VALIDATION OF MICROBIOLOGICAL CONTROL METHOD OF HERBAL DRUGS

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Abstract

With the purpose to standardize microbiological quality control twelve different sample treatments were performed.

Melissa officinalis and *Mentha piperita* were used as medicinal plant samples.

Based on statistical analysis presented, addition of 0.1% polysorbate 20 to Diluent Solution (0.1% peptone water- 0,85% sodium chloride) and blender homogenization improve Mesophilic Aerobic Bacteria Count Technique when sample suspension was assayed. Prefilter membrane filtration treatments were less proper.

1. Introduction

In Argentine, herbal drugs are widely consumed in their natural forms to prepare herbal infusions, or as tablets, capsules, extracts, oils, creams and lotions. Pharmaceutical argentine market includes autochthonous and foreign species. Due to their natural source, medicinal herbs often carry a great number of bacteria and moulds. Around the world, it is well established the necessity to determine microbial contamination of herbal drugs. European Pharmacopoeia provides microbiological criteria (1). Argentine Pharmacopoeia does not establish microbiological specifications for this kind of products. World Health Organization accounts to determine specific microorganisms and different sample pre-treatments for microbiological analysis of medicinal plant materials (2). Sanitary controls demonstrate if Good Manufacture Practices during harvesting and handling were performed. *Staphylococcus aureus* presence indicates a probable contamination by the operator during production. Due to their physical characteristics these products show problems for sample homogenization.

In our laboratory, we have validated different sample treatments with the purpose to standardize microbiological quality control of herbal remedies.

2. Materials and methods

10 gr. samples of *Mentha piperita* and *Melissa officinalis* previously irradiated with a dose of 25 kGy and inoculated with 10^3 CFU of *Staphylococcus aureus* were assayed by Pour Mesophilic Aerobic Bacteria Count Technic (P M A B C T) in Tryptic Soy Agar (TSA) (3). Twelve different sample treatments were assayed. Samples were suspended in:

A) Diluent solution (0.1% peptone water- 0,85% sodium chloride) with 0.1% polysorbate 20.
B) Diluent solution (0.1% peptone water- 0,85% sodium chloride).
C) Diluent solution (0.1% peptone water- 0,85% sodium chloride) with 0.1% polysorbate 20 and homogenization in blender 1 min at 20,000 rpm.
D) Diluent solution (0.1% peptone water- 0,85% sodium chloride) and then homogenized in blender 1 min at 20,000 rpm.

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Suspension (1), supernatant when suspension was allowed to sediment for 30 min at room temperature (2) and filtrate of suspension through a prefilter membrane (3) were assayed by P M A B C T for A, B, C, and D.

Control with *Staphylococcus aureus* suspension was performed. Initial contamination of the samples, prior to be inoculated, was also investigated.

3. Results

Initial contamination of *Mentha piperita* and *Melissa officinalis* was less than 10 micro-organisms per g.

Melissa officinalis: Counts were performed by triplicate. Mean counts and coefficients of variation for each treatment by suspending the sample in Diluent Solution (B) and in Diluent solution with 0.1% polysorbate 20 (A) are showed in table 1.

Data presented in Table 2 shows mean counts (triplicated assay) and coefficients of variation for each treatment by suspending the sample in Diluent Solution (D) or in Diluent solution with 0.1% polysorbate 20 and by blender homogenization 1 min at 20.000 rpm (C).

Mentha piperita: mean counts (duplicated assay) for each treatment are shown in table 3.

4. Discussion

Microbiological quality control contributes with medicinal herbs safety and inocuity. For our purpose, quality control is the testing activities used to determine that herbal drugs meet predetermined microbiological requirements. WHO guidelines recommend, for microbiological method validation, that the count for the test micro-organism in the sample should not differ by more than a factor of 10 from the calculated value of the control suspension (2). Validation is a documented evidence providing a high degree of assurance that an analytical method performs as expected.

Variance analysis was done, for the results of both raw herbal materials, assuming independent variables, a normal distribution with variance homogeneity and control suspension as ideal count.

Melissa officinalis: All of the treatments assayed fulfil WHO recommendations. Variance analysis showed no significative difference between count means ($p< 0,05$) of the treatments performed. Tukey's Multiple Comparison Test did not show difference between treatments and control. According to % coefficient of variation C1 treatment was selected for this raw herbal material.

Mentha piperita: All of the treatments assayed fulfill WHO recommendations. Variance analysis showed no significative difference between mean counts for all of the treatments performed ($p< 0,05$). A comparison by Tukey method did not show difference between treatments and control according to the compared parameter. Sample suspension in Diluent solution (0.1% peptone water- 0,85% sodium chloride) with 0.1% polysorbate 20 with or without blender homogenization 1 min at 20,000 rpm. (A1 and C1 treatments) were more accurate.

Prefilter membrane filtration treatments were less appropriate for both herbal drugs.

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6. References

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Table 1 - *Melissa officinalis* suspended in Diluent solution (0.1% peptone water-0,85% sodium chloride) with and without 0.1% polysorbate 20

Treatment	mean (CFU/g) n:3	% coefficient of variation
A1	$1{,}69 \times 10^4$	49
A2	1.91×10^4	33
A3	$7{,}50 \times 10^3$	87
B1	$2{,}02 \times 10^4$	35
B2	$1{,}45 \times 10^4$	22
B3	$1{,}03 \times 10^4$	32
CONTROL	$1{,}65 \times 10^4$	20

Table 2 - *Melissa officinalis* suspended in Diluent solution (0.1% peptone water-0,85% sodium chloride) with and without 0.1% polysorbate 20 and blender homogenizated.

Treatment	mean (CFU/g) n:3	% coefficient of variation
C1	$1{,}71 \times 10^4$	27
C2	2.25×10^4	42
C3	1.19×10^4	78
D1	$2{,}50 \times 10^4$	43
D2	$1{,}87 \times 10^4$	43
D3	$1{,}44 \times 10^4$	52
CONTROL	$1{,}65 \times 10^4$	20

Table 3 - *Mentha piperita* treatments

Treatment	mean (CFU/g) n:2	Treatment	mean (CFU/g) n:2
A1	9.8×10^3	C1	1.1×10^4
A2	7.2×10^3	C2	7.0×10^3
A3	3.7×10^3	C3	2.1×10^3
B1	7.1×10^3	D1	5.7×10^3
B2	7.4×10^3	D2	6.2×10^3
B3	1.7×10^3	D3	1.1×10^3
CONTROL	9.2×10^3		