Analysis of Preservative Capacity for Contemporary Iranian Shampoos

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ABSTRACT

Preservative capacity is measured for seventeen currently accessible Iranian made shampoo products. Upon purchase, bacterial counts show no trace of any microbial contamination. The shampoo bottles are distributed to seventeen female graduate students, residing at the time in different dormitories of Tarbiat Modares University (TMU). After 40 days, the allocated bottles are returned and their bacterial counts repeated, under the same conditions. To our surprise, no trace of any microbial contamination is observed. Three possibilities are considered. 1) The factory employed preservative(s) act very efficiently. 2) The shampoo component(s) are themselves bactericidal. 3) Existence of sanitary conditions and the absence of any microbial contaminants at TMU! Evidently, the third possibility is ruled out. The second choice is also discarded, because practically no significant antimicrobial activity is demonstrated by any of the four main ingredients of the shampoos including sodium lauryl ether sulfate (SLES), betaine, coconut fatty acid, and glycerin. Hence, the most likely possibility is the first one, since upon deliberate contamination of the seventeen brands; the factory employed preservatives [5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (KATHON CG)] prove highly bactericidal against four major contaminants: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and the methicillin-resistant *Staphylococcus aureus* (*MRSA*).

GRAPHICAL ABSTRACT

Cultured samples contaminated with the four bacteria: Shampoos (1-17) and their constituents (18-21) *vs.* the control (22)

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Introduction

Soaps are among surfactants that make up detergents which appear in forms of liquid, powder, paste, bar, cake, molded piece, *etc.* [1]. Surfactants may be applied in household laundry products, domestic and industrial cleaners, cosmetic products, shampoo formulations, *etc*. They are capable of forming micelles because of their hydrophilic and hydrophobic groups [2, 3]. As a result, they help achieve cleaning, rinsing, and fabric softening due to the above mentioned surface-active properties [4, 5].

About 70–80% of the entire weight of most detergents is water. The rest is the primary surfactant, followed by the foam boaster, thickeners, conditioning agents, modifiers, and desired additives [6, 7]. While, the sterility of shampoo is not often required; contamination with pathogenic microorganisms and microbial contaminants appear unavoidable [8]. Microorganisms such as *E. coli*, *S. aureus*, and *P. aeruginosa* easily grow in places that are not often completely dried, for instance: bathtubs, shower areas, kitchen counters, sinks, *etc*. [9]In such places, microbes can easily be transmitted to shampoos, soaps, towels, and the like. A number of shampoo preparations employed in this work are based on sodium laurylsulphate surfactants

that separate or discolor upon proliferation of *Pseudomonas* species [10]. Of special concern is the detection of the latter opportunistic pathogen that has spoilage potentials and is the most common microorganism associated with recall of many cosmetic formulations in the United States and Europe [7, 11-12]. As a rule of thumb, bacterial count in the products must be low with no pathogen. What isso alarming to usis the report on the 43% rate of microbial contamination of shampoo brands marketed in some countries [13].In one instance, three babies in a nursery became infected with *Serratia marcescens*, purulent conjunctivitis (two cases) and *omphalitis* (one). Thirteen of fourteen babies recovered fully but one died from *S. marcescens* meningitis and *septicaemia*. All infections were traced to intrinsically contaminated baby shampoo introduced to the units five days before the first reported case [14-15].

In another instance, the microbiological quality of sixteen different shampoo formulations (manufactured by sixteen different factories) was studied to determine the preservative capacity of these products at a time of sale and after use. Procedures used were according to those described in ISO technical standards. Thirteen (81.25%) of the formulations studied were found to be free of

contamination. One product harbored *E. coli* and two contained *P. aeruginosa* [15-16].

In another instance, 8 commercial brands of shampoo were studied and none of them harbored microorganisms in access of 10^4 CFU/mL. Only 15% of the products revealed bacterial count between 10^2 to 10^3cells/mL [13-15].

