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A Microbiological View of Raw Drugs Safety

Hamsaveni Gopal R, Ramkumar V,
Radha Krishnareddy and, Saraswathy A.

Introduction

Traditional healing through herbs has been the practice of many countries since ages, as they were generally believed to be non-toxic natural products. According to World health organization, the use of herbal drugs exceeds 2-3 times that of allopathy. Despite its continuous usage over many centuries, the herbal remedies face a very low profile. Why?

The probable reason may be due to the lack of data available on the quality and quantity of the efficacy and safety of the herbal drugs.

The quality of a herbal drug depends on the following parameters :

1.Botanical-authenticity, proper seasonal collection and storage conditions.

2.Chemical-presence of organic and inorganic chemicals, water soluble and solvent soluble extractives, volatile matter, ash value etc,.

3.Microbiological- total bacterial and fungal content and absence of pathogenic orgamsms.

What is microbiological contamination? Generally plant's outer surface is loaded with bacteria and fungi whereas the inner tissues were found to be free or contain very few organisms. For example the outer layer of cabbage was found to contain 1-2 million bacteria whereas the inner layer was found to contain only few 100s-1000s [1].

What are the sources of contamination? The natural sources of contamination are from air, water and soil.

CSMDRIA, Arumbakkam, Chennai -600106.

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Air carries dust laden with microbes. Checking of air samples revealed that sample on a windy and dusty day showed a huge load of microbes of more than 5 million organisms whereas sample collected after a shower had very few organisms as the dust settles in soil due to rain. The dust particles in the air are one of the sources of contamination.

Water is inevitable for the growth of the plant and dirty water can contribute its share to the contamination as evidenced in a finding that plants growing near untreated sewage water were found to contain lot of gastro intestinal pathogens.

Soil is a very rich source of both saprophytic and pathogenic organisms. So roots get contaminated from the soil and the feacal matter of human and animals of soil are an addition.

Other than these natural sources contamination is possible from defective storage conditions and due to personal handling.

Most of the indigenous practitioners use their own formulations prepared in a small scale. So if the handling person is unhygienic, he can contribute his share to the contamination. A survey in food industry has revealed that each individual sheds 10^3 - 10^5 organisms per minute. [2].

Now, why importance should be given to the contamination?

Contaminating bacteria may be either pathogenic or non pathogenic. Even when the organism is a saprophyte, it can spoil a preparation by converting the active principle of the drug to another useless compound, reducing the therapeutic value of the drug. When the organism involved is pathogenic, it is all the more critical in public health point of View.

Safety of the herbal drug

The pathogenesis of a herbal drug depends on the toxins present - either of plant tissues or of contaminating microbes .The microbial toxins include toxins of bacteria and fungi.

Bacterial toxins

The bacterial toxins are metabolites of bacteria which may be either filterable [exotoxin] or non filterable, [endotoxin]. Generally they may be categorized as

1.Enterotoxin -*Staphylococcus aureus* produces an enterotoxin, which causes symptoms ranging from nausea, vomiting headache, abdominal cramps, fever, diarrhea with blood and mucus to death.[3]

2.Neurotoxin- *Clostridium botulinum* produces a powerful neurotoxin which can

paralyze all the involuntary muscles of the body and whose lethal dose in mice was found to be 0.33 X 10^{-7} mg. per Kg. body weight.[4]

3.Toxins interfering with carbohydrate metabolism- Man depends on carbohydrate for his energy and toxins interfering with that may hamper his health. Examples of such toxins are
Cytotoxin of *Salmonella* sp.,
Diarrhogenic toxin of *Bacillus cereus*
Entero toxin of *Escheria coli* and
Toxins of *Shigella dysentery* and
Vibrio parahemolyticus.

Mycotoxins

Mycotoxins are fungal metabolites which are found to be toxic to man and animals. The predominant genera producing toxins are:-
Penicillium and Aspergillus. A mass death of l-lakh turkey poults in 1960 and 14,000 ducklings in 1970 were reported in U.S.A and the common cause in both these episodes were found to be a peanut meal contaminated with *A. flavus.*

A. flavus yielded aflatoxin G and aflatoxin B, that are highly oxygenated heterocyclic compounds and named G and B, due to the fluorescence- green and blue on exposure to long wavelength ultraviolet light. The recent concern of aflatoxins is due to their carcinogenic properties in man and animals. [5] Other toxins include

Patulin from *P. expansum, P. urticae, A. clavatus.*
Ochrotoxin from *A. ostianus. P. commune* etc.
Luleoskyrin from *P. islandicum*
Sterigmatocystin from *A. nidulans, A. versicolor, P. thomii* etc
Penicillic acid from *P.puberulum, A. sulpheureus* etc.
Alimentary toxic aleukeia [ATA] from *Mucor. Fusarium* etc
This is a brief prelude on the importance of microbiological testing in safety point of view.

