Research Article

The study of using Tannin extracts from Indian almond leaves to produce acne patches from discarded silkworm cocoons

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Abstract: *This research focuses on utilizing Tannin extract from Indian Almond leaves to develop acne patches made from discarded silkworm cocoons. The goal is to explore the antibacterial properties of Tannin in addressing acne and to create a natural, eco-friendly skincare product. The process began by extracting Tannin from the Indian Almond leaves, which were boiled in distilled water and then diluted to different concentrations (100%, 50%, 25%, and 12.5%). The antibacterial effectiveness was tested using the disc diffusion method under both aerobic and anaerobic conditions. For comparison, antibiotics like Azithromycin, Roxithromycin, and Vancomycin were used as controls, while distilled water served as the negative control. The results indicated that the 100% Tannin extract had the highest antibacterial efficiency under anaerobic conditions, comparable to Vancomycin. Roxithromycin was the most effective in both oxygen conditions, but the Tannin extract, particularly at 100% concentration, showed promising results as well. In the next phase, discarded silk fibers were processed to extract Fibroin, which was used to create acne patches. Two types of patches were made: one with just Fibroin and the other with a 1:1 mix of Fibroin and Tannin extract. These patches were tested under the same oxygen conditions to assess their antibacterial properties, with Roxithromycin as the control. The results showed that the Fibroin-Tannin acne patch performed similarly to Roxithromycin in inhibiting acne-causing bacteria, demonstrating its potential as a natural alternative for acne treatment. In conclusion, the combination of Fibroin from discarded silkworm cocoons and Tannin extract from Indian Almond leaves can produce an effective, environmentally friendly acne treatment. This study not only highlights a sustainable approach to reducing waste from the silk and agricultural industries but also provides a viable option for natural skincare solutions that benefit both consumers and the environment.*

Keywords: Indian Almond leaves ; Tannin extract ; Roxithromycin

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1. INTRODUCTION

Acne is a skin condition that occurs on the face or other parts of the body that contain numerous sebaceous glands. It is influenced by two main factors: internal factors, such as hormonal changes, and external factors, including medication, diet, and pollution. The size and severity of acne lesions depend on various factors, such as the type of acne (e.g., blackheads, whiteheads, cysts) and bacterial involvement. Acne affects individuals of all ages, but it is particularly common among teenagers due to hormonal fluctuations during puberty (Thararin Clinic, 2021).

In the present days, there are many ways to treat acne, for example, using topical medicine or oral medicine in which the medicine used will have to depend on the severity of the acne. Most of these medicines often use antibiotics that are effective against acne-causing bacteria. This may result in many side effects such as irritation from topical application or a problem which causes the acne in that area to atrophy and cause marks and atrophic acne scars (De med clinic, 2562). Therefore, we have an idea to develop acne patches as an alternative for treating acne using natural substances.

During the dry season in Thailand, especially in the northern region of Thailand, there is a problem with PM 2.5 which is caused by combustion (Phanthawat setthawilai, 2021). Most fuels are from combustion of agricultural waste and fallen dry leaves. From observing the community areas around Varee Chiang Mai school and San Pa Liang village, Nong Hoi subdistrict, Mueang district, Chiang Mai, it was found that tropical Almond trees, which have a large amount of fallen leaves, people usually dispose them by burning them. Interestingly, Indian Almond leaves, often burnt, contain Tannin, a compound known for its skin bacterium inhibition properties. Through research, we've discovered that Tannin's effectiveness can be tested using the disk diffusion method and can easily be extracted through a simple boiling process.

Meanwhile, Thailand, renowned for its silk production, faces a unique challenge. From a field trip to the Queen Sirikit Sericulture Centre (Chiang Mai), it was found that there is a process of selecting and discarding the imperfect shaped cocoons. Moreover, the remaining silk cocoons from the production that are left from that weaving factory will be discarded causing leftover waste and spoilage problems, affecting the community and the surrounding areas. However, silk contains two proteins: Sericin and Fibroin. Fibroin, comprising 70-75% of the cocoon, exhibits natural compatibility with human skin and is extensively used in the medical and cosmetic industries. Therefore, we have the idea to use such cocoon waste to make Fibroin that has the properties of flexibility and good moisture retention and does not cause skin irritation.