Finally, *Staphylococcus*, *Pseudomonas*, *Klebsiella*, *Achromobacter* and *Alcaligene* were detected in shampoo, hand and body lotion, facial cleanser, and liquid soaps [15-17].

So, microbial contamination of shampoo products is very common and appears of great concern. Microorganisms can cause health hazards because their metabolic products may be toxic and/or mutagenic. Hence, in order to guarantee long-term stability, shampoo formulations need protection against microbial spoilage. This may be in the form of preservatives which play a vital role in the product formulations [12, 17].

Among preservatives KATHON CG is the most common. Its active ingredients are two isothiazolinones, including 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one [18].

As a service to the community, in this work durability of Iranian shampoo formulations are probed at the time of sale and after 40 days (or usage of 2/3 of their bottles, whichever comes first). In addition, the shampoo products are tested against deliberate contaminations to *E. coli*, *S. aureus*, *P. aeruginosa*, and *MRSA*.

Experimental

Materials and Methods

The probed shampoos are purchased directly from their Iranian manufactures. In conformity with the publisher non-solicitation regulations, each brand is given a numerical code (1-17) prior to the experimental work. Bacteria sues are obtained from the Bacteriology Department, Faculty of Medical Sciences, TMU. Muller-Hilton agar is purchased from Liophil Chem Company, Italy. Brain-Hearb Infusion (BHI) agar is purchased from Merck Company, Germany. The seventeen human subjects are selected from volunteer female graduate students residing at the TMU dormitories (ages 22-28).

To determine whether 1-17 are initially germ-free, four 100 μL specimens of each brand are probed: one at the normal concentration and three others with serial dilutions at 0.1, 0.01, and 0.001*v/v* in normal saline (NaCl 93%). Hence, a total of sixty eight samples are simultaneously cultured. Each sample is transferred to a sterile Petri dish containing 20 mL BHI agar and incubated at 35 °C for 48 hours. Surprisingly,

all the latter shampoo samples prove totally germ-free.

Consecutively, these seventeen microbial free shampoo samples, 1-17, are assigned to seventeen female graduate students, residing at the time in different dormitories of TMU. The samples are recollected after 40 days, or as soon as the residual quantity in each container becomes approximately 1/3 of its original volume. Repeating the above microbial test for each sample shows no microbial contamination under normal conditions.

To expand on the preservative capacity of the above normally durable shampoos, 1-17 are tested against deliberate bacterial contaminations with *E. coli*, *S. aureus, P. aeruginosa*, and *MRSA*. Similarly the four major constituents of shampoos including: SLES, betaine, coconut fatty acid, and glycerin (18-21) are contaminated. Specifically, each of the above mentioned bacterial strains is cultured overnight on Muller-Hilton agar (ATCC, WDCM1). A suspension of each is prepared in 5 mL normal saline containing 10^9 cells/mL. These suspensions (100 µL) are mixed and homogenized separately with 500 µL shampoo products 1-17 and their constituents 18-22. After a specific time, 5 mL sterile normal saline is added to these mixtures and vortexed for one minute. Dilutions of each tube are cultured on Muller-Hilton agar. The plates are maintained at 37 °C for 18 hours then number of colonies per sample is counted.

Results and Discussion

Codes of standards on Iranian manufactured shampoos (No. 3271, ICS: 07.100.99; 71.100.70) require total absence of *E. coli*, *S. aureus*, and *P. aeruginosa* with no more than 10^2 CFU/mL of mesophilic aerobic bacteria [19]. One of our main objectives in this work is to find if all seventeen contemporary Iranian manufactured shampoos (1-17) comply with the above regulation.

First of all, to determine whether the shampoo products are germ-free at purchase, four 100 µL specimens of each brand are simultaneously cultured in agar. They include one at normal concentration and three others with serial dilutions at 0.1, 0.01, 0.001*v/v* in normal saline (NaCl 93%). Hence, in this part of our work, a total of 68 samples are simultaneously incubated. Specifically, each sample is transferred to a sterile Petri dish containing 20 mL BHI agar and incubated at 35 °C for 48 hours. The results of the first part of our survey come out rather surprising, because samples appear totally germ-free!

