ANTIMICROBIAL EFFICACY OF THE ORGANIC GREASY OILS COMBINATION - SEA BUCKTHORN OIL AND MAIZE GERMS OIL

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Abstract

Maize-germ oil is obtained from seedlings of *Zea mays* L. (*Gramineae*) by pressing (cold-pressed maize-germ oil). Maize-germ oil is highly valued in nutritional terms on account of the palmitic acid content and, primarily, of the high oleic and linoleic acid content. In the food industry, maize-germ oil is used in the production of foodstuffs (diet products, baby foods, margarine, and mayonnaise).

Sea Buckthorn oil (*Hippophae rhamnoides*) supports healthy skin growth by high amounts of polyunsaturated fatty acids, tocopherols, and beta carotene. Sea buckthorn seed oil promotes cell tissue growth, restoring skin tissue, supports wound healing, protects skin from anti-inflammatory effect, UV damage and absorption. Sea Buckthorn oil can actually improve metabolism and retard skin maturation, thus slowing the aging process, possibly because of the effects of its high levels of Vitamins A and E.

In this study we evaluated the possible useful combination of fatty oil from corn germ and the Sea Buckthorn oil as both vehicle, and active ingredient, in pharmaceutical products for oral use, due to their primarily intrinsic antimicrobial properties reflected in the quality and safety of finished products.

These studies were carried out according to the methodology of the European Pharmacopoeia monograph namely "Efficacy of antimicrobial preservatives", demonstrating the antimicrobial effect both in terms of quality and quantity, on the following standard test microorganisms: *Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Escherichia coli ATCC 8739*, *Candida albicans ATCC 10231* and *Aspergillus brasiliensis ATCC 16404.*

This study aimed to demonstrate the antimicrobial efficacy of organic fatty oils combination- without added preservatives in its manufacturing formulation.

Both ingredients were 100% organic certified. The ingredients were pure and did not contain any additives or chemicals; they were not hydrogenated and were free of genetically modified organisms (GMOs).

Rezumat

Uleiul de germeni de porumb este obținut din germeni de *Zea mays* L. (*Gramineae*) prin presare la rece. Uleiul de germeni de porumb este extrem de apreciat din punct de vedere nutrițional datorită conținutului ridicat de acid palmitic, acid oleic și acid linoleic. În industria alimentară, uleiul de germeni de porumb este utilizat în fabricarea produselor alimentare (produse dietetice, alimente pentru copii, margarină și maioneză).

Uleiul din fructe de cătină (*Hippophae rhamnoides*), susține o creștere sănătoasă a pielii datorită unei cantități mari de acizi grași polinesaturați, tocoferoli, și beta-caroten. Uleiul de cătină promovează creșterea țesutului celular, refacerea țesutului pielii, susține vindecarea rănilor, protejează pielea. Uleiul de cătină poate îmbunătăți metabolismul și întârzie maturarea pielii, încetinind astfel procesul de îmbătrânire, probabil datorită efectelor unor niveluri ridicate de vitamine A și E.

În acest studiu am arătat posibila destinație a combinației de ulei gras din germeni de porumb și ulei de cătină, atât ca vehicul, cât și ca ingredient activ în produse farmaceutice pentru uz oral, datorită proprietăților antimicrobiene intrinseci ale acestui amestec, proprietăți care se vor reflecta atât în calitatea cât și în siguranța produselor finite.

Acest studiu a fost efectuat în conformitate cu metodologia din Farmacopeea Europeană și anume din monografia „Eficacitatea conservanților antimicrobieni", demonstrând efectul antimicrobian, atât din punct de vedere calitativ cat și cantitativ, pe următoarele microorganisme standard: *Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Escherichia coli ATCC 8739, Candida albicans ATCC 10231* și *Aspergillus niger ATCC 16404.*

Acest studiu a avut ca scop demonstrarea eficacității antimicrobiene a combinației de uleiuri grase organice, combinație fără conservanți în formula de fabricație.

