to obtain pure culture. The pure cultures were tentatively identified based on microscopic, morphological and biochemical tests (Holt, 1981).

Antibacterial Activity of herbal medicines on indicator bacteria

Six sterile culture tubes containing pre-sterilized nutrient broth (5ml) were provided for each indicator bacteria respectively. These were labeled 0 hour to 5 hour where 0 hour serves as control. One gram (1g) of herbal medicine A and 1 ml of herbal drug B were added to the culture tubes containing the indicator bacteria. The suspension of the indicator bacteria and herbal medicine were thoroughly mixed and held at room temperature (28±2°C). Antibacterial activity was determined by plating 0.1ml of the suspension at every one hour interval up to the 5th hour. The plates were incubated and the colony forming units counted.

Phytochemical Screening of herbal products

Herbal medicine A and B were screened for the presence of the following phytochemicals: alkaloids, tannins, saponins, cardiac glycosides, anthraquinones and phlobatannins. The methods described by Herborn (1998) were used to ascertain the presence of alkaloids, cardiac glycosides and phlobatannins. The presence of saponins was detected using the method of Odebiyi and Sofowora (1978), while tannins and anthraquinones were screened for using the method of Trease and Evans (1985).

Results

A total of 7 microorganisms were isolated from the two herbal products. The distribution of the isolates in the herbal products is shown in Table 1. A total of 4 fungi (*Basidiobotrytis* sp, *Oedocephalum* sp, *varicosporium* sp and *Articulospora inflata*) and 3 bacteria (*Bacillus subtilis*, *Bacillus coagulans* and *Bacillus cereus*).

Qualitative phytochemical screening reveal the presence of saponins, tannins, alkaloids and cardiac glycosides in herbal product A, and tannins, anthraquinones, alkaloids and cardiac glycosides in herbal medicine B (Table 2).

Antimicrobial analysis shows that the herbal products possess bacteriostatic effect on the indicator bacteria. The herbal remedies did not show any sign of inhibition of the indicator bacteria when the agar diffusion method was used. However, using the rate of killing, the herbal preparation show bacteriostatic effect on the indicator bacteria as growth rate decreased from the first hour to the fifth hour (Figures 1 and 2), except for *E. coli* and *Salmonella typhi* suspended in herbal product A, and *Salmonella typhi* and *Bacillus cereus* suspended in B, whose growth increased from the 4th hour. *Staphylococcus aureus* was found to be more susceptible to the antibacterial actions of herbal preparation A and B.

Table 1: Microorganisms isolated from two Nigerian herbal preparations

Microbial isolates	Herbal preparation A	Herbal preparation B
<i>Basidiotrytis</i> sp	+	-
<i>Oedocephalum</i> sp	+	-
<i>Varicosporium</i> sp	+	-
<i>Articulospora inflata</i>	-	+
<i>Bacillus cereus</i>	+	+
<i>Bacillus subtilis</i>	+	-
<i>Bacillus coagulans</i>	-	+

+: present, -: absent

Table 2: Qualitative analysis of phytochemical constituents of two Nigerian Herbal preparations

Phytochemical Constituents	Herbal preparation A	Herbal preparation B
Saponins	+	-
Tannins	+	+
Phlobatinnins	-	-
Anthraquinones	-	+
Alkaloids	+	+
Cardiac glycosides		
i. Keller Killiani's test	+	+
ii. Salkowski's test	+	+
iii. Liberman's test	-	-

+: present, -: absent

Discussion

Herbal plants have been used as source of medicine in virtually all cultures (Baquar, 1995). In the last two decades, the numbers of herbal medical practitioners in Nigeria had increased and this has made the National Agency for Food and Drug Administration and Control (NAFDAC) to check their activities. Most of these alternative medical practitioners always claim that their herbal preparations can cure all manners of ailments. This is definitely worrisome. This study was designed to verify the claims by herbal medical practitioners that herbal their preparations can cure all manners of diseases. Moreover, the study will also verify the microbial quality of the herbal preparations.

One of the major shortcomings of herbal preparations in the developing countries is the unhygienic conditions under which they are produced. In the present study, it was observed that herbal preparation A and B are not sterile (Table 1). Bacteria that are of health importance such as *Bacillus* species were isolated from the herbal products. The presence of *Bacillus* species may be as a result of inadequate heat processing, improper handling of products and contaminated processing equipment (Frazier and Westhoff, 2003).