Consecutively, in the second part of our work, these seventeen microbial free shampoo samples 1-17 are assigned to seventeen 22-28 years old female graduate

students, residing at the time in different dormitories of TMU. The samples are recollected after 40 days, or as soon as the residual quantity in each container becomes approximately 1/3 of its original volume. Repeating the above microbial test for each sample results in another surprise for us, sinceno microbial contamination is found under normal conditions! Looking for good explanations for the above observations one finds several factors which prevent microbial contamination of shampoos. For example, Branna and Dille established that the dispensing closure used for shampoo and cosmetic containers play an important role in protecting them from in-use microbial contaminations [20]. All things considered, we adoptthe following three possibilities: 1) The factory employed preservative(s) may act very efficiently. 2) The shampoo component(s) (18-21) maybe bactericidal. 3) TMU dormitories are perhaps free of microbial contaminants. Evidences, at least in two universities similar to TMU, rule out the third possibility [21, 22]. The second possibility is also discarded, because the four main ingredients of the shampoos including sodium lauryl ether sulfate (SLES), betaine, coconut fatty acid, and glycerin show no significant antimicrobial activity. The only possibility left among the three goes back

the first choice: The factory employed preservative(s) may act very efficiently.

This brings us to the third part of our work, which is to probe the above possibility. Hence, the seventeen shampoo brands (Samples 1-17), plus their four main constituents (Samples 18-21) are separately contaminated with four strains of bacteria including *E. coli*, *S. aureus*, *P. aeruginosa,* and the *MRSA.* Prior to such contaminations, each of the above mentioned bacterial strains is cultured overnight on Muller-Hilton agar, from which four suspensions are made, each in 5 mL normal saline containing 10^9 cells/mL. Then 100 μL of each of the latter suspensions of bacteria is separately added to 500 μL of each of the seventeen shampoo samples (1-17), as well as to 500 μL aliquots of each of the four shampoo constituents (18-21). Consequently, the above samples are homogenized. After a 24 hours, 5 mL sterile normal saline is added to these mixtures and vortexed for one minute. Three dilutions of each sample (1, 0.1, and 0.01 *v/v*) are cultured on Muller-Hilton agar. The plates are maintained at 37 °C for 18 hours then the number of colonies per sample is counted. The overnight vicinity (24 hours) shampoo samples extirpate all of the bacteria. Hence, vicinage time is gradually decreased and experiments are repeated down to 12 and then 6 hours, where all the bacteria are

similarly extirpated. So the time is decreased again. After 30 minutes and in dilution of 0.01 *v/v* alterations are observable and results are recorded (Table 1). Quantitatively, these results are based on the percent bacterial growth (X), which is shown by X= (1-((B-T)/B))*100. Here, B is the number of colonies in a bacterial assay used as the "Control" (Sample 22). "Control"

is attributed to every one of the four strains of bacterial directly cultured on agar, without being subjected to the shampoo formulations (1-17) or their constituents (18-21). Finally, T is the number of colonies found in either one of the seventeen shampoo samples (1-17), or the four shampoo constituents (18-21).

Table1. *E. coli*, *S. aureus*, *P. aeruginosa*, and *MRSA* growth percentages (X), upon thirty minutes adjacency to seventeen shampoo samples (1-17), along with four shampoo constituents (18-21), in comparison to the corresponding control bacterial assays not exposed to the shampoo formulations or their constituents (Sample 22)

