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PREPARATION OF VANISHING CREAM CONTAINS CURCUMIN FREE CHIMICAL PRESERVATIVE WITH PROPOLIS AS A MODEL OF NOVEL NATURAL PRESERVATIVE

Katayoun Drakhshandeh[1], Kaveh Berenjian[2*], Elham Arkan[3]

1. *Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Department of Pharmaceutics, Phd Candidate, Tehran University of Medical Sciences, Tehran, Iran*
3. *Nano Drug Delivery research center, Kermanshah University of Medical Sciences, Kermanshah, Iran*

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ABSTRACT

Introduction: adding preservative is necessary for the production of topical medications such as vanishing creams and also adding chemical preservative is often accompanied by different side effects. Therefore, the need to use a natural compound with preservative properties that could be a suitable and low-cost alternative for chemical preservative with no side effects is felt. Propolis is a natural compound that its pharmacologically active contents are flavonoids and flavone. It also has some of the phenolic including: cinnamic alcohol, cinnamic acid, vanillin, benzyl alcohol, benzoic acid, caffeic acid and ferulic acid and the main factor for germicidal effects of propolis is caffeic acid.

Materials and methods: preparation of anti-inflammatory vanishing cream containing curcumin and propolis and investigating preservative effects of propolis is done during the four stages of preparing curcumin extract from Curcuma longa rhizome by soxhlet, preparing vanishing cream formulation using stearic acid and eucerin as the fat phase and triethanolamine as water basis, phenytoin and sodium lauryl sulfate as the absorption enhancer and Emulsifier and hydroxyanisole as antioxidants, preparing propolis extract by crushing the propolis samples, addition of 96% ethanol, stirring the mixture one or two times for 3 days, and keeping in a warm and dark place for one to two weeks and smoothing for one day in 1-4 centigrade temperature, contracting and separating alcohol by Rotary devices, and performing microbial tests through pour plate method.

Findings: at the start of microbial testing, zero time of the vanishing cream containing preservatives (methyl paraben + propyl paraben) and the vanishing cream without preservatives and the vanishing cream containing propolis were all resistant to the microorganisms. After a week, microorganisms grew in the vanishing cream without preservatives, but no microorganism was still able to grow in the vanishing cream containing propolis and preservatives.

Discussion and conclusion: this study, like other studies, indicates the preservative activity of propolis. In addition, due to lack of toxicity, the natural composition of propolis and availability of this composition as a preservative, it can be a good alternative to the usual preservatives in vanishing creams.

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Introduction

Vanishing creams are a suspension mixture of oil and water and adding pharmaceuticals is also possible. However, adding preservative is necessary because of counteracting the damaging effects of toxins produced by bacteria and fungi in topical medications that are caused by water reservoirs of ointments, gels and vanishing creams. Adding chemicals preservative is

Corresponding Author: Kaveh Berenjian, Department of Pharmaceutics, PhD candidate, Tehran university of Medical Sciences, Tehran, Iran Email: Kaveh.Berenjian@gmail.com

often accompanied by different side effects. One of the challenges for manufacturers of topical medication is the type and amount of preservative required for each drug and its effects on the skin of different people, especially in cases where the drug is used for damaged skin. The necessity to apply a natural preservative compound without side effects that can be a suitable and low-cost alternative to chemical preservative is felt. Curcumin is an analgesic and anti-inflammatory natural compound that is obtained from the extract of curcuma longa. Like other plants, it belongs to the family of zingiberaceae that is a source of materials with interesting Phytochemistry and various pharmacological effects and a number of plants of this family are used in traditional medicine [1]. Propolis is a sticky and pasty form material that is produced by worker bees for various uses including closing the pores of the hives and preventing the effects of light and moisture and coping with external factors as well as decontamination and adjustment of ambient temperature of hives. This non-toxic sticky material is divided into 12 types on the basis of physical and chemical properties and geographical location the substance is prepared from. Propolis is like a wax compound that is softened at 25 to 45 ° C, but in terms of strength, it hardens at less than 15 ° C and at temperatures above 45 ° C, it increasingly becomes sticky and resinous. It will become fluid at 60 to 70° C and some cases my reach the melting point of 100 ° C. The most common solvent for business extraction includes ethanol, ether, glycol, and water and Propolis is dissolved in organic solvents such as ethanol, acetone, and benzene in different amounts (2].

