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# COMPARATIVE EVALUATION OF MICROBIOLOGICAL QUALITIES OF FORMULATIONS CONTAINING ASHWAGANDHA MARKETED IN YAVATMAL DISTRICT OF INDIA

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**ABSTRACT**

*Withania somnifera Linn.* is used daily by the patients suffering from general disability and to promote vitality. In the present study microbial content of formulations containing *Withania somnifera Lin* (Ashwagandha) marketed in Yavatmal district in India were determined. The presence of *Escherichia coli*, *Staphylococcus aureus*, and *P. aeruginosa* were determined by using methods described as per WHO guidelines. The total ten formulations containing Ashwagandha marketed under various brands were selected randomly and tested for presence of microbial contamination. The results revealed that six samples were contaminated with *P. aeruginosa and Escherichia coli*. Whereas four samples were contaminated by *Staphylococcus aureus*. It can be considered that the Good Manufacturing Practice was not followed properly while manufacturing and storage condition may not be maintained during transit of formulations.

## 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 plant.[1] People use herbal medicines to try to maintain or improve their health.

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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. 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 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.

Table No.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 |

## 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]

## MARKETED FORMULATION SELECTED FOR STUDY

*Withania somnifera (*L.) 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. The main constituents of Ashwagandha are alkaloids withanine. The other alkaloids like withananine somniferinine, somnine, pseudo-withanine, tropine, somniferine pseudotropine, cuscohygrine, anferine and anhydrine are also present in the plant. Ashwagandha is reported to have anti-carcinogenic effects in animal and potentiating apoptotic signaling cancerous cell lines[5]

## EXPERIMENTAL WORK:

### SAMPLE COLLECTION

The ten marketed herbal formulations containing Ashwagandha were collected from the retail medical stores of Yavatmal (Vidarbha region, India). The formulation were given code i.e. H1 to H10

### MATERIALS & 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 *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

### DETERMINATION OF *ESCHERICHIA COLI*

Ten grams of the specimen in lactose broth, was suspended which has no antibacterial effect to make 100 ml (may adjust pH at 7). It is called pretreatment material. Incubated 100 ml of pretreatment material at 30-37°C for 2-5 hrs. The amount of above homogenized pretreatment material containing 1gm of

the material being examined was transferred to 100 ml of MacConkey broth and was incubated at 43-45°c for 18-24 hrs Prepare subculture on a plate with MacConkey agar and incubate at 43-45°c for 18-24 hrs 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.2

## DETERMINATION OF *STAPHYLOCOCCUS AUREUS*

Ten 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.3

## RESULTS

Table No.2 Comparative detrmination of microbial contamination in herbal formulations containing Ashwagandha.

| Formulation code | *Pseudomonas aeroginosa* ($10^3$ cfu /gm) | *Escherichia coli* (10cfu/gm) | *Staphylococcus aureus* ($10^5$cfu/gm) |
|---|---|---|---|
| H1 | $6 \times 10^2$ | - | $9 \times 10^4$ |
| H2 | $12 \times 10^2$ | - | $13 \times 10^4$ |
| H3 | $13 \times 10^2$ | 2 x 10 | $15 \times 10^4$ |
| H4 | $16 \times 10^2$ | 2 X 10 | $3 \times 10^4$ |
| H5 | $6 \times 10^2$ | | $12 \times 10^4$ |
| H6 | $6 \times 10^2$ | - | $15 \times 10^4$ |
| H7 | $7 \times 10^2$ | | $8 \times 10^4$ |
| H8 | $5 \times 10^2$ | 1 x 10 | $7 \times 10^4$ |
| H9 | $3 \times 10^2$ | | $5 \times 10^4$ |
| H10 | $11 \times 10^2$ | 3 x 10 | $3 \times 10^4$ |