The bogus claim that herbal preparation A is a complete antimicrobial remedy with 99.9% efficacy may not also be true. The results shows that herbal preparation has bacteriostatic and not bacteriocidal effect (Figure 1). More so, a higher concentration of the product (1g/ml) was used before a slight antibacterial effect was observed. The same

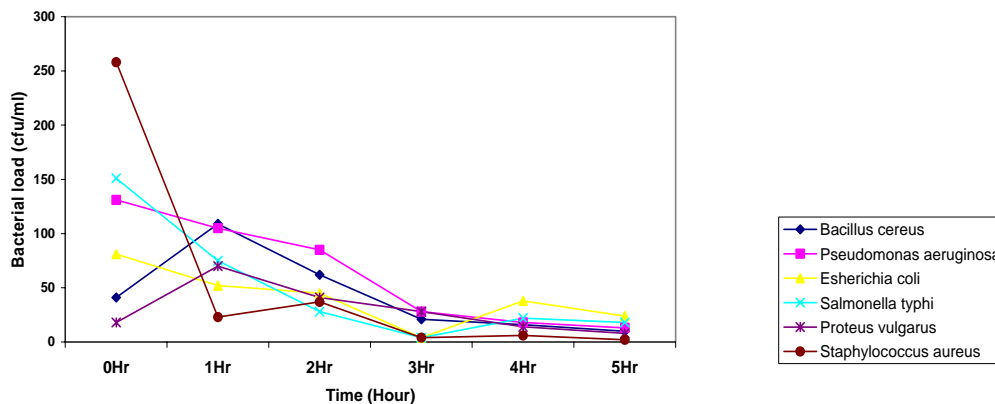


Fig. 1: Antibacterial Activity of herbal drug A on indicator bacteria

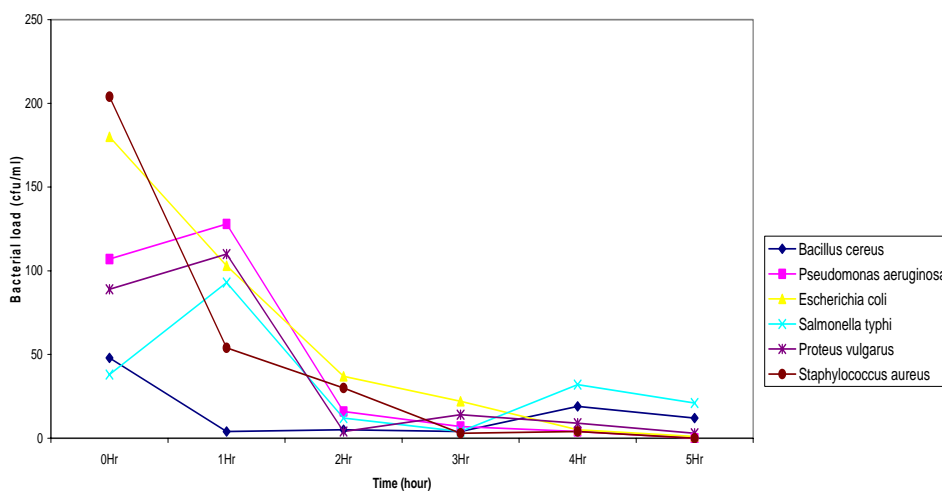


Fig. 2: Antibacterial Activity of herbal drug B on indicator bacteria

observation was noticed for herbal preparation B (Figure 2), which the producers claimed to be very effective against *Salmonella typhi*.

The herbal preparations were found to contain phytochemicals such as: saponin, tannins, alkaloids, anthraquinone and cardiac glycosides. These phytochemicals are known to have antimicrobial effects (Ming, 1999, Oyetayo and Oyetayo, 2006). Most antibacterial substances have the following modes of action: inhibition of cell wall synthesis, disruption of cell membrane function, inhibition of protein synthesis, inhibition of nucleic acid synthesis and also by acting as antimetabolites (Madigan et al., 2000; Russell, 2002).

In conclusion, this study revealed that herbal preparation A and B are not sterile and may serve as source of infection to the end users. Though, the herbal preparations contained some important phytochemicals (Table 2), these phytochemical components may not be at an effective concentration to make the products efficacious in antagonizing the pathogenic activities of invading microorganisms. Herbal medicine practitioners should be educated on the need to get appropriate concentration of the bioactive components in plants instead of using the whole infusion. Moreover, the regulatory agency, NAFDAC, is advised to carry out more detailed and regular analysis of these herbal preparations to prevent the unsuspecting and uninformed end users from buying what may aggravate their ailments.

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