The most important pharmacologically active contents of propolis are flavone and flavonoids. Flavonoids play an important role in plant pigments the most notably of which is 7 and 5 Dihydroxytry flavon. The role of flavonoids has been approved in biological activity of propolis. At least 38 types of flavonoids is identified in propolis that include: Galangine, Kamenpool, quercetin, pinocembrin, and Pynobangine. Some of the phenolic include: cinnamic alcohol, cinnamic acid, vanillin, benzyl alcohol, benzoic acid, ferulic acid, and caffeic acid. The main preservative effect of propolis is caffeic acid. It is also found that propolis contains high amounts of vitamins, especially vitamin B complex, vitamin C, vitamin E and provitamin A and minerals and trace elements such as calcium, magnesium, iron, zinc, silica, potassium, phosphorus, manganese, cobalt and copper [3]. It should be noted that the overall quality and color of propolis depends on plant sources used by bees per hive [4, 5]. Ethno pharmacology review of this material in Iran is the healing properties of this compound such as treating coughs, colds, chest pains, burns, wounds, dental and gum problems as an preservative [6]. Healing features of propolis are anti-hepatotoxicity, anticancer, antioxidant, anti-inflammatory, antimicrobial and antiviral properties and immune-enhancing activity [2, 7, 8, 9].

Materials and methods

Preparation of anti-inflammatory vanishing cream containing curcumin and propolis and investigating preservative effects of propolis is done in four stages. The first stage is extracting curcumin from Curcuma longa through Soxhlet. The mechanism of this process is in fact the leaching process or extracting from the solid by liquid (solvent) and is based on the principle that the oil is dissolved in the soluble solvent, which is usually n-hexane, until it is saturated and gets out of the pores of oil seeds. When dissolution reaches saturation in hexane, a balance is established between liquids outside the solid (Mysla) and liquid inside the solid (oil and Mysla) and to the same number that the oil molecule gets out of the oil seed particles, oil molecules gets into the solid phase. Factors such as temperature, extraction time, solvent content, grain moisture content, particle size, shape and size of the crushed particle affect the extraction process.

Methodology of the first stage (curcumin extraction):

1) 100 grams of rhizome of turmeric is powdered, the smaller the particles, the better.
2) A filter paper is shaped as a tube with closed bottom and diameter of the tube should be such that it is easily inserted into the soxhlet tube.
3) 200 cc n-Hexane is poured into a round bottom flask and attached to a clamp and a few pieces of conglomerate is added to the flask.
4) Soxhlet and condenser are installed on the flask.
5) The faucet is opened so that the water flows in the condenser.
6) A wire mesh is placed under the flask and Bunsen burner is lighted under it.
7) When the first drop of solvent is distilled and leaked from the condenser, the time is recorded.
8) Extraction will continue for five hours.
9) Heating is stopped after five hours and the system is let to cool down slightly and all vapors in the condenser are cooled down and entered into the liquid phase.
10) Then, the condenser is removed and Soxhlet is separated. In the final stage, flask is separated from clamp.
11) The content of the flask is transmitted to the beaker and solvent is let to evaporate.

Curcumin powder is obtained after extraction and drying. The curcumin can be used in a variety of topical medications such as vanishing creams.

Second stage: preparing anti-inflammatory vanishing cream containing curcumin

To prepare the vanishing cream containing an extract of the rhizome of turmeric (curcumin), stearic acid and ocerine are first used as the fat phase to produce the method base of vanishing cream. This means that stearic acid is melted at a temperature of 70-80 °C to be fully transparent, then solid ocerine is added to stearic acid at the melting point of stearic acid so that this

section of the fat base is also melted and completely uniform and transparent. For the construction of water base, triethanolamine is used. First, it is dissolved in a given volume of water in a beaker and sodium lauryl sulfate and Tween 20 are dissolved in another beaker (tween and sodium lauryl sulfate are used as absorption-enhancer and Emulsifier). Para-hydroxyanisole is added to the water base as antioxidants. Then, water base is heated at 70 ° (bain-marie). At the end, it is added to the fat phase maintaining the 5 degrees difference in temperature (temperature of the water phase is greater). In this stage, curcumin obtained from extraction is softened with a liquid paraffin and added to the vanishing cream base.

Third stage: propolis extraction and curcumin addition to the vanishing cream

Propolis samples are crushed and 10 grams of it are carefully weighed, poured in a flask of 250 ml, then the sample size reaches 100 ml by 96% ethanol, and the mixture is blended well to prepare the extract. This is repeated for one or two times for 3 days, then the mixture is kept in a warm and dark place for one to two weeks. After that, the mixture is softened and kept at 1-4 degrees centigrade in the refrigerator for one day. The liquid is then filtered again and extract obtained is kept at impenetrable and opaque glass. The remaining alcohol in the suspension that is obtained by Soxhlet is completely separated and pure alcohol extract is obtained. Alcohol is separated by Rotary devices and pure extract is obtained.

The extract is then melted and crashed with liquid paraffin and then added to the vanishing cream containing curcumin created in the previous step.

Fourth stage: carrying out microbial tests

10 g of desired samples are separately uniformly mixed with 90 ml diluent so that the initial dilution is .1. In addition, 1 g of each sample is uniformly mixed with 49 ml of diluent so that the dilution of .02 is obtained. Dilutions .1 and .01 are also prepared and 1 g of them is poured in the sterile plates (three plates for ach dilution) under sterile conditions and is pour plated by Soybean Casein Digest Agar medium and then incubated at 30-35 degrees centigrade for 10 days. In order to count Pseudomonas aeruginosa, Staphylococcus aureus, and Candida-albicans, surface method is used in the intended medium.

The reason for cultivation in different dilutions is the possible contamination that precisely determines the amount of pollution. Incubation condition of cultivation, except in the case of Candida-albicans in the second phase (cultivation in solid medium of Sabouraud Dextrose Agar), is at a temperature of 35-30 ° C (48 hours) up to seven yesterday [11 , 10].

Finally, the presence or absence of colonies on the plate is reviewed.

Findings

The results of investigating the preservative effects of alcoholic extract of propolis compared with the usual preservatives (methyl paraben and propyl parabens in a ratio of 9: 1) show the effectiveness of the herbal blend. Preservative activity of propolis on some pathogenic bacteria such as streptococcus sirquets, Streptococcus pyogenes, Streptococcus mutans, Streptococcus sobrinus, Staphylococcus aureus, and Salmonella is well described, both in vitro and in clinical conditions. In fact, research in this field has shown that the multiple activity of propolis makes glycans synthesized by bacteria insoluble and thus, Glycosyl transferase enzyme activity, which is an enzyme involved in glucose transport and growth and reproduction of bacteria, is stopped [12].

Propolis also prevents synthesis of bacterial protein and changes the nature of the cytoplasmic membrane and cell wall and eventually cause lysis of bacteria. On the other hand, it has been shown that propolis destroys sporulation bacteria causative agent of Luke American disease in bees, and also prevents the reformation of new spores and their growth in cultivation medium. This finding indicates the presence of the substances in propolis that impact spore bacteria and destroy them in living environment [13] in **[Table 1]**.

Table 1: comparing preservative effect of propolis with preservative (methyl paraben and propyl parabens) in the anti-inflammatory vanishing cream containing curcumin

he results of cultivating vanishing cream formulation without preservative using pour plate method at zero time			
Type of the microorganism under study	Dilution .1	Dilution .02	Dilution .001
Mesophilic aerobic bacteria	Negative	Negative	Negative
Molds and yeasts	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative
Pseudomonas aeruginosa	Negative	Negative	Negative
Escherichia coli	Negative	Negative	Negative

The results of cultivating vanishing cream formulation without preservative using pour plate method after one week			
Type of the organism under study	Dilution .1	Dilution .02	Dilution .001
Mesophilic aerobic bacteria	Positive	Positive	Positive
Molds and yeasts	Positive	Positive	Positive
Staphylococcus aureus	Positive	Positive	Positive
Pseudomonas aeruginosa	Positive	Positive	Positive
Escherichia coli	Positive	Positive	Positive