Ambele ingrediente au fost 100% certificate organic. Ingredientele au fost pure și nu conțineau aditivi sau substanțe chimice; nehidrogenate și lipsite de organisme modificate genetic (OMG).

Keywords: Maize-germ oil, Sea Buckthorn oil, European Pharmacopoeia, Efficacy of antimicrobial preservatives.

Introduction

Maize-germ oil is obtained from seedlings of *Zea mays* L. (*Gramineae*) by pressing (cold-pressed maize-germ oil) and is highly valued in nutritional terms on account of the palmitic acid content and, of the high oleic and linoleic acid content. In the food industry, maize-germ oil is used in the production of foodstuff (margarine, mayonnaise, diet products, and baby foods). In the cosmetics industry, maize-germ oil is also used in the production of soaps and hair-care products [3,12].

Sea buckthorn seed oil is useful, highly prized, and a specific oil typically used to treat damaged skin, ulcerations, cancer, acne, dermatitis, scar tissue, wrinkles, eczema, burns, obtained from whole Sea buckthorn berries (*Hippophae rhamnoides*). It supports healthy skin growth by high amounts of polyunsaturated fatty acids, tocopherols, and beta carotene. Sea

buckthorn seed oil promotes cell tissue growth, restoring skin tissue, supports wound healing, protects skin from anti-inflammatory effect, UV damage and absorption. Sea buckthorn extracts can actually improve metabolism and retard skin maturation, thus slowing the aging process, possibly because of the effects of its high levels of vitamins A and E. Current researches indicate that not only it enhances the immune activity and disease resistance, but it also destroys harmful free radicals found at the skin level. This oil is exceptionally rich in essential fatty acids, carotenes, tocopherols, and phytosterols [5, 9, 10, 13].

Sea buckthorn fruit (berry) and seed oil possess more than 190 key bio-active nutrients. These key bio-active nutrients include vitamins, omega fatty acids and minerals. Over the past few decades extensive studies have been conducted in China, the former USSR and Scandinavia into the properties and constituents of sea buckthorn. These studies revealed that sea buckthorn contains over 190 active minerals and nutrients, including powerful antioxidants, omega fatty acids, vitamins C, A and E, beta-carotene, free amino acids, flavonol, and carotenoids. Sea buckthorn is a potent source of rare omega 7 fatty acids, which is credited with many of its exceptional healing properties. The plant is used worldwide in many forms to treat a huge range of conditions and complaints, including: stomach and gastro-intestinal diseases, sunburn and sun damage, asthma and chest congestion, night blindness, dry eye, arthritis, radiation burns, gout, bedsores, burns, cuts, high blood pressure, wrinkles, blood vessel diseases, acne, high cholesterol, dermatitis, dry skin, eczema, obesity, skin rashes, ulcers. It's also used as a general tonic to modulate the immune system.

The study of this combination was performed: with the objective of demonstrating its own antimicrobial activity in addition to the benefic effect on the human body, in order to scientifically demonstrate the maintenance of the microbiological quality for a multidose oral product, particularly for multidose containers during normal conditions of use and to provide additional scientifically proofs supporting the product stability in general and prevention of proliferation or limitation of microbial contamination which, during normal conditions of storage and use could occur in a product and present a hazard to the patient from infection and spoilage of the combination. Antimicrobial preservative properties of the formulation must not be used as a substitute for good manufacturing practice [2, 4], this product is the result of sustained and prolonged research and development activity, a continue care for the product's development and improvement.

A further object was to demonstrate that the antimicrobial efficacy may be enhanced by one compound or more from the natural organic, and

also for sustaining the current green policy in force: natural products, without preservatives, in the fight for the human's health and safety, for a clean environment, in respect for the nature.

Materials and Methods

The two organic fatty oils were obtained by cold pressing technique from organic plant materials, by the authorized manufacturer, SC Aroma Plant SRL.

