

Quality Control of Polyherbal Drugs for the Detection of Microbial Agents

Mariyah Yacoob Bawa¹*, Zeba Parween Imran¹, Muhammad Daniya², Khan Usmanghani²

¹Department of Microbiology, Federal Urdu University, Karachi, Pakistan

²Department of Research and Development, Herbion Pharmaceutical (Pvt.) Limited, Karachi, Pakistan

Keywords: Herbal drugs, raw materials, bulk, extracts, microbial contamination, bacteria, molds, mycotoxins.

Author's Contribution

All the authors contributed significantly to the research that resulted in the submitted manuscript.

Article info.

Received: June 5, 2017

Accepted: July 12, 2017

Funding Source: Nil

Conflict of Interest: Nil

Cite this article: Bawa MY, Imran ZP, Daniya M, Usmanghani K. Quality Control of Polyherbal Drugs for the Detection of Microbial Agents. *RADS J. Pharm. Pharm. Sci.* 2017;5(3): 51-56.

***Address of Correspondence Author:**
mariyahyacoob@gmail.com

ABSTRACT

Objective: Herbal drugs have been utilized for the treatment of infectious diseases as medicines since ancient times. Plant and their extracts used for the manufacturing of different dosage form design. Medicinal plant is comprised of different parts that include root, leaf, stem, flowers, bark that have a high level of microorganisms, bacteria, yeast and especially fungal molds.

Methods: The antimicrobial analysis conducted on herbal raw materials, Bulk, Extracts, we found less CFU/gm. and percentages of Total Aerobic Count in bulk and extracts because these are heated at extreme temperatures.

Results: We have observed no fungal growth in bulk and extracts so it is devoid of any spore and toxin formation. In Raw materials, some molds were found that is similar with nonpathogenic strain that will not produces any toxin. It is understood, if the toxin producing mold growth is present in raw materials so the herbs, extract and excipient when combined together and converted into bulk products, by this technique all the toxins and dormant spores are killed at an extreme temperature i.e. 18°F to 20°F. The bulk is the semi-finished product and combination of herbal raw materials, extract, and excipient after testing it will go for final packaging and we get the finished product. The supplier/manufacturer of raw materials will reveal its purity that is accepted or rejected by quality control (Microbiology and chemical lab), Herbion Pakistan (Pvt.) Limited, Karachi, Pakistan.

Conclusion: It was concluded that Polyherbal Drugs has effective and safe to consume for eradication of diseases.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Plants are being used as material medica for the treatment of ward off malaise since ancient times. The substances that are extracted from medicinal plants can be utilized in variety of drugs as dosage form design as tablets, capsule, syrup etc. [1]. Plants drugs as compared to allopathic medicine used for medicinal purposes are one of the safest ways

for treatment of self-limiting and life-threatening human diseases especially to eliminate bacterial, fungal and yeast infections [2].

The aim of this research is to check the antimicrobial activity of the herb itself that will not hinder/interfere the growth of the different herbs. The growth of antimicrobial agents includes Total viable count and Total fungal count identified by Pour plate method and Membrane filtration method. The detection of

pathogenic species is also the aim of this research to find out the either any Gram positive, Gram negative bacteria, and Fungal, yeast species in herbs for e.g. *Staphylococcus aureus*, *Salmonella* spp, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, Bile tolerant Gram negative bacteria includes the family *Enterobacteriaceae* encompasses approximately 20 genera, including *E. coli* and all members of the coliform group; in addition it includes foodborne pathogens *Salmonella*, *Shigella*, and *Yersinia*. Different plants used as a medicinal source available after transformation of herbs convert into raw materials and these combined together to get an appropriate polyherbal medicinal product. These medicinal herbs are formulated in variety of products e.g. Ayurvedic, Chinese, Unani, Homeopathy, Siddha and modern herbal medicines. There are different forms of herbal drugs used as a therapeutic agent as parts of a plant example leaves, fruits, root, stem and wood. Herbal remedies consisting of different parts of plants, purified plants extract constituents, which often work together synergistically to combat ailments [3].

