Study on An Enzyme Shampoo of Traditional Chinese Medicine

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Abstract. This research rationally exploits Chinese medicine resources and develops a kind of Chinese traditional medicine enzyme shampoo, which is developed by utilizing the antioxidant and bacteriostatic properties of black soybean enzyme, and combining with nine-flavored plant ingredients including mulberry leaf, Semen Cuscutae, astragalus mongholicus, cacumen platycladi, angelica sinensis, fructus kochiae, cortex dictamni, fructus psoraleae, and folium artemisiae argyi. At present, the problem of traditional Chinese medicine enzyme preparation has been effectively solved, and preliminary experiments on external treatment of the scalp have been carried out, which proves that it has a better anti-dandruff and nourishing effect.

1. Research Background

Enzyme is the Japanese name for enzymes, mainly refers to a low-salt liquid containing biologically active substances extracted from plants after deep fermentation. It has a strong catalytic ability, can speed up the chemical reaction, but will not affect or change the products of chemical reaction. It has been proved that the enzyme has the function of anti-oxidation, bacteriostasis and anti-inflammation[1]. Shampoo is one of the fastest growing products in the personal care market, which has the functions of removing Dandruff, dyeing hair and baking oil. The ancients used natural soap to clean their hair. Later, Surfactant-based Shampoos became popular. Now, more and more Chinese herbs, plant extracts, etc. are used in shampoos, shampoo is also moving toward cleaning, maintenance and other functions of the multi-direction development. Enzyme shampoo refers to a functional shampoo prepared with enzymes, which can effectively improve hair quality problems.

The Chinese Medicine Enzyme Shampoo developed in this paper is prepared by adding, subtracting and cutting Qibaomeirandandan and combining with black soybean enzyme. It uses technical means to fuse the Chinese herbal medicine liquid with the original enzyme solution, and externally apply it to the scalp to realize the integration of washing and protecting and achieve an effective anti-dandruff effect.

1.1 Causes of scalp problems

Existing studies have shown that problems such as dandruff, itching, and oily head are caused by Malassezia and other harmful colonies that use oil as a source of nutrients. Its abnormal reproduction destroys the scalp barrier, and toxic substances invade the scalp to cause an inflammatory reaction, which leads to excessive cell proliferation, and more fully developed cell populations are produced on the scalp surface, which forms excessive dandruff after shedding. From a medical point of view, the problem of dandruff is not serious, but from the perspective of beauty and people's daily life, it will bring certain troubles. In traditional Chinese medicine, "hair is the manifestation of blood, and hair is the master of the Kidney". Thinning or dry hair is mostly a symptom of insufficient blood essence,
liver and kidney[2-4]. At present, the treatment of dandruff is mainly based on ZPT, ketoconazole and other anti-fungal agents. If it is used for too long, it is easy to develop drug resistance, and it will destroy the scalp barrier and cause subsequent hair problems.

1.2 Research Progress of Enzyme Shampoo Products
The source of raw materials for enzymes is very wide, and it can be fermented with fruits, flowers, grains, etc. as raw materials. In the fermentation process, the microorganism uses the nutrients in the raw material to carry on the complex material metabolism, spurs many kinds of metabolite and the secondary metabolite. Nowadays, many plants and fungi are used as raw materials for enzyme production, which contain bioactive components after fermentation by microorganisms. The fermentation mode can be divided into natural fermentation and inoculation fermentation. The former is based on the raw materials and the dominant bacteria in the environment for fermentation, while the latter is based on one or more microorganisms for fermentation, mainly lactic acid bacteria, yeast and so on. The domestic enzyme research mainly concentrates on the plant enzyme: such as flower enzymes, fruit enzymes, etc., which are mostly used for moisturizing and laxative functions for weight loss, hangover and liver protection.

In recent years, plant enzymes have been widely used in shampoo, and the technology has developed rapidly. In Chenggang et al.’s patent (CN 108938548 A), “Plant Enzyme Nutrition Shampoo”, uses ginseng enzyme, trichosanthin enzyme, and Ligusticum chuanxiong enzyme to form a nutritive shampoo to actively supplement human nutrition and promote detoxification[5], the equipment method is complicated and the cost is high. The Li Yinta et al.’s patent (CN 107854393 A), "An enzyme shampoo and its preparation method", develops a method for preparing an enzyme shampoo from eggshell membranes, which is mainly used to supplement nutrition and reduce split ends [6]. In the domestic research and development of enzyme shampoos, there are few researches on effective anti-dandruff and realization of maintenance, and lack of reasonable ancient prescription support.

