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Comparative evaluation microbiological qualities of Ashwagandha formulations marketed in Yavatmal district of India

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Abstract

Ashwagandha formulations are used daily by the patients suffering from general disability and to promote vitality. In the present research work Ashwagandha formulation marketed in Yavatmal India were determined for the presence of microbial content. The specific Medias were used for determining the presence of Escherichia coli, Staphylococcus aureus, and P. aeruginosa. The W.H.O limits for microbial content is followed while determination. The total ten Ashwagandha formulation of various brands were selected randomly and tested for presence of microbial contamination as per W.H.O. The data indicated suggest that there was presence of Escherichia coli (3 samples), Staphylococcus aureus (4 samples), and P. aeruginosa (4 samples) were contaminated. It can be considered that the GMP was not followed properly while manufacturing and storage condition may not be maintained during transit of formulations.

Keywords: Microbial contamination; Ashwagandha formulation; Specific medias

Introduction

Herbal medicines are nothing but the formulations used for treatment of different diseases. They are sold as tablets, capsules, powders, teas, extracts, and fresh or dried plants. People use herbal medicines to try to maintain or improve their health. These medicines are either manufactured by maintaining ratio of herbs given in Herbal Pharmacopeia and created by the modern pharmaceutical industry. Nowadays, they are manufactured and sold most widely in 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 careIn 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 and fungus entry in raw materials. There is a possibility of loss of efficacy of raw materials by microorganisms before harvesting, and during handling and storage [3].

The microbial quality of pharmaceuticals is influenced by the environment and quality of the raw materials used which may be affected because of use of heavily contaminated raw materials of natural origin.

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 the processes like storage, transit, growth condition, unhygienic use of medicines by patients. The manufacturing processes when the ready-made medicinal products are produced [4].

Marked Formulation selected for Study

Withania somnifera Linn., also known as Ashwagandha, Indian ginseng, The plant is said to have a potential property of pacifying 'Vata'in herbal drugs compared therapeutic value of its roots with Panax ginseng. The main constituents of Ashwagandha are alkaloids and, withanine is the main constituent. The other alkaloids are withananine somniferinine, somnine, pseudo-withanine, tropine, somniferine pseudotropine, cuscohygrine, anferine and anhydrine.Ashwagandha is reported to have anti-carcinogenic effects in animal and potentiating apoptotic signaling cancerous cell lines [5].

Experimental Work

Sample collection: The ten herbal formulation of Antidiabetic churna marketed by various herbal manufacturers were collected from the retail medical stores of Yavatmal (Vidarbha region, India). The formulation were given code i.e. H1 to H10

Materials and Methods

Serial dilutions were made and viability assessed using the pour plate method by incubating plate at 37°C for 24 hours.

The plate was placed on a colony counter and the numbers of colony forming units were taken. The specific media utilized were Nutrient agar, Cetrimide Nutrient agar, Salt Nutrient agar, MacConkey agar as per the growth of microbes in the media [6].

Pathogen determination

Determination of Staphylococcus 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 resteaked on Mannitol salt agar and incubated at 37°C for 24 hours. After the incubation, the colonial morphology was observed [7]. The results are expressed in Table No.2 and Figure No.1

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 43-45°C for 18-24hrs Prepare subculture on a plate with MacConkey agar and incubate at 43-45°C for 18-24hrs Growth of red generally non-mucoid colonies of Gram negative rods were surrounded by reddish zone of precipitation shows that there is possibility of presence of Escherichia coli [8].

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

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 sub cultured into Triple sugar iron medium allowed the microbe to grow and the growth of bacteria and the reaction results were observed [9]. The results are expressed in Table No.2 and Figure No.1

Discussion

The present study reports microbial contaminations in herbal products widely distributed over the country. It was found that the formulations code H2, H3, H4 and H10 were contaminated by Pseudomonas aeruginosa and H3, H4, and H10 were contaminated by Escherichia coli whereas the formulations having code no H2, H3, H5 and H6 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

Table 1: W.H.O. Limits for microbial contamination.

Microorganism	Finished product Cfu
Escherichia coli	10^1/gm
Staphylococus.aureus	10^5/gm
Pseudomonas aeruginosa	10^3 /gm
Salmonella species.	Nil

Table 2: Comparative detrmination of microbial contamination in Antidiabetes churna.

Sr.No	Formulation code	Pseudomonas aeroginosa (10^3 cfu /gm)	Escherichia coli (10cfu/gm)	Staphylococcus aureus (10^5cfu/gm)
1	H1	6×10^2	-	9×10^4
2	H2	12×10^2	-	13×10^4
3	H3	13×10^2	2 x 10	15×10^4
4	H4	16×10^2	2 X 10	3×10^4
5	H5	6×10^2		12×10^4
6	H6	6×10^2	-	15×10^4
7	H7	7×10^2		8×10^4
8	H8	5×10^2	1 x 10	7×10^4
9	H9	3×10^2		5×10^4
10	H10	11×10^2	3 x 10	3×10^4

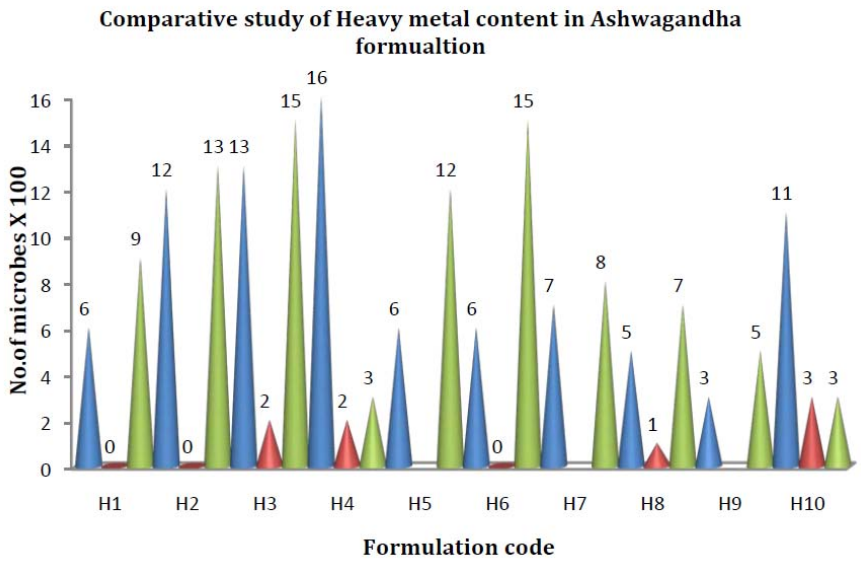

Figure 1: Comparative determination of Pseudomonas aeruginosa in Antidiabetic churna.

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.

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