The mixture of fatty organic oils was tested for antimicrobial effectiveness by European Pharmacopoeia A (EP-A) standards, the most stringent standards of the three major compendia, Japanese Pharmacopoeia, United States Pharmacopoeia and European Pharmacopoeia [2, 7, 11], which specify two early sampling time points (6 and 24 hours) not required by the United States Pharmacopeia or Japanese Pharmacopoeia. Culture media were inoculated with between 10^5 and 10^6 colony-forming units of the test organisms: *Staphylococcus aureus ATCC 6538*, *Pseudomonas aeruginosa ATCC 9027*, *Escherichia coli ATCC 8739, Candida albicans ATCC 10231* and *Aspergillus brasiliensis ATCC 16404*. Sampling and enumeration were conducted at protocol-defined time points throughout 28 days. The efficacy of antimicrobial preservation was tested by adding to the studied product an established quantity of a suitable micro-organism, which presents a risk for infection or spoilage. Then the infected product was maintained at the prescribed temperature and there were taken samples with a specified periodicity to count the number of bacteria or/and fungus, on or in a specified culture media.

The tests were carried out under standard conditions, in compliance with European Pharmacopoeia requirements: agar medium B for bacteria, agar medium C without the addition of antibiotics for fungus, recently grown stock culture of each of the specified test micro-organisms, a concentration about 10^8 micro-organisms per milliliter [1, 2, 4, 7].

The incubation of the bacterial cultures was performed at 30-35 ^{0} C for 18-24 h, the culture of *Candida albicans* at 20-25 ^{0} C for 48 h and the culture of *Aspergillus niger* at 20-25^{0}C for one week or until good sporulation was obtained. The determination of the number of colony-forming units (CFU) per milliliter in each suspension was performed by plate count or membrane filtration method.

The suspensions were used immediately after preparation. The volume of the suspension from the *inoculum* did not exceed 1% of the volume of the product and was mixed thoroughly to ensure homogeneous distribution. The elimination of any residual antimicrobial activity of the

product was made by dilution, filtration or the use of a specific inactivation method. The inoculated product was maintained at 20-25 ^{0}C, protected from light.

The appointed times were, according to the European Pharmacopoeia requirements for the *"Efficacy of antimicrobial preservatives of Oral preparations":*T_0= time 0 of contact between sample (preparation) and control (culture medium and micro-organism) for bacteria and fungus; T'= after 14 days of contact, for bacteria and fungus; T"= after 28 days of contact, for bacteria and fungus.

At these periods of time it follows the decrease of the initial viable micro-organisms number comparative with the initial time (T_0).

The preservative properties of the preparation are adequate if, in the conditions of the determinations, there is a significant decrease or no increase, as appropriate, in the number of micro-organisms into the inoculated preparation after the specified time and at the prescribed temperatures [2].

Table I

Timing of practical testing [2, 4, 6, 7, 8]

Crt. No.	Time of contact between sample and control	Type of time
1	T_0- time 0 of contact between sample and control	immediately after sample inoculation with mico-organism test = pharmacopoeias time
2	T_1- at one hour from the sample inoculation with standard micro-organism	intermediate time
3	T_2 - at three hours from the sample inoculation with standard micro-organism	intermediate time
4	T_3- at six hours from the sample inoculation with standard micro-organism	intermediate time
5	T_4- at 24 hours from the sample inoculation with standard micro-organism	intermediate time
6	T_5- at 48 hours from the sample inoculation with standard micro-organism	intermediate time
7	T_6- at seven days from the sample inoculation with standard micro-organism	intermediate time
8	T_7- at 14 days from the sample inoculation with standard micro-organism	Pharmacopoeias time T'
9	T_8- at 21days from the sample inoculation with standard micro-organism	intermediate time
10	T_9- at 28 days from the sample inoculation with standard micro-organism	Pharmacopoeias timeT''

The criteria of acceptance, in terms of decrease in the number of micro-organisms with time, vary for different types of preparations according to the degree of protection intended: Oral preparation. The evaluation criteria of antimicrobial activity are given in terms of the log

reduction of the number of viable micro-organisms against the value obtained for the *inoculum*. For the oral preparation the accepted criteria are presented in table II.