In 2016, Guanqun [7] mentioned the possibility of preparing functional green products from plant enzymes, but the following domestic scholars have little empirical research on the treatment of scalp problems with external use of enzymes. However, in the market, there are many types of shampoo products with various effects, and most shampoos are synthesized by adding chemical substances. These products use alkaline to clean the hair, and then use conditioner or essential oil to neutralize the PH (the PH value of healthy hair is 5.0-5.6). The cycle repeats, the hair can only be temporarily protected, and the scalp cannot be removed Conserve at the root. More serious, long-term use will cause certain damage to the scalp environment and may cause other problems. With the improvement of national health awareness, the requirements for shampoo are gradually increasing, and a natural, green, and effective shampoo and hair care product is urgently needed.

1.3 Mechanism of Action of Chinese Medicine
Studies have shown that Chinese traditional medicine enzyme can make the liquid of large molecules into small molecules, which are more easily absorbed by the body[8]. After fermentation, black beans are rich in amino acids and enzymes, which can play an anti-oxidant effect and effectively inhibit the proliferation of Malassezia on the scalp and solve the problem of dandruff.

This shampoo uses nine medicinal materials, including mulberry leaf, Semen Cuscutae, astragalus mongholicus, cacumen platycladi, angelica sinensis, fructus kochiae, cortex dictamni, fructus psoraleae, and folium artemisiae argyi, which are sterilized and made into Chinese medicine extract; Combining the black soybean enzyme stock solution, adding mild surfactants, a strong traditional Chinese medicine shampoo is made. The product has a good effect of anti-dandruff maintenance and strong hair root. Among them, Semen Cuscutae and fructus psoraleae have the functions of tonifying kidney, nourishing essence and anti-oxidation; mulberry leaf and folium artemisiae argyi can dispel wind and stop itching; cacumen platycladi prompts to produce dark hair; cortex dictamni and fructus kochiae can clear heat, remove dampness and stop itching, and inhibit the growth of bacteria; angelica sinensis can invigorate blood and nourish blood, rich in micronutrient, can expand the scalp capillary;
astragalus mongholicus contains saponins, amino acids, etc., which increase skin resistance, improve skin blood circulation, and promote hair growth.

2 Production process

2.1 Instruments
Food grade plastic bucket, multi-function mixer, Roche foam meter, TGL-6C desktop centrifuge, NGL rotary viscometer.

2.2 Chinese medicine enzyme extraction process
1. Weigh 15-20 parts of black beans, wash and break them;
2. Add 500-800 parts of water to the raw material of black bean, boil it for 20-40 min, cool it to 30-32 °C, inoculate 0.1-0.3 g/l active dry yeast in the fermenter, and stir it slowly and evenly;
3. In a temperature environment of 30-40°C, carry out alcohol fermentation for 20-40 days. During fermentation, check the fermenter periodically to remove excess gas from the fermentation process. When a large amount of raw enzyme liquid is produced in the fermentation tank, obtain the filtrate by filtering with a plate-frame filter;
4. Inoculate 0.1-0.3 parts of probiotics, perform aerobic fermentation at 32°C for 60 days, and centrifuge to obtain the supernatant. Carry out ultrasonic sterilization on the enzyme stock solution under the conditions of 25°C, 25KHz for 40s to obtain black soybean enzyme.

2.3 Shampoo formula

2.3.1 Raw material.
Ingredient A: Enzyme stock solution (3-15 parts), mulberry leaf (5-10 parts), Semen Cuscutae (3-5 parts), astragalus mongholicus (3-5 parts), cacumen platycladi (15-25 parts), angelica sinensis (10-20 parts), fructus kochiae (5-10 parts), cortex dictamni (5-10 parts), fructus psoraleae (3-5 parts), folium artemisiae argyi (5-10 parts), soap base (60-90 parts), glycerin (10-20 parts).
Ingredient B: Guar gum (viscosity regulator), propyl betaine (surfactant), methyl isothiazolinone (preservative), etc.

2.3.2 Chinese herbal shampoo preparation.
1. Mix Ingredient A (the original solution of enzyme removal) in order, add 8 times of water and cook for 30 minutes, collect the filtrate, then add 6 times of water and cook for 30 minutes, and combine the two decoctions. Add 70% absolute ethanol for overnight to alcohol precipitation, filter, and heat in a water bath at 100°C to volatilize the filtrate until there is no alcohol.
2. Add the prepared soap base to the Beaker, place in the sink, and keep at 60 °C in a water bath. After the plant soap base is melted to clear, mix the Chinese medicine solution, the black soybean enzyme solution and the soap base in proportion and heat it in a water bath. Add the Ingredient B, pour the milk soap into the mould, stir quickly, cool to 60 °C, stir slowly, then wait 30 - 60 minutes, wait to cool, ultrasound to remove the bubbles, put it in the refrigerator overnight to see if it is layered, filling.

