# Develop Of Solid Soap Using Terfezia Claveryi Extract And Evaluation Of Its Antioxidant Activity

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**KEYWORDS**
Soap, Antioxidants, Saponification, Foam Stability, Mushroom Extract, Cosmetics.

**ABSTRACT**
Recently, studies have become increasingly interested in natural antioxidants extracted from Terfezia claveryi due to their ability to enhance body health and prevent common modern diseases. They meet most of the body's needs for prevention, treatment, tissue and cell restoration, and treatment of oxidative stress, which is a common use in the current time. T. claveryi has been identified as a potential fresh cosmetic source due to its biologically active compounds such as phenols, flavonoids, beta-carotene, and tannin. The aim of the current study was to develop solid soap using T.claveryi extract and evaluate its antioxidant activity. The solid soap was manufactured using the traditional cold method.
The results showed the effect of T. claveryi extract in modifying the acidity (pH) of the soap, which reached 7.83. It indicates that the ability of T. claveryi extract to reduce the pH value of the soap compared to soap that does not contain extracts. T. claveryi soap has a foam stability of 47.29% and the control soap has a foam stability of 45.16% and after one hour the stability rate was 95%, so the T. claveryi extract soap has better foam stability compared to the control soap. It enhances the possibility of using T.claveryi extracts to improve the foam properties of transparent solid soap. The antioxidant activity test showed that T.claveryi soap has an antioxidant activity at the highest concentration of 500 mg/ml and a rate of 25.39% compared to the control soap 0.508%. It supports the effectiveness of using T.claveryi extract soap in skin care products such as whitening, moisturizing and anti-aging, while the control soap does not have any antioxidant activity.

## 1. Introduction

*Terfezia claveryi* are known as a rich source of natural biologically active compounds. They are exploited in the cosmetics industry due to their multiple benefits for the skin and hair. These compounds include phenols, polyphenols, terpenoids, selenium, sugars, vitamins, and volatile organic compounds. The compounds are ideal for use in cosmetics due to their antioxidant, anti-aging, anti-wrinkle, and skin-lightening properties, in addition to their moisturizing effects (Hayati et al., 2020). The example of is *Terfezia claveryi*, a large, edible belonging to the Ascomycota family, which grows naturally after rainfall (more than 200 ml from September to October) in arid and semi-arid areas. Truffles are found in the Arabian Peninsula and Iraq, North Africa, the Mediterranean Basin and Western Asia (Al Obaydi et al., 2020).

The main components of mushrooms distributed in three different environments in the Kurdistan Region were studied. Carbohydrates were the most abundant macronutrients, ranging from 24.5% to 37%, proteins 4.4-5.0%, and fats were 2-2.5% (Yousif et al., 2020). It also contains chemical components responsible for antioxidant effects such as antioxidant activity and ascorbic acid. Phenolic, flavonoids, β-carotene, tannin, lycopene, and anthocyanins were the main antioxidant compounds compared to β-carotene and ascorbic acid content (Yousif et al., 2020).

*T. claveryi* species produce common dietary antioxidants including tocopherol (α and δ), butylated hydroxyanisole (BHA), hydroxytoluene (Tejedor-Calvo et al., 2021), and unsaturated fatty acids such as oleic acid and linoleic acids (Tejedor-Calvo et al., 2021). Cosmetology is a growing scientific field that has a direct impact on society, as the cosmetics sector focuses on the search for new biological alternatives to improve the properties of products and replace chemical compounds. Many of the compounds used in this sector are based on biological sources such as bacteria, fungi, and algae. These biological materials are characterized by their biosurface activity and contain vitamins, antioxidants, pigments, enzymes, and peptides that have promising properties and multiple benefits. In addition, these products can be easily produced commercially (Gupta et al., 2019). Therefore, the aim of the current study was to develop solid soap using *T.claveryi* extract and evaluate its

antioxidant activity.