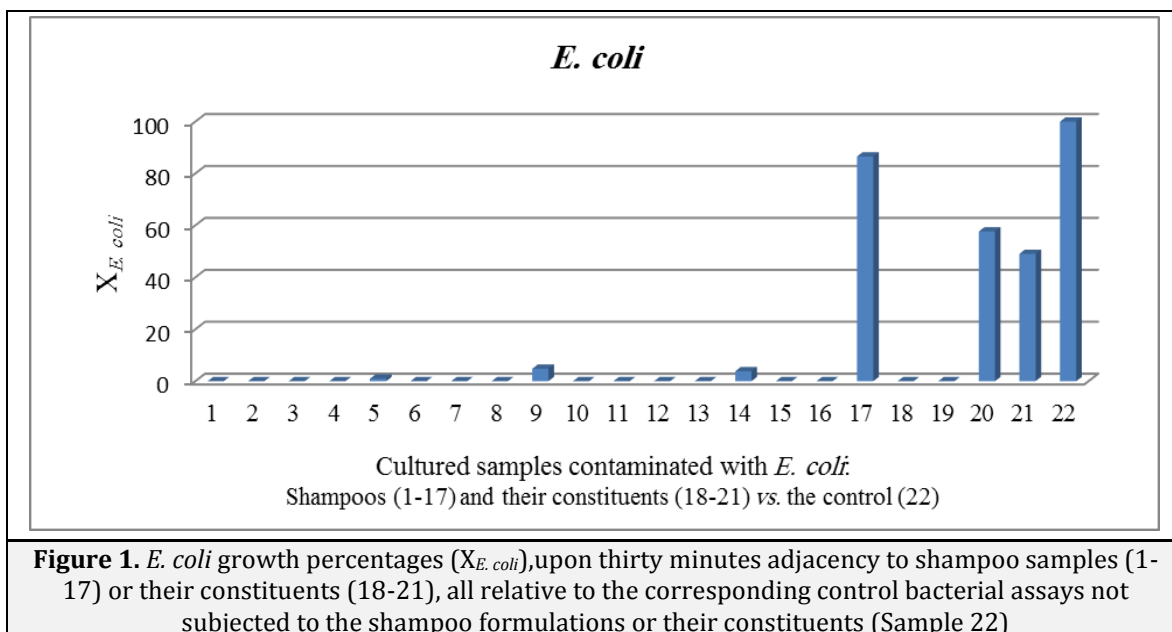| Cultured samples		X			
		E.coli	*S.aureus*	*P.aeruginosa*	*MRSA*
Seventeen contaminated shampoo formulations	1	0	113.64	0	71.70
	2	0	89.65	0	74.21
	3	0	88.38	0	91.82
	4	0	143.69	0	83.65
	5	0.96	93.69	0	84.28
	6	0	115.15	0	171.70
	7	0	49.24	0	50.31
	8	0	86.62	0	79.25
	9	4.81	30.81	0	48.43
	10	0	46.72	0	44.65
	11	0	6.31	0	10.07
	12	0	17.93	0	49.06
	13	0	111.11	0	100.63
	14	3.85	0	0	0
	15	0	41.41	0	30.19
	16	0	42.42	0	30.82
	17	86.54	7.07	0	1.26
Four contaminated shampoo constituents	18	0	175.76	0	0
	19	0	9.09	0	10.69
	20	57.69	117.68	69.13	71.07
	21	49.04	54.04	57.72	61.64
Control pure bacteria	22	100.00	100.00	100.00	100.00