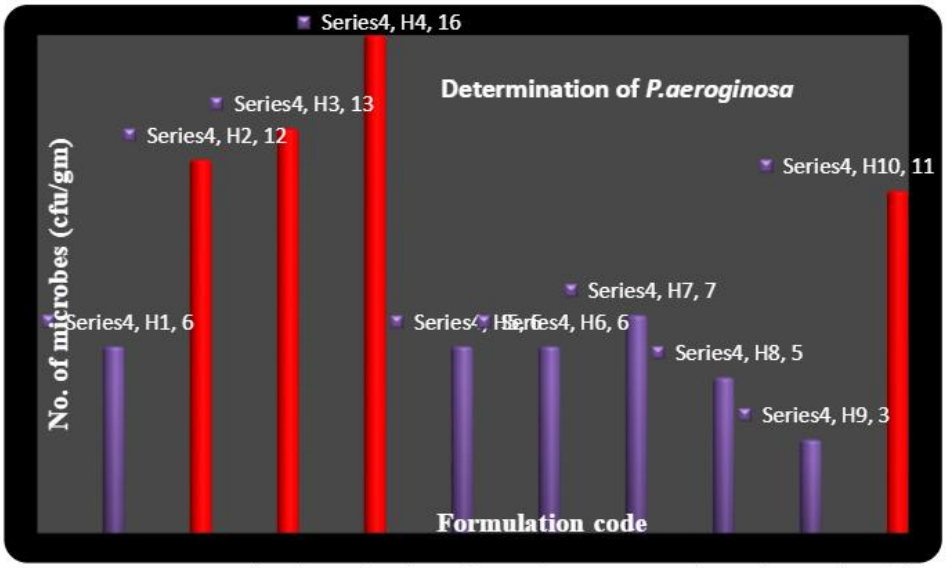


Figure No.1: Comparative determination of *Pseudomonas aeruginosa* in marketed formulations containing Ashwagandha.

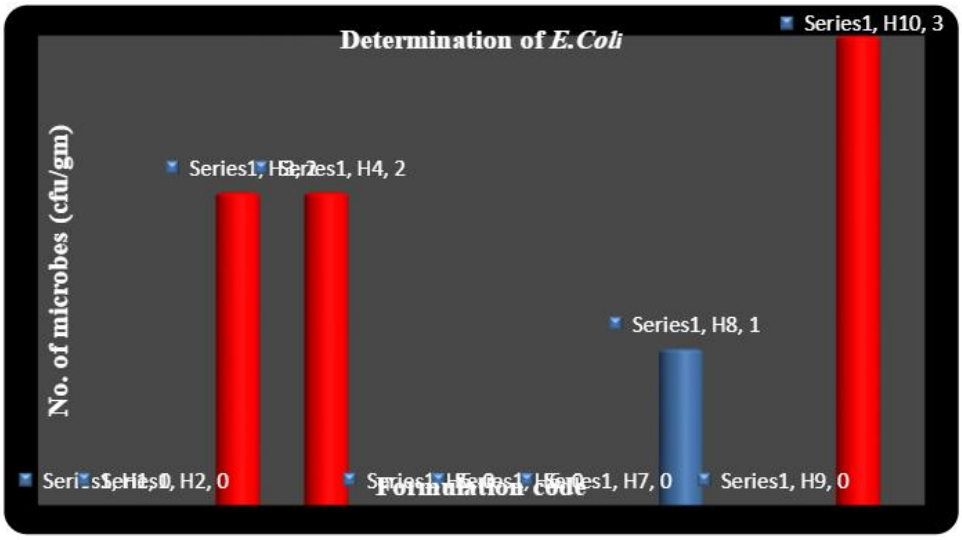


Figure No.2: Comparative determination of *Escherichia coli* in marketed formulations containing Ashwagandha.

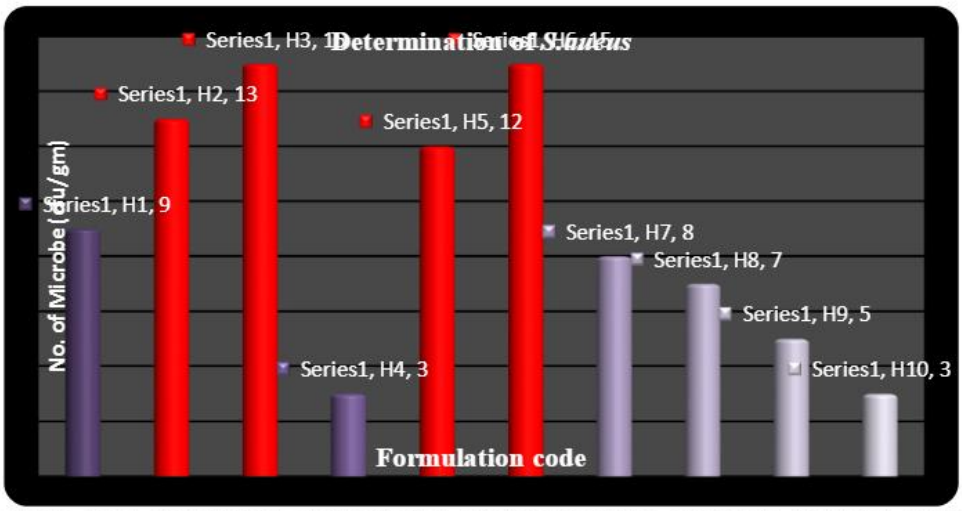


Figure No.3: Comparative determination of *Staphylococcus aureus* in marketed formulations containing Ashwagandha.

## 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 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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