Herbal drugs normally can carry a number of bacteria and molds, often originating in the soil. Predomination of a vast range of bacteria and fungi are from naturally occurring microflora and aerobic spore formers [4, 5].

Research has been carried out on medicinal plants parts as products the microbial contamination of plant drug. The total aerobic microbial count, the total fungal count, together with tests for the presence of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella* spp is the requirement of quality product. According to European Pharmacopoeia, all the above-mentioned pathogens should be absent in herbal preparations because these are responsible for severe medical problems [5]. The rate of microbial contamination is significantly higher in herbal remedies as

compare to synthetic products, chemical drugs and pharmaceuticals in European Pharmacopoeia and care should be taken to detect the presence of fungal toxins i.e. mycotoxins, aflatoxins as these minutes quantity of mycotoxins on human subjects to produce lethal effects of drugs [6, 7].

Different herbal remedies may also be contaminated with dangerous microbial toxins, includes exotoxins, mycotoxins and endotoxins, toxins are very injurious to health [7-9].

Different plant used in herbal medicine include *Panax ginseng*, *Ginkgo biloba*, *Eurycoma longifolia*, *Epimedium* spp, *Withania somnifera*, *Tribulus terrestris*, *Tanacetum parthenium*, *Ulmus rubra*, *Mallotus philippensis*, *Foeniculum vulgare*, *Fallopia japonica*, *Vaccinium macrocarpon*, *Boswellia serrata*, *Curcuma longifolia*, *Zingiber officinale*, *Smilax chinensis*, *Pansinystalia johimbe*, *Lavendula officinalis*, *Matricaria chamomilla*, *Moringa oleifera*, *Aloe vera*, *Helix hedra*, *Glycyrrhiza glabra*, *Thymus vulgaris*, *Filipendula ulmaria* etc. All of the herbs along with their important plants parts have investigated for the identification of antimicrobial activities.

METHODOLOGY

Quality Control of Poly Herbal Drugs to Evaluate Microbes and Pathogens

Quality control and assurance is the method of standardization of the herbal medicines (Figure 1) to assure its qualitative, quantitative analysis and microbiological analysis to detect and ensure the presence of microorganisms in their standard range or not. The microbial assessment is required for and the detection of harmful pathogens safety, efficacy, and reproducibility in the herbal extracts samples. Herbal drug preparations with no apparent microbial agents adjusted by addition to a required content of an important constituent or of substances with known therapeutic activity and adding excipients, different herbal raw

materials and extracts of herbs. A part or product of any herb use normally in the product should be capable of meeting specified requirements for the quality control examination.

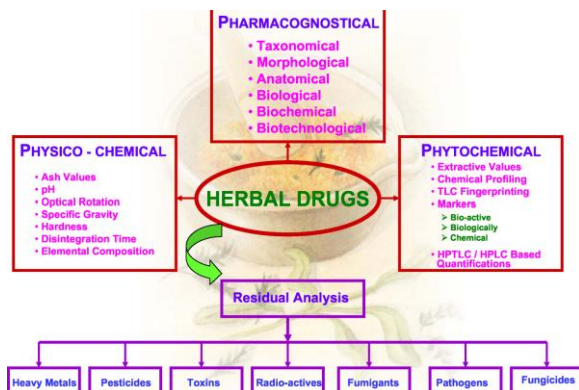


Figure 1. Herbal drugs – standardization.