Contamination with *E. coli*

E. coli growth percentages ($X_{E.\ coli}$) are assessed after thirty minutes adjacency with shampoo samples (1-17) as well as their constituents (18-21). These are all measured against to the "Control", which is an *E. coli* assay, not subjected to the shampoo formulations or their constituents (Sample 22) (Figure 1, Table 1).
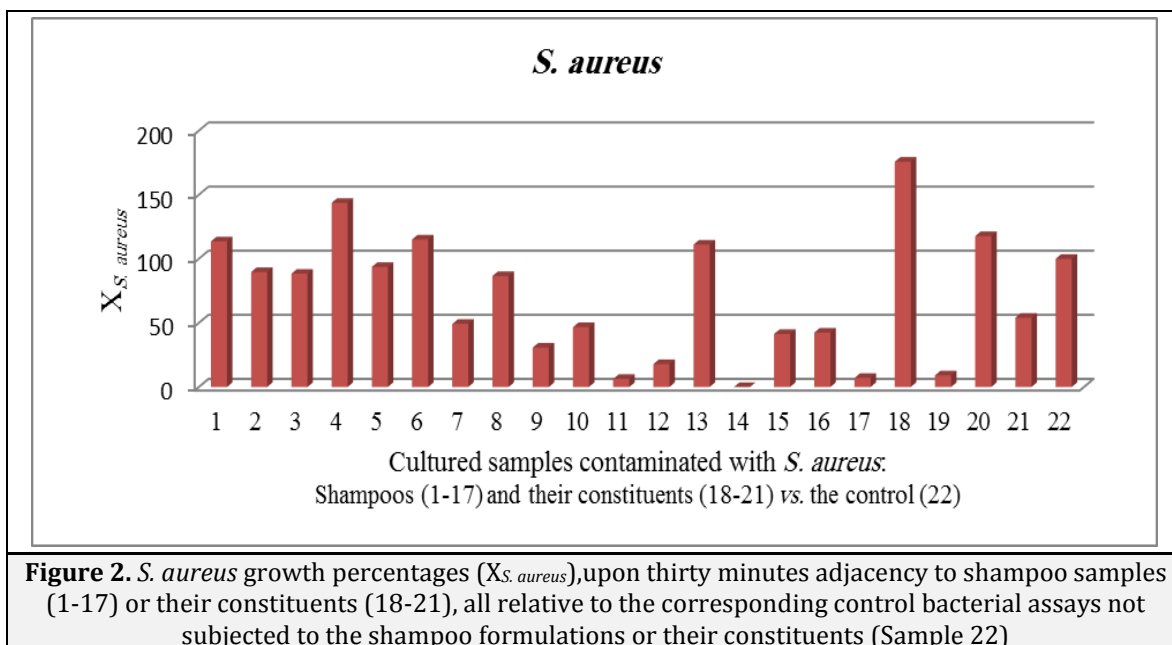
All Iranian manufactured shampoo formulations appear resistant to *E. coli* except for brands 5, 9, 14, and 17 which show $X_{E.\ coli}$ of 1, 5, 4, and 86, respectively. From four Iranian manufactured shampoo constituents, only SLES (Sample 18) and coconut fatty acid (Sample 19) appear resistant to *E. coli.* Hence, it is likely that shampoo 17 is composed mainly of betain and glycerin (Sample 20 and 21 respectively). If so, decreasing concentration of the latter may help its durability against *E. coli*. Therefore, we caution against using shampoo 17 in places contaminated with a high concentration of *E. coli*. Such places includes wimming pools with untreated water, contaminated surface water and groundwater, water-reservoirs polluted by animals and humans, everywhere that animals, particularly cows, sheep, and goats, livestock, *etc*. [23].

Contamination with *S. aureus*

S. aureus growth percentages ($X_{S.\ aureus}$) are evaluated after thirty minutes adjacency to shampoo samples (1-17) as well as their constituents (18-21). These are all measured relative to the "Control", which is a *S. aureus* assay not subjected to the shampoo formulations or their constituents (Sample 22) (Figure 2, Table 1).

Figure 1. *E. coli* growth percentages ($X_{E.\ coli}$),upon thirty minutes adjacency to shampoo samples (1-17) or their constituents (18-21), all relative to the corresponding control bacterial assays not subjected to the shampoo formulations or their constituents (Sample 22)

Figure 2. *S. aureus* growth percentages (X$_{S. aureus}$), upon thirty minutes adjacency to shampoo samples (1-17) or their constituents (18-21), all relative to the corresponding control bacterial assays not subjected to the shampoo formulations or their constituents (Sample 22)

Comparing *E. coli* with *S. aureus*, one finds the latter more potent for inducing rather high X$_{S. aureus}$for all samples except 14. Interestingly, SLES (Sample 18), which is one of the four constituents of shampoo formulations, totally eliminates *E. coli* while not only it fails to eliminate *S. aureus,* butit stimulates its growth. Therefore, among 1-17 we recommend shampoo 14 to be employed in places contaminated with high concentration of *S. aureus*. Such places include wimming pools, gyms, wrestling locker rooms, *etc.* [24].
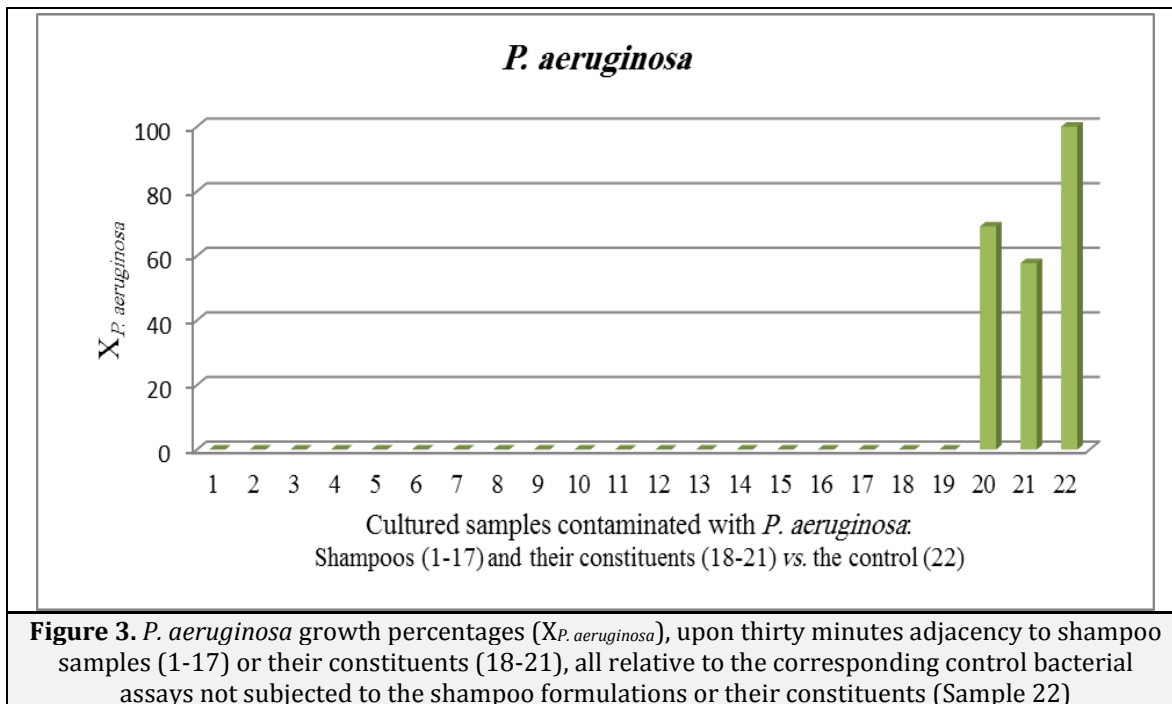
Contamination with *P. aeruginosa*

P. aeruginosa growth percentages (X$_{P. aeruginosa}$) are assessed after thirty minutes adjacency to shampoo samples (1-17) as

well as their constituents (18-21). These are all assessed relative to the "Control", which is a *P. aeruginosa* assay not subjected to the shampoo formulations or their constituents (Sample 22) (Figure 3, Table 1).

Perhaps *P. aeruginosa*is the least potent among the probed bacteria, because all Iranian manufactured shampoo formulations emerge resistant to it. From the constituents, SLES (Sample 18) and coconut fatty acid (Sample 19) appear resistant to it. So, all seventeen samples (1-17) score the best against *P. aeruginosa*. Hence, Iranian shampoos are recommended to be used in areas contaminated with *P. aeruginosa.* These areas include: Hospitals, swimming pools,

whirlpools, water-reservoirs polluted by

animals and humans, *etc*. [25].

Figure 3. *P. aeruginosa* growth percentages ($X_{P.\ aeruginosa}$), upon thirty minutes adjacency to shampoo samples (1-17) or their constituents (18-21), all relative to the corresponding control bacterial assays not subjected to the shampoo formulations or their constituents (Sample 22)

Contamination with the *MRSA*

The *MRSA* growth percentages (X_{MRSA}) are measured after thirty minutes adjacency to shampoo samples (1-17) as well as their constituents (18-21). These are all assessed relative to the "Control", which is a *MRSA* assay not subjected to the shampoo formulations or their constituents (Sample 22) (Figure 4, Table 1).

Between the samples (1-21) probed only shampoo 14 and constituent 18 show resistance to *MRSA*. Interestingly, SLES (18) cannot eliminate *S. aureus* but demonstrates significant bactericidal ability against the more potent *MRSA* (Figure 5). Similarly and to our surprise,

1-2, 4-5, 8, 10, 13, 15-16, and 20 eradicate *MRSA* more than *S. aureus*. Hence, we recommend usage of shampoo 14 in places contaminated with *MRSA*. Such places include hospitals, prisons, and nursing homes, *etc.* [26].

Finally, deliberate contamination of Iranian shampoo formulations and their constituents with 10^9 bacteria (*E. coli*, *S. aureus*, *P. aeruginosa,* and *MRSA*) illustrate the products can eliminate contaminants during 1 hour and the constituents cannot act efficiently to eradicate all bacteria strains. So, preservatives including 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (KATHON

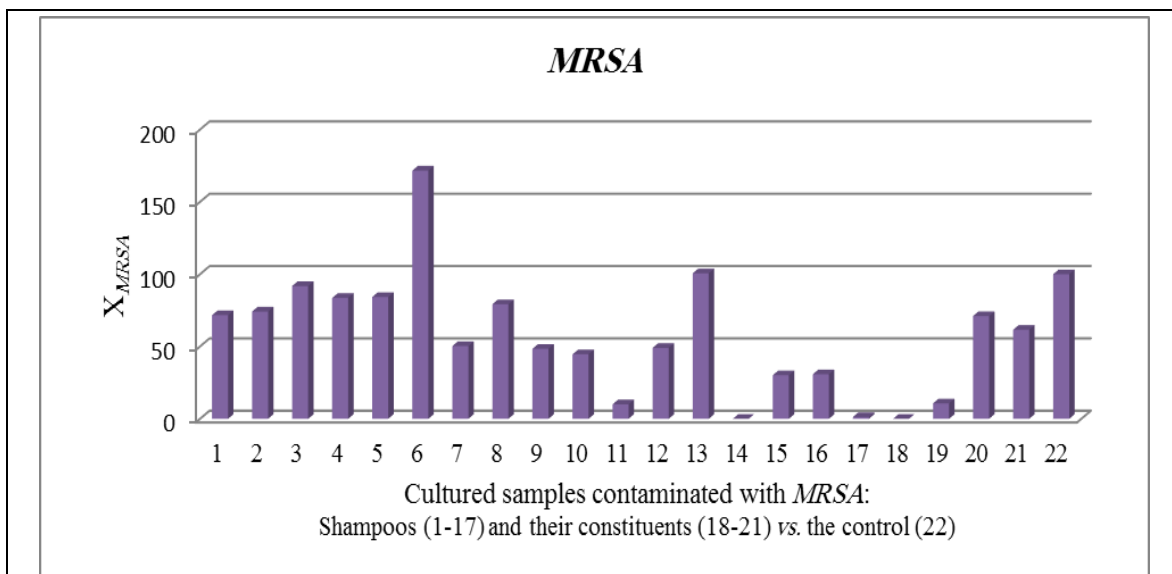CG) prove highly bactericidal against   contaminants.