## 2. Methodology

### Sample Collection

Fifteen kilograms of desert truffles were obtained from the Samawa desert in Muthanna Province and the desert of Rutba city in Anbar Province, Iraq during the period from November 20 to January 10, 2023. The samples were then transferred to the laboratory of the Department of Life Sciences at the College of Education, Al-Qadisiyah University to be carefully washed with running water, dried in the laboratory on absorbent paper and cut into small pieces using an electric mixer.

The samples were then dried in a hot oven at a temperature of 43 °C and ground with a mechanical grinder to store the obtained powders in clean, dry, tightly sealed containers until they are used in extraction

### Preparation of Terfezia claveryi extracts using a Continuous Soxhlet Apparatus

The extraction process of T. claveryi was applied according to the method of (Al-Daody, 1998). It is using polar solvents where 100 g of truffle fruiting body powder was weighed and ground by an electric grinder into a fine powder instantly, the powder was filled into a paper thimb tube. Then, it was placed in the extraction tube or the main chamber of the Saxlet device. The device was installed in the boiling flask containing alcohol (ethanol 70%) with a volume of 150 ml accompanied by the installation of the condenser with a connection to a cold water source, to start the extraction process and continue for 3 hours.

After that, the process of separating the solvent (70% ethanol) from the extract was using the Rotary Vacuum Evaporator (RVE) under low pressure and at a temperature not exceeding 40 °C. The T. claveryi extracts were stored in opaque bottles and left in the refrigerator at 4 °C until use.

### Soap making

The coconut oil soap with the T. claveryi extract is made by dissolving 5 g of stearic acid at 70 °C and then heating 30 g of coconut oil using a magnetic stirrer at 550 rpm at 70-80 °C for five minutes. Then, the dissolved stearic acid is mixed with 0.2 g of sodium chloride and 0.3 g of citric acid until homogeneous at 70-80 °C. 5 g of sodium hydroxide NaOH is dissolved in 15-20 ml of distilled water and stirred well until the effect (saponification) appears, taking into account the temperature of the oil mixture Citric acid with NaOH solution at 50 ° C.

The appearance of the effect is a state in which soap is formed as a result of the completion of the chemical reaction between the oils and the sodium hydroxide solution, where it begins to condense in the form of a mixture, after which about 19 g of ethanol are added and mixed with the mixture until it becomes homogeneous for 5 minutes. The process of adding ethanol is used to remove the bubbles resulting from pouring the soap into the molds. Then, 5 g of sugar solution are added to increase transparency, then 5 g of glycerin are added and stirred until the soap dissolves, then 0.5% extract, T.claveryi is added. The temperature is reduced to 40 °C after the mixture is poured into a mold. The samples stored at room temperature for a full day until the soap solidifies.

### Biochemical tests for soap

A pH meter was used to measure the pH of the soap and a pH 7 buffer solution was used for calibration. Measuring the pH of soap is done well by diluting 1 g of soap containing the T. claveryi extract and the control soap in 10 ml in a glass beaker.

Foam stability: According to Anggraini et al (2015), one gram of the sample was added to 10 ml of water and then placed in a glass beaker using a magnetic stirrer for 15 minutes. The foam was poured into 50 ml graduated measuring cylinders, first measuring the initial foam height. Second, foam height after settling for 5 minutes.

Foam stability% = Initial foam height * 100% * (2)/ Final foam height

**Measure the biological antioxidant activity of distinct compounds**

The antioxidant activity of the T. claveryi extracts present in soap was tested using the DPPH radical capture T. claveryi. It is according to the method of (Patel Rajesh & Patel Natvar, 2011). The following steps were followed: A DPPH solution was prepared with a weight of 0.04 g of Diphenyl Picryl Hydrazine (DPPH) and dissolved in 100 ml of methanol to obtain a concentration of 400 µg/ml. The control solution (ascorbic acid) was also prepared by dissolving 0.5 g of standard ascorbic acid in 100 ml of methanol and water in a ratio of 1:1. A series of concentrations were prepared for each of the T. claveryi extracts present in soap and the standard antioxidant ascorbic acid VitC and ranged between (31.2-800) