Comparative evaluation of microbiological quality of Hepatoprotective Herbal formulations marketed in Yavatmal District of India

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Abstract

In the present study herbal products marketed in Yavatmal India were determined for the presence of microbial. Microbial contents in herbal products were examined as suggested in as per W.H.O. The total of ten herbal products of various brands were selected randomly and tested for microbial contamination. Of which 3 samples did not conform to the W.H.O guidelines. The formulations are used daily by the patients suffering from Liver diseases. The specific media were used to determining the presence of Escherichia coli (4 samples), Staphylococcus aureus (3 samples), and P. aeruginosa (4 samples). The data indicated suggest that there is requirement of in process improvement to provide better quality for consumer health in order to be competitive in international markets.

Keywords: Microbial contamination, Hepatoprotective Herbal formulations, Specific Media

1. Introduction

Herbal medicines are plant derived materials and preparations with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants, inorganic materials or animal origin. Herbal medicine preparations are developed and created drugs by the modern pharmaceutical industry. Nowadays, they are manufactured and sold most widely on the pharmaceutical market for curing diseases and promoting public health worldwide.1

Herbal drugs have been used since ancient times as remedies and treatment for a range of diseases. Western pharmaceutical drugs play a major role in modern medicine, but traditional medicine are used by approximately 60% of people in rural areas still make an important contribution in health care.2

In India, the unscientific methods of collection, storage, transportation and congenial climatic conditions make the raw materials of herbal drugs prone to fungal infestations. The raw materials are collected using unscientific methods and are commonly exposed to many microbial contaminants. The raw materials are often deteriorated by microorganisms before harvesting, and during handling and storage.3

The liver performs the normal metabolic homeostasis of the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including pharmaceutical and environmental chemicals. Drug-induced hepatotoxicity is a major cause of iatrogenic diseases, accounting for one in 600 to one in 3500 of all hospital admissions.4

The microbial quality of pharmaceuticals is influenced by the environment and quality of the raw materials used during formulation. Some infectious outbreaks have been associated with the use of highly contaminated raw materials of natural origin.

Table No.1 W.H.O. Limits for microbial contamination5

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Finished product Cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>10³/gm</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10³/gm</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>10¹⁴/gm</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>Nil</td>
</tr>
</tbody>
</table>

1.1 Sources of contaminations in herbal products.

The practices of most ethnic herbal medicine include the use crude or raw herbs that are collected from the wild or from cultivated fields and their prepared or ready-made products.

Toxic contaminants may come from:

- Environments and conditions that the medicinal plants are grown or collected
- The conditions under which they are dried and processed
- The storage conditions and conditions during transport
- Unhygienic use of medicines by patients
- The manufacturing processes when the ready-made medicinal products are produced.6

2.0 Marketed Formulations Selected for Study

Hepatoprotective formulations ie churna of 10 different marketed brands were selected for study. It consist of Cardaus marianus, Chelidonium majus, Taraxacum officinale, hionanthus virginica, Quassia amara, Heparbovinum, Ceanothus americanus, Colocynthis, Leptandra virginica, Natrium sulphuricum, Nux vomica, Phosphorus,Teucrium marum

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3. Experimental Work

3.1 Sample collection

The ten herbal formulations of Hepatoprotective formulations marketed by various herbal manufacturers were collected from the retail medical stores of Yavatmal (Vidarbha region, India). The formulation code were given to selected formulation as HP1 to HP10.

3.2 Materials & methods

Serial dilutions were made and viability assessed using the pour plate method. The plates were incubated at 37°C for 24h. The plate was placed on a colony counter and the number of colony forming units was taken. The microbial content was taken as the mean of duplicate determinations. The media utilized were Nutrient agar, Cetrimide Nutrient agar, Salt Nutrient agar, MacConkey agar.

3.3 Pathogen determination

3.3.1 Determination of S. aureus

10 mg of the sample was added into Tryptic soya broth and incubated at 37°C for 24 hours. The sample was then streaked on Vogel-Johnson agar and incubated at 37°C for 24 hours. A single colony on each plate was then restreaked on Mannitol salt agar and incubated at 37°C for 24 hours. After the incubation, the colonial morphology was observed.

3.3.2 Determination of Escherichia coli

Suspend 10 gm of the specimen in lactose broth or any other broth, which has no antibacterial effect to make 100ml (may adjust PH at 7). It is called pretreatment material Incubate 100ml of pretreatment material at 30-37°C for 2-5 hrs. Transfer amount of above homogenized pretreatment material containing 1gm or 1ml of the material being examined to 100ml of MacConkey broth and incubate at 43-45c for 18-24hrs Growth of red generally non-mucoid colonies of Gram-negative rods, sometimes surrounded by a reddish zone of precipitation, indicates the possible presence of E.coli.

The results are expressed in Table No.2 and Figure No.1.

3.3.3 Determination of P. aeruginosa

The diluted sample was streaked onto Cetrimide agar plate. After the incubation at 37°C for 24 hours, the green colonies were tested for oxidase reaction and subcultured into Triple sugar iron medium. Growth of bacteria and the reaction results were observed.

The results are expressed in Table No.2 and Figure No.3.

4.0 Results

Table No.2 Comparative determination of microbial contamination in Hepatoprotective formulations

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Formulation code</th>
<th>Pseudomonas aeruginosa (10^7 cfu/gm)</th>
<th>Escherichia coli (10^7cfu/gm)</th>
<th>Staphylococcus aureus (10^8cfu/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HP1</td>
<td>5 x 10^2</td>
<td>-</td>
<td>8 x 10^5</td>
</tr>
<tr>
<td>2</td>
<td>HP2</td>
<td>7 x 10^2</td>
<td>-</td>
<td>7 x 10^5</td>
</tr>
<tr>
<td>3</td>
<td>HP3</td>
<td>6 x 10^2</td>
<td>-</td>
<td>5 x 10^5</td>
</tr>
<tr>
<td>4</td>
<td>HP4</td>
<td>3 x 10^2</td>
<td>-</td>
<td>6 x 10^5</td>
</tr>
<tr>
<td>5</td>
<td>HP5</td>
<td>12 x 10^2</td>
<td>2 X 10</td>
<td>13 x 10^2</td>
</tr>
<tr>
<td>6</td>
<td>HP6</td>
<td>6 x 10^2</td>
<td>-</td>
<td>15 x 10^5</td>
</tr>
<tr>
<td>7</td>
<td>HP7</td>
<td>13 x 10^2</td>
<td>4 X 10</td>
<td>9 x 10^4</td>
</tr>
<tr>
<td>8</td>
<td>HP8</td>
<td>14 x 10^2</td>
<td>1 x 10</td>
<td>7 x 10^4</td>
</tr>
<tr>
<td>9</td>
<td>HP9</td>
<td>15 x 10^2</td>
<td>3 x 10</td>
<td>14 x 10^3</td>
</tr>
<tr>
<td>10</td>
<td>HP10</td>
<td>6 x 10^2</td>
<td>-</td>
<td>6 x 10^4</td>
</tr>
</tbody>
</table>

Figure No.1: Pseudomonas aeruginosa content in Hepatoprotective formulations
5.0 Discussion

The present study reports microbial contaminations in herbal products widely distributed over the country. It was found that the formulations code HP5, HP7, HP8 and HP9 were contaminated by Pseudomonas aeruginosa and HP5, HP7, HP8 and HP9 were contaminated by Escherichia coli whereas the formulations having code no. HP5, HP6 and HP9 were contaminated by Staphylococcus aureus more than the limit prescribed by WHO if such product was consumed by patient there was possibility of infection. Medicinal plants have been generally used for decades. Consumers can easily acquire pathogenic microorganisms by consuming contaminated products. The results from this study suggest that the production of herbal products is still in critical situation in terms of quality and safety. Very low product quality can be derived from many factors such as cultivation, harvest, manufacturing procedure, transportation, and storage. The good handling must be carried out starting from raw materials to finished products.

Acknowledgements

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References

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