2. METHOD & MATERIAL

Preparation of Fibroin silk

First, clean the silk by removing any visible dirt. Next, soak 40 grams of silk in a solution of sodium carbonate (concentration 0.02M) for 30 minutes to degum the silk. Rinse the silk 5-6 times until the wash water is clear. Then, sterilize the silk in an oven at 60 degrees Celsius for 4 hours. After drying at room temperature, shred the silk into small pieces and sterilize again in the autoclave for 60 minutes. Dissolve the degummed silk in a solution of $CaCl_2$ mixed with 20% ethanol (V/v), heating at 75 degrees Celsius for 72 hours. Perform dialysis for 3 days, and finally, centrifuge the dialyzed solution to obtain the final product of pure fibroin solution.

Preparation of Tannin

Wash the Indian Almond leaves to remove dust and dirt with clean water, then sterilize them by autoclaving for 20 minutes. Next, grind 20 grams of the sterilized leaves into a fine powder. Boil the leaves in 1000 milliliters of distilled water for 30 minutes. Filter out the remaining leaf debris using cheesecloth and store the liquid in a container. Finally, prepare the Indian Almond leaves extract by diluting it to the desired concentrations of 100%, 50%, 25%, and 12.5%.

Preparation of P. acnes disk

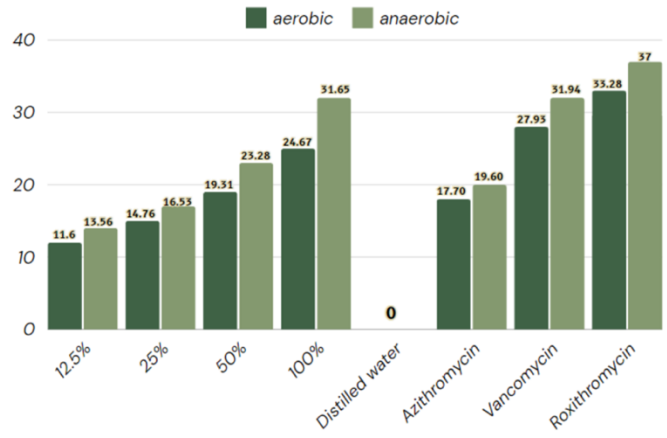
Cultivate acne-causing bacteria on culture plates under both aerobic and anaerobic conditions for 1-3 days. Spread the bacteria onto 12 agar plates. Prepare these 12 plates by dividing them into four sections. On six of these plates, apply Tannin extract in concentrations of 100%, 50%, 25%, and 12.5% under both aerobic and anaerobic conditions. For the remaining six plates, apply Azithromycin, Vancomycin, Roxithromycin, and distilled water (100%) under both conditions. Evaluate the effectiveness of the Tannin extract at 100%, 50%, 25%, and 12.5% concentrations, as well as the antibiotics Azithromycin, Roxithromycin, and Vancomycin in inhibiting the growth of acne-causing bacteria.

Preparation of Fibroin acne patches

Prepare a solution containing gelatin, pure Fibroin, and Tannin extract. Mix these components until homogeneous mixture is achieved. Then, autoclave the mixture for 2 hours. Pour the mixture into pre-prepared molds and allow it to set into the desired shapes. Finally, evaluate the effectiveness of the Fibroin-based acne patches, both with and without Tannin extract in inhibiting acne-causing bacteria under both aerobic and anaerobic conditions. Finally, test the material properties of the Fibroin acne patches.

3. FINDINGS

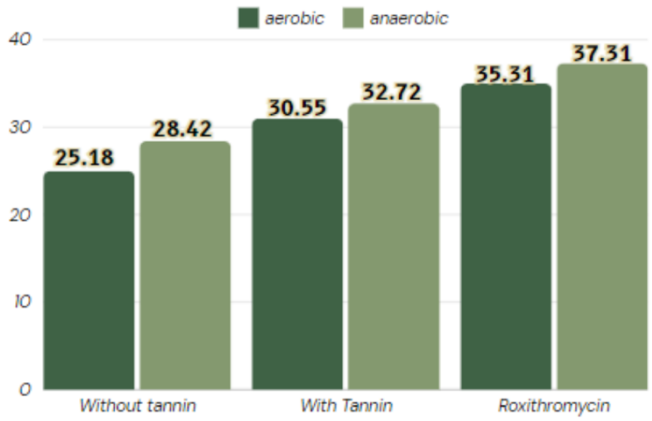
3.1 Experimental Result 1



Testing the ability to inhibit the acne-causing bacteria of Tannin in various concentrations and antibiotics.

Tannin extract at 100% concentration was found to be most effective in inhibiting acne-causing bacteria. In anaerobic conditions, it was more effective than in aerobic conditions. Roxithromycin was the most effective drug in both conditions. While the 100% tannin extract did not match the efficacy of Roxithromycin, it was comparable to Vancomycin in inhibiting acne-causing bacteria.

3.2 Experimental Result 2



Testing the ability to inhibit the acne-causing bacteria of Fibroin acne patches with and without Tannin.

Roxithromycin was the most effective in inhibiting acne-causing bacteria in both conditions. Although the Fibroin-based acne patches with Tannin extract did not match the efficacy of Roxithromycin, they were still nearly as effective in inhibiting the bacteria.

3.3 Experimental Result 3 : Materiality measurement of the fibroin acne patches Fibroin acne patches are translucent with brown tint from the tannins. They have a smooth surface and a low rate of degradation.

Swelling degree	Degradation degree	Elasticity	Gel fraction
28.20%	1.67%	7cm	78.00%

4. DISCUSSION

From experiment 1, it was found that the 100% concentrated Tannin extract was the most effective in inhibiting the growth of acne-causing bacteria. When comparing the two conditions aerobic and anaerobic, the 100% concentration was more effective in anaerobic conditions. Roxithromycin was the most effective antibacterial agent under both conditions. In summary, the 100% concentrated Indian almond leaf extract had antibacterial efficacy against acne-causing bacteria that was not as high as Roxithromycin but was comparable to Vancomycin.

From experiment 2, it was found that Roxithromycin can inhibit acne-causing bacteria the most in both conditions. Even though the Fibroin acne patches containing Tannins are not as effective as the antibiotic, Roxithromycin, their ability to inhibit the growth of acne-causing bacteria is similar to the antibiotic, Roxithromycin.

5. CONCLUSION

The extract from Indian Almond leaves has properties that can inhibit acne-causing bacteria. It is hypothesized that the higher the concentration of the solution, the greater its ability to inhibit the growth of "P. acnes". From the experiments, it was found that Tannin extract from Indian Almond leaves at concentrations of 100%, 50%, 25%, and 12.5% could inhibit the growth of acne-causing bacteria. However, the 100% concentration was the most effective, as indicated by the largest diameter of the clear zone, measuring 31.67 mm in anaerobic conditions and 24.67 mm in aerobic conditions. When compared to the Vancomycin, the 100% Tannin extract had similar antibacterial effects in both conditions.

Comparing the antibacterial efficacy of Fibroin acne patches and antibiotics, Roxithromycin was found to be the most effective in both conditions. When comparing to Fibroin acne patches with and without Tannin extract in both conditions, Roxithromycin consistently showed the highest inhibition of acne-causing bacteria. The Fibroin acne patches with Tannin extract had the effectiveness that can inhibit the growth of acne-causing bacteria close to Roxithromycin.

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