Total aerobic count and total yeast and mold count by microbial limit test pour plate method. All herbal supplements were tested as follows: Wore gloves and mask before starting microbiological testing and handling of media. Disinfected the gloves, S.S testing table, media bottles with Isopropyl alcohol (IPA). Transferred 10ml / 10gm of the sample in 250ml glass bottle containing 90ml /100ml of sterilized Fluid Soybean Casein Digest Medium (TSB) or Buffered sodium chloride peptone water (pH 7.0) or Phosphate buffer (pH 7.2) or normal saline to make 1:10 dilution (i.e. master dilution). If product to be examined has antimicrobial activity, this so far as possible removed or neutralized (Soy lecithin 0.5% polysorbate 20 4.0%). Diluted further if necessary, the fluid so that it was expected to yield between 30 - 300 colonies. Pipette 1ml of last prepared dilution into each of for sterile Petri plates with the help of sterile pipette/ micropipettes. Promptly poured in to two Petri plates about 15 - 20ml of sterilized Tryptone Soy Agar (TSA) and Sabouraud Dextrose Agar Medium (SDA) previously had been melted and cooled to approximately 45 °C. Cover the Petri plates and mixed the sample with the media by

tilting or rotating the plates and allowed the contents to solidify at room temperature. Inverted the Petri plates, and incubated Tryptone Soy Agar (TSA) containing plates for 72 hours at 32.5 ± 2.5 °C and Sabouraud Dextrose Agar Medium (SDA) for 5 to 7 days at 20° to 25 °C. After incubation period, examined the plates for growth, counted the number of colonies i.e. average no of colonies \times dilution factor and expressed the results in terms of the colony-forming unit per ml of sample. If no microbial colonies are recovered from the plates representing the first 1:10 dilution of sample, dictated the results as less than 10 colony forming unit per ml of sample.

Membrane Filtration Technique

Used membrane filter having a nominal pore size not greater than 0.45 μm and 47 - 50mm of diameter whose effectiveness was not affected by component of sample to be investigated. Placed filter paper in filtration assembly with the help of sterilized forceps. Suspended 10ml of Liquid sample in the filtration assembly under LFH. After filtration of 10ml sample, washed the membrane filter by pouring 100ml of rinsing fluid at the membrane with pH 7.2 phosphate buffer or pH 7.0 peptone water (fluid A). Transferred one of the membrane filters, intended primarily for the enumeration of Total aerobic microbial count (TAMC) to the surface of a plate of Soybean Casein Digest Agar medium (TSA) and enumeration of combine yeast and mold count (TYMC), at the surface of a plate of Sabouraud Dextrose Agar (SDA) with the help of sterilized forceps. Incubated the plate of TSA at 30 ± 2.5 °C 72 hours and SDA at 20 ± 2.5 °C for 5 days; counted the number of colonies per ml.

Detection of Pathogens

We have examined presence or absence of pathogenic species along with their positive and negative controls in raw materials, extracts and bulk, for example *Staphylococcus aureus*,

Escherichia coli, *Pseudomonas aeruginosa*, Gram negative bacteria, *Salmonella* species, *Candida albicans*, *Aspergillus niger*.

RESULTS

Identification of TAC from Raw materials in terms of CFU/gm

The count of viable bacteria has been checked from raw materials and found count under range. The limits of TAC in raw materials should be $<10^3$. The highest bacterial growth in Raw material was observed in *Gingko biloba*, *Veegum*, *Fallopia japonica*, *Ulmus ruba*, *Tanacetum parthenium* 900CFU/gm.

Identification of TYMC from Raw materials in terms of CFU/gm

The count of viable fungal and yeast count has been checked from raw materials and found count under range. The limits of TYMC in raw

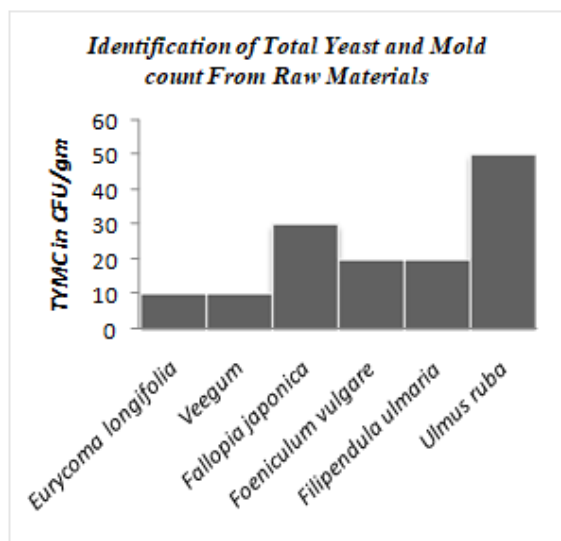


Figure 2. Identification of TAC and TYMC from Raw materials in terms of CFU/gm.