The results of cultivating vanishing cream formulation with usual preservative (Methyl paraben Propyl paraben 9: 1) using pour plate method at zero time			
Type of the microorganism under study	Dilution .1	Dilution .02	Dilution .001
Mesophilic aerobic bacteria	Negative	Negative	Negative
Molds and yeasts	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative
Pseudomonas aeruginosa	Negative	Negative	Negative
Escherichia coli	Negative	Negative	Negative

The results of cultivating vanishing cream formulation with usual preservative (Methyl paraben Propyl paraben 9: 1) using pour plate method after one week			
Type of the organism under study	Dilution .1	Dilution .02	Dilution .001
Mesophilic aerobic bacteria	Negative	Negative	Negative
Molds and yeasts	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative

The results of cultivating vanishing cream formulation with propolis at zero time			
Type of the microorganism under study	Dilution .1	Dilution .02	Dilution .001
Mesophilic aerobic bacteria	Negative	Negative	Negative
Molds and yeasts	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative
Pseudomonas aeruginosa	Negative	Negative	Negative
Escherichia coli	Negative	Negative	Negative

The results of cultivating vanishing cream formulation with propolis after one week			
Type of the microorganism under study	Dilution .1	Dilution .02	Dilution .001
Mesophilic aerobic bacteria	Negative	Negative	Negative
Molds and yeasts	Negative	Negative	Negative
Staphylococcus aureus	Negative	Negative	Negative
Pseudomonas aeruginosa	Negative	Negative	Negative
Escherichia coli	Negative	Negative	Negative

Discussion and conclusion

In this study, preservative activity of propolis extract as a natural herbal compound was investigated and it was found that the extract of propolis has an antibacterial activity similar to usual preservatives (methyl paraben + propyl paraben) in the vanishing cream production.

In the study of Kujumgiev et al. (1999), the antibacterial properties of propolis against Staphylococcus aureus and E. coli was investigated and the results showed the positive effect of propolis on bacteria, which is in line with the results of this study. He even demonstrated that simultaneous use of propolis with some antibiotics increases its effects. In fact, propolis with antibiotics tested also has a synergistic effect in terms of antibacterial properties [14].

However, studies have shown that propolis is more effective against Gram-positive bacteria than Gram-negative bacteria. The difference in effects is often caused by different structure of the cell wall of Gram-positive and gram-negative bacteria [13]. The reason is the composition of the cell wall of these bacteria, which has only a thin layer of mocopeptide and the majority of cell wall structure consists of outer membrane that is mainly of lipopolysaccharide. This is while the cell wall of gram-positive bacteria is composed of a large amount of complex composition and is largely mocopeptide resistant and that is why they are more resistant to antibacterial substances [13, 15].

Brumfitt et al (1990) investigated antibacterial effect of propolis on Staphylococcus aureus bacteria and growth inhibition zone diameter turned out to be 14 mm in antibiogram tests [16].

Velazquez et al (2007) found that the amount of MIC of ethanol extract of propolis on Staphylococcus aureus bacteria is 1.0 mg per ml [17].

In a study in 1985, it was found that Propolis was effective in 209 strains of Staphylococcus aureus and its MIC is 1 mg per ml and its MBC is 12 mg per ml [18].

Antibacterial effect of propolis on some of the microorganisms tested in this study can be related to the type of active ingredients present in the extract and extraction methods and solvents [19]. Specifically, this is more important when the structure and composition of propolis varies from region to region and even propolis produced in one region in different seasons is different to the other seasons [20].

It should be noted that several studies have reported that antibacterial effect alcoholic extract of propolis is much better, which is because of freeing and better purification of flavonoid that is indeed the main active component of propolis [21]. In this regard, it has been shown that ethanol extract of propolis has several features, including antibacterial, antiviral, anti-inflammatory properties and is also immune stimulant [22].

So given that propolis is a safe and yet non-toxic antimicrobial substance, propolis can be used as a good alternative in pharmaceutical production, especially topical medications, and advances in medical science and safe pharmaceutical compounds.

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