Table II

Efficacy of antimicrobial preservation-Criteria of acceptance for Oral preparations

	Decrease, Logaritmic reduction	
	14 days	**28 days**
Bacteria *Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Aspergillus niger ATCC 16404*	3	NI*
Fungs *Candida albicans ATCC 10231*	1	NI*

NI* = no increase

The test of the efficacy of the antimicrobial activity is not intended to be used for routine control purposes, batch by batch, of finished product [2].

Results and Discussion

Antimicrobial properties of the studied product by the test for efficacy of antimicrobial preservation were demonstrated and could be emphasized by the curves' trend from Figures 1 (*Staphylococcus aureus ATCC 6538*); 2 (*Pseudomonas aeruginosa ATCC 9027*); 3 (*Escherichia coli ATCC 8739*); 4 (*Candida albicans ATCC 10231*) respectively 5 (A*spergillus niger ATCC 16404*).

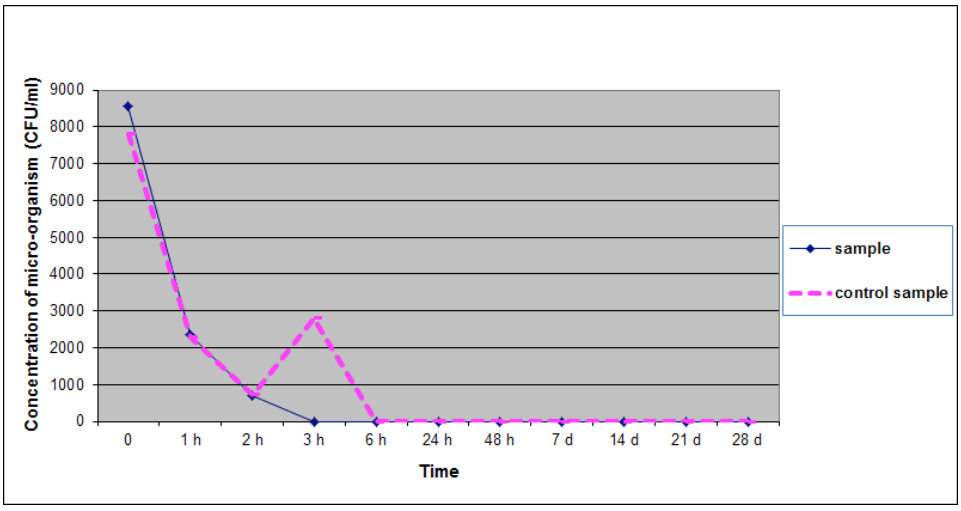

Figure 1

Test for efficacy of antimicrobial preservation for the mixture of Sea buckthorn oil with Maize germs oil, on *Staphylococcus aureus ATCC 6538*