materials should be $<10^2$. The fungal growth is detected in few Herbal Raw Materials only that's include *Eurycoma longifolia*, *Veegum*, *Fallopia japonica*, *Foeniculum vulgare*, *Filipendula ulmaria*, *Ulmus ruba* (Figure 2) but in acceptable range while other did not show any fungal growth.

The limits of TAC and TYMC in bulk should be $<10^3$ and $<10^2$ respectively. The fungal count was not observed in bulks. The bacterial count (CFU/gm) was found in acceptable limit of bulk samples and could not observe any fungal count because of no growth of microbial colony. The polyherbal Verona capsules showed highest CFU/gm (Figure 3).

Identification of Total Aerobic count From Bulk

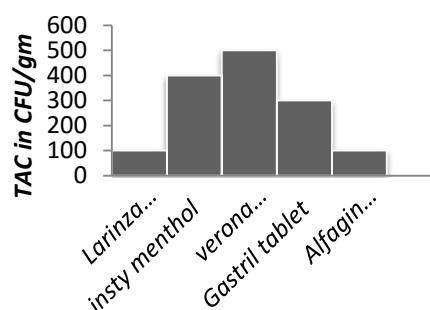


Figure 3. Identification of TAC and TYMC from Bulk in terms of CFU/gm.

The limits of TAC and TYMC in extract should be $<10^3$ and <50 respectively. The fungal count was not observed in extracts. The bacterial count found in acceptable limit of extracts samples. We could not see any fungal count because of no growth of microbial colony (Figure 4).

Identification of Total Aerobic count From Extracts

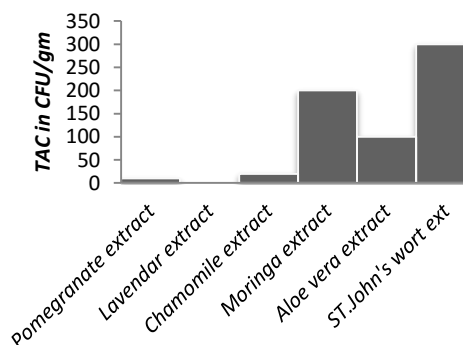


Figure 4. Identification of Total aerobic count from extracts.

Detection of Pathogens

Each sample was subjected for microbial growth for the detection of pathogens, consequently no detection or find any traces of pathogenic species *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, Gram negative bacteria, *Salmonella* species, *Candida albicans*, *Aspergillus niger*.

DISCUSSION

The colony forming unit/ grams or milliliters of total aerobic and fungal were matched the BP and USP standards, raw materials should be less than 10^3 for bacterial count and less than 10^2 for fungal count. We found growth under the range of an acceptable limit from raw materials. We identified limited colonies of bacteria in 1:100 dilution TSA plates and some moulds were detected in 1:10 dilution SDA plates on few herbs of raw materials. Fortunately, all the raw materials we tested for detection of pathogenic species along with their positive and negative controls but we could not find any pathogens for example *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, Gram negative bacteria, *Salmonella* species, *Candida albicans*, *Aspergillus niger*. The results are very much satisfactory and fulfilled the criteria of acceptable limit which allow the production for addition of multiple raw materials at one time to manufacture a complete batch of finished product. The highest bacterial growth in Raw material was observed in *Gingko biloba*, *Veegum*, *Fallopia japonica*, *Ulmus ruba*, *Tanacetum parthenium* 900CFU/gm. The Fungal growth is detected in few Herbal Raw Materials only that's include *Eurycoma longifolia*, *Veegum*, *Fallopia japonica*, *Foeniculum vulgare*, *Filpendula ulmaria*, *Ulmus ruba* but in acceptable range while other did not show any fungal growth.