3 Efficacy analysis and evaluation
The evaluation of the efficacy of antidandruff shampoo focuses on the condition of Dandruff, grease and Malassezia after use. As the evaluation of traditional Chinese Medicine Enzyme antidandruff shampoo, it is required to be scaleable, repeatable and comparable, which has statistical significance.

3.1 Evaluation method
1. Randomized, parallel controlled efficacy study. In the experiment, 50 volunteers aged 20-35, half of them male and half of them female, were selected to try the enzyme shampoo. All the volunteers had
flaky white scales on their heads, or excessive Dandruff, and had problems with scalp oil and itching. In order to ensure the accuracy of the experimental results, volunteers are not allowed to use any drugs, health products and other shampoos that affect the results during the experiment, and cooperate with the experimental arrangements to maintain regular life during the study.

2. Treatment method: Wash your hair with this herbal enzyme shampoo and massage your scalp for 2 minutes before rinsing. Wash every 2 days for 4 weeks. The clinical efficacy was evaluated before use, 2 weeks and 4 weeks after use.

3. Evaluation of dandruff: The AFSF score method is used for dandruff detection. Setting: 0 points for no visible scaling; 1 point for occasional small scaling at the hair root; 2 points for visible small scaling at the hair root; 3 points for a small amount of large scaling or a large amount of small scaling; 4 points for a lot of large scale desquamation. Use a digital camera to take pictures of scalp and dandruff and record them.

4. Quantitative cultivation method of Malassezia: Use scrubbing method to inoculate 1cm2 of dandruff from 5 places near the scalp into Sabouraud medium, place it in a constant temperature incubator at 28°C for 1 week, observe and record the distribution of Malassezia colonies.

5. Self-evaluation: Using a questionnaire survey, the subjects were asked to evaluate the improvement degree of Dandruff, hair oil and itching before and after using the product, and recorded by 0-3 points, of which 0 points = little improvement, 1 point = slight improvement, 2 points = significant improvement, 3 = basic recovery or complete improvement.

3.2 Test data and analysis
Using spss statistical software, t test was used for the measurement data which accorded with normal distribution and homogeneity of variance, and rank sum test was used for the measurement data which did not accord with normal distribution and homogeneity of variance, P<0.05 indicates a significant difference, P<0.01 indicates a very significant difference. Table 1 shows the evaluation results of dandruff and Malassezia colonies before and after using this product (persons/cm2). The following figure shows the experimental data:

| Evaluation item | Average dandruff score | Average number of Malassezia colonies |
|-----------------|-----------------------|-------------------------------------|
| Average dandruff score | 2.41±0.35 | 1.78±0.51 | 1.43±0.26 |
| Average number of Malassezia colonies | 6.58±1.21 | 1.52±0.55 | 0.23±0.19 |

Table 2 shows the results of evaluation and analysis: 50 subjects showed significant improvement in Dandruff, grease and itching after treatment. The mean scores of self-evaluation of Dandruff, grease and itching after 2 weeks were 1.46,1.36 and 1.34 respectively, and the mean scores of self-evaluation of improvement of Dandruff, grease and itching after 4 weeks were 2.08,1.94 and 1.78 respectively; there was significant difference between the two groups (p < 0.05).

| Time | 2 weeks after use | 4 weeks after use |
|------|------------------|------------------|
| Scalp problems | Dandruff | Grease | Itching | Dandruff | Grease | Itching |
| Complete improvement (3 points) | 4 | 5 | 7 | 14 | 12 | 11 |
| Significant improvement (2 points) | 19 | 17 | 15 | 26 | 24 | 20 |
| Slight improvement (1 point) | 23 | 19 | 16 | 10 | 13 | 16 |
| Little improvement (0 points) | 4 | 9 | 12 | 0 | 1 | 3 |
4 Conclusion
At present, many stubborn scalp problems have not been effectively solved. The products researched and developed in this paper can solve the problems from the point of view of bacteriostasis, provide effective scalp care and contribute to the inheritance and innovation of traditional Chinese medicine. In this paper, the experimental base is not large enough, the innovation of experimental methods and enzyme preparation technology is insufficient. Therefore, the follow-up research should make specific optimization attempts for process development and formula improvement.

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