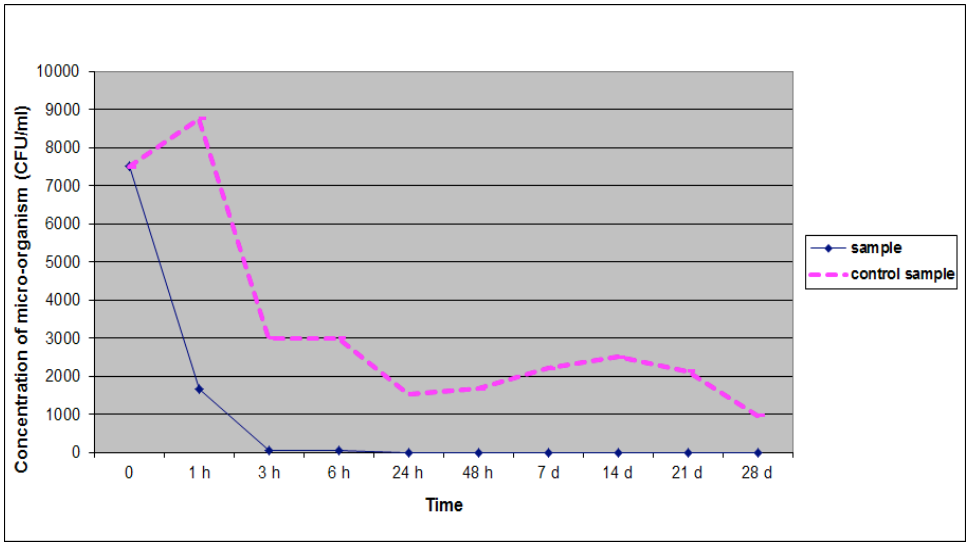

Figure 2

Test for efficacy of antimicrobial preservation for the mixture of Sea buckthorn with Maize germs oil, on *Pseudomonas aeruginosa ATCC 9027*

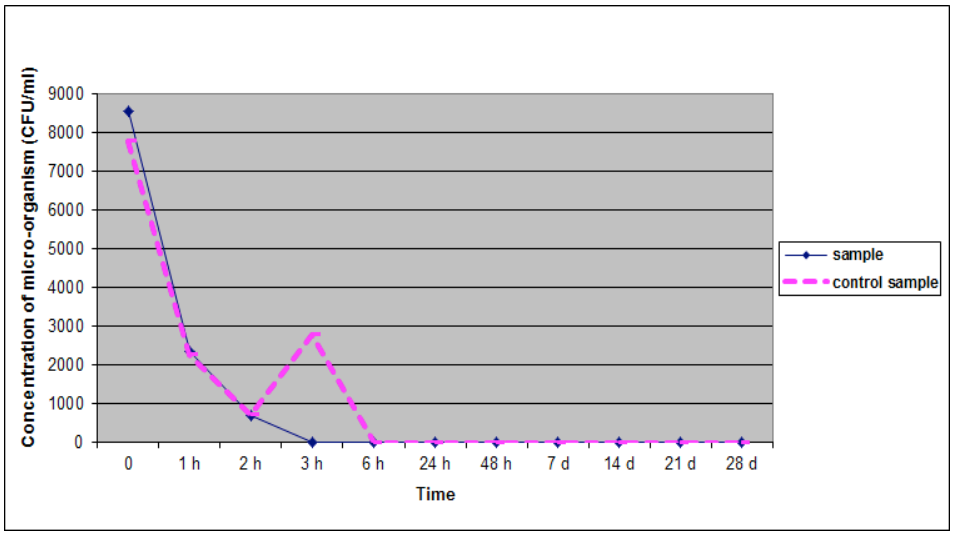

Figure 3

Test for efficacy of antimicrobial preservation for the mixture of Sea buckthorn with Maize germs oil, on *Eschericia coli ATCC 8739*