Figure 4. *MRSA* growth percentages (X_{MRSA}), upon thirty minutes adjacency to shampoo samples (1-17) or their constituents (18-21), all relative to the corresponding control bacterial assays not subjected to the shampoo formulations or their constituents (Sample 22)
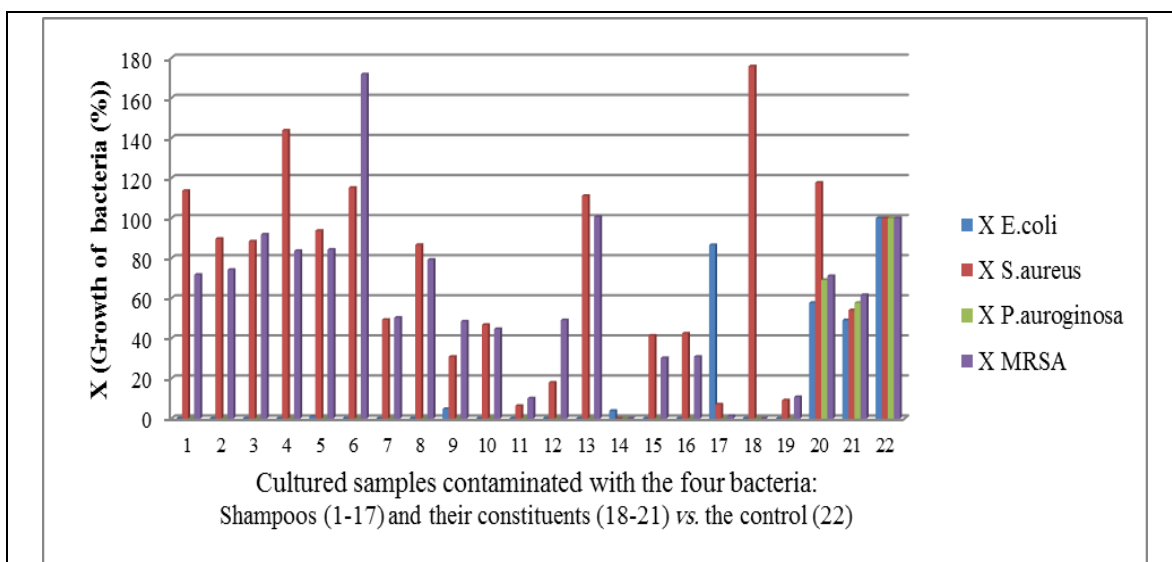

Figure 5. *E. coli*, *S. aureus*, *P. aeruginosa*, and *MRSA* growth percentages (X), upon thirty minutes adjacency to seventeen shampoo samples (1-17), along with four shampoo constituents (18-21), all relative to the corresponding control bacterial assay not subjected to the shampoo formulations or their constituents (Sample 22)

Conclusion

Preservative capacity is measured for seventeen currently accessible Iranian made shampoo products 1-17. Upon purchase, bacterial counts show no trace of any microbial contamination. After 40 days

normal usage by seventeen volunteers, the allocated bottles are returned and their bacterial counts are repeated, under the same conditions. Surprisingly, no trace of any microbial contamination is observed. No antimicrobial activity is practically demonstrated by any of the four main ingredients of the shampoos including SLES, betaine, coconut fatty acid, and glycerin. Upon deliberate contamination of the seventeen brands; the factory employed preservatives [5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (KATHON CG)] immerge highly bactericidal against contaminants: *E. coli*, *S. aureus*, *P. aeruginosa,* and *MRSA.* Actually, all samples 1-17 are fully capable of killing a 10^8 population of the above bacteria in one hour. The highest antibacterial activity is demonstrated by shampoo 14 after 30 minutes of contamination. Hence, shampoo 14 proves to be the most resistant against high concentration of the above mentioned bacteria.

Acknowledgments

Ethical approval

In conformity with the publisher non-solicitation regulations, each brand is given a numerical code (1-17) prior to the experimental work.

Conflict of Interest

The authors declare that they have no conflict of interest.

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