The colony forming unit/ grams or milliliters of total aerobic and fungal were matched the

limits of USP and BP, bulk should be less than 10^3 for bacterial count and less than 10^2 for fungal count. We found growth under the range of an acceptable limit from bulk in terms of CFU/gm. We identified limited colonies of bacteria in 1:100 dilution TSA plates and some moulds were detected in 1:10 dilution SDA plates on few herbs of raw materials. Fortunately, all the raw materials we tested for detection of pathogenic species along with their positive and negative controls but we could not find any pathogens for example *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, Gram negative bacteria, *Salmonella* species, *Candida albicans*, *Aspergillus niger*. The results are very satisfactory and fulfilled the criteria of standard limit which allow the production for addition of bulk which was heated at optimum temperature to manufacture a complete batch of finished product. The count of viable Aerobic count has been checked from extracts and found count under range. The count of viable Aerobic count has been checked from bulk and found limits under range. The 500 Colony forming unit/ grams of bacterial count was observed in Verona capsules manufactured from the mixture of different herbs that included *Tribulus terrestris*, *Eurycoma pansinystalia johimbe*, *Withania somnifera* etc. The test results are compiling with the limits of WHO guidelines while the count of Larinza granules is 100 CFU/gm, Insty menthol 400 CFU/gm, Verona capsules 500 CFU/gm, Gastril tablet 300 CFU/gm, Alfagin capsules 100 CFU/gm.

The colony forming unit/ grams or milliliters of total aerobic and fungal were matched the limits, Extracts should be less than 10^3 for bacterial count and less than 50 for fungal count. Fortunately, was found growth under the range of an acceptable limit as mentioned from Extracts in terms of CFU/gm. We identified limited colonies of bacteria in 1:100 dilution TSA plates and some moulds were detected in 1:10 dilution SDA plates on few herbs of raw

materials. All the raw materials we tested for detection of pathogenic species along with their positive and negative controls but we could not find any pathogens for example *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, Gram negative bacteria, *Salmonella* species, *Candida albicans*, *Aspergillus niger*. The results are very satisfactory and fulfilled the criteria of acceptable limit which allow the production for addition of extract which was heated at optimum temperature to manufacture a complete batch of finished product. The 300 CFU/gm of bacterial count was observed in ST John's wort extract, 10CFU/gm in Pomegranate extract, <10CFU/gm in Chamomile extract, 200CFU/gm Moringa extract, 100CFU/gm in Aloe vera extract. The active was extracted from the *Hypericum perforatum*, *Punica granatum*, *Lavandula officinalis*, *Matricaria chamomilla*, *Moringa oleifera*, *Aloe vera*.

REFERENCES

1. Bandaranayake WM. Quality control, screening, toxicity, and regulation of herbal drugs. Modern phytomedicine: turning medicinal plants into drugs. 2006; 25-57.
2. De Smet PA, D'Arcy PF. Drug interactions with herbal and other non-orthodox remedies. In: Mechanisms of drug interactions 1996 (pp. 327-352). Springer, Berlin, Heidelberg.
3. De PS. Health risks of herbal remedies. Drug safety. 1995; 13(2):81-93.
4. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. Journal of applied microbiology. 1999 Jun; 86(6):985-90.
5. World Health Organization. WHO monographs on selected medicinal plants. World Health Organization; 1999.
6. World Health Organization. (2002). WHO traditional medicine strategy 2002-2005.
7. Parekh J, Karathia N. Screening of some traditionally used medicinal plants for potential antibacterial activity. Indian Journal of Pharmaceutical Sciences. 2006; 68(6).
8. Pharmacopoeia, European. (2002). European pharmacopoeia. Strasbourg: Council of Europe.
9. Safety, International Program on Chemical, Chemicals, Inter-Organization Programme for the Sound Management of, & Organization, World Health. (2010). WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2009: World Health Organization.