Aim of the experiment

The department of ISM under ministry of health and family welfare has awarded CSMDRIA with a project of standardization allotting 13 plant drugs in 1997.The plan design was to procure at least 3-5 samples of each plant from various regions of the country and carry out standardization work as per methods suggested.

Total 38 samples of 11 plant species were carried out during that year and this paper deals with the microbiological work carried out on those plants. WHO has suggested various methods for testing of

which pour plate technique was adopted in this institute.

Methodology

1 gm.of the powdered drug was ground in a sterile mortar and pestle with 10-ml.of lactose broth and homogenized with polysorbate 20R or polysorbate 80R.

This solution was made up to 100 ml.and treated as stock solution.

From the stock, different dilutions were made from 10^{-1}. to 10^{-7}.

The first and last two dilutions were unchecked, as first 2 diln. may contain too many organisms beyond counting and the last 2 may contain too less a number and there is a possibility of missing the important pathogens.

The dilutions chosen were 10^{-3} - 10^{-5} Pour plate method was adopted [6] using nutrient agar for counting bacteria, sabourad agar for fungi, macconkey agar for enterobacteriaceae and brilliant green agar for ***Salmonella.***

Total count was made for each dilution, calculated per gram and average taken for tabulation.

Allowable limits

WHO has suggested tentative allowable limits for raw drugs that are being used in formulations of internal and external use.

They are:-

Allowable limits of drugs meant for external use

5.0×10^7 bacteria /gm.
5.0×10^3 molds and yeasts/gm
5.0×10^4 enerobacteriaceae/gm and *No Salmonella.*

Allowable limits of drugs meant for internal use

5.0×10^5 bacteria /gm.
5.0×10^3 molds and yeasts/gm
5.0×10^3 entrobacteriaceae/gm and *No Salmonella.*

An assessment of the microbial quality status of the tested drugs was made considering the WHO limits as standards. Table below shows the results obtained.

TOTAL VIABLE COUNT OF BACTERIA AND FUNGI

S.No.	Name of the plant	Total no. Checked	External use			Internal use		
			A	B	C	A	B	C
1.	Alteranthera triandra Lam.	5	5	3	3	2	3	2
2.	Aristolachia bracteolata Lam	4	4	3	3	2	3	2
3.	Calamus thwaitseii Becc	2	2	2	2	0	2	0
4.	Coldenia procumbens L.	5	4	2	1	0	2	0
5.	Coleus amboinicus Lour	3	3	2	2	0	2	0
6.	Cretava magna Lour.	5	5	2	2	0	2	0
7.	Dryopteris filix Mas	2	2	0	0	0	0	0
8.	Enicostemma littorale Blume.	5	5	1	1	1	1	1
9.	Euphorbia nivulia Buch Ham.	1	1	0	0	0	0	0
10.	Garcinia pedunculata Roxb.	4	4	2	2	2	2	1
11.	Pavonia odorata Willd.	2	2	1	1	0	1	0
	Total	**38**	**37**	**18**	**17**	**7**	**18**	**6**
	%		**97**	**47**	**45**	**18**	**47**	**16**

A-number of samples within limits of bacteria
B-number of samples within limits of fungi
C-number of samples within both limits of bacteria and fungi

Results and discussion

Table 1 shows that out of 38 samples tested 37 were within limits of bacterial count [97%] and 18 within limits of fungal count [47%]. But a drug should pass in both limits if they are to be used in formulations for external use. In that case only 17 out of 38 were fit enough for use, which amounts to 45%.

If the same drugs were to be used for formulations of internal use,

Only 7 drugs out of 38 were within limits of bacteria [18%] and 18 within limits of fungal count [47%]. Drugs well within limits of both bacteria and fungi were found to be only 6, which amounts to 16%. Fitness of 45% for external use and 16% for internal use is definitely not a good microbial status for herbal drugs in the international standards of safety.

Suggestions

The microbial status should be improved to get better drugs in safety point of view. There are many methods available like Sterilization with moist heat [autoclaving], dry heat [hot air oven] Ultraviolet irradiation, use of ethylene oxide, antimicrobial spray etc,. So trials should be conducted to evolve methods, which can cause chemical damage to a minimum extent and microbial damage to a maximum extent in a drug. Example of such method is pasteurization of milk in which application of heat at 72 degrees for 15 min.kills most of the bacteria and totally destroys important pathogens like tubercle bacilli, at the same time keeps the flavour, texture, fat content and taste intact. Similar methods should be evolved for herbal preparations that can improve the quality and safety of the drugs, which in turn can boost the image of Indian herbal drugs in the international market.

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