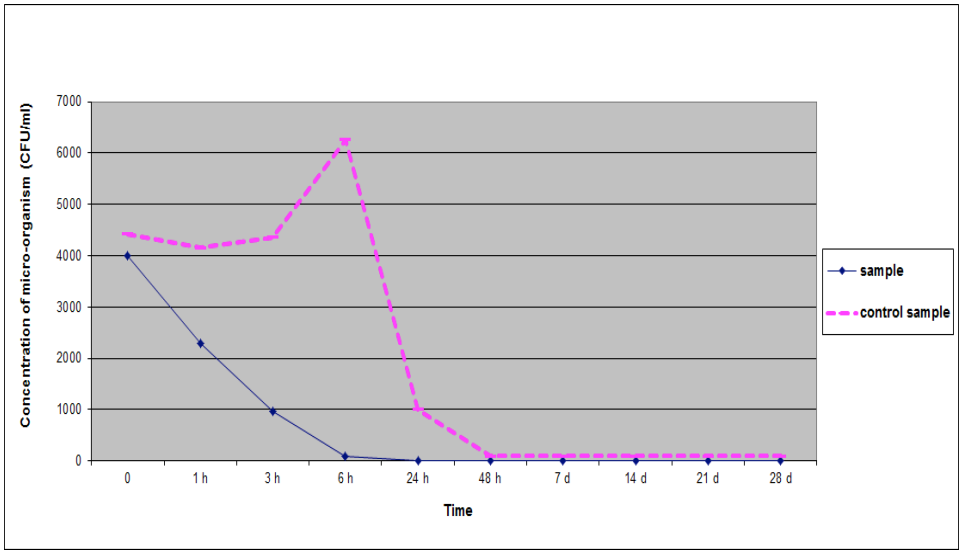

Figure 4
Test for efficacy of antimicrobial preservation for the mixture of Sea buckthorn with Maize germs oil, on *Candida albicans ATCC 10231*

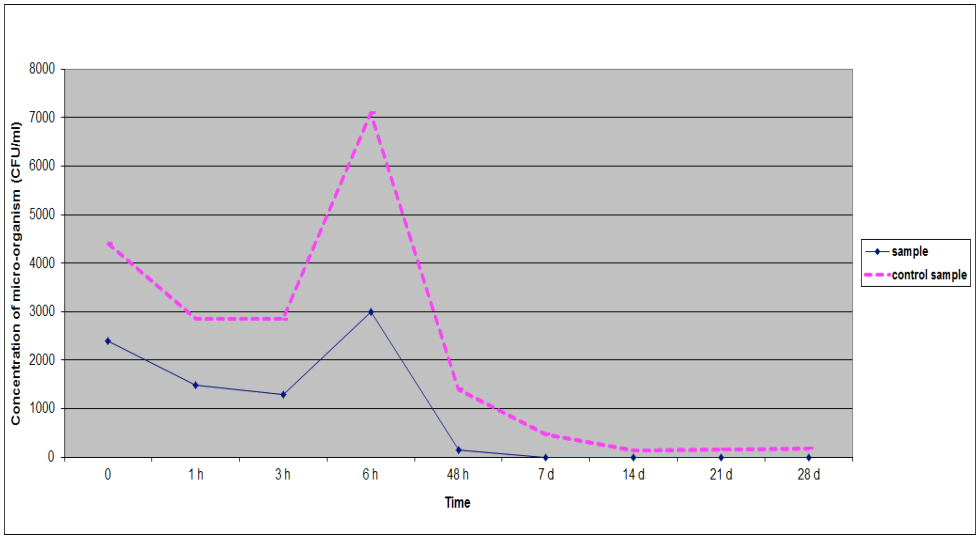

Figure 5
Test for efficacy of antimicrobial preservation for the mixture of Sea buckthorn with Maize germs oil, on *Aspergillus niger ATCC16404*

The studied product, a mixture of maize-germ oil and sea buckthorn oil, presents antimicrobial activity on the *Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Eschericia coli ATCC 8739, Aspergillus brasiliensis ATCC 16404* and *Candida albicans ATCC 10231*

because it could be observed a decrease of the bacteria number by a factor of at least 10^2/mL product within 14 days, the logarithmic expression being 5. After this time the micro-organism registered no increase, NI (= No Increase).

With these results it can be concluded that the product could achieve the criteria and justify the efficacy of antimicrobial preservation, in compliance with the acceptance criteria requested by the current edition of European Pharmacopoeia [2].

Conclusions

The natural antimicrobial activity was demonstrated by the test for efficacy of antimicrobial preservation in compliance with European Pharmacopoeia. The existence of these properties was demonstrated, according to the criteria of acceptance for oral use products.

The obtained results are due to both extracts from the mixture of natural organic fatty oils (Sea buckthorn oil and maize germs oil), optimal ratio between those two ingredients from the mixture and by the kind of primary packaging.

The rapid microbial inhibition, along with the complete reduction of all microbial challenges with combination oral solution, demonstrates its antimicrobial activity in this formula without preservative system and will afford greater protection against contamination and subsequent exposure to microbial damages during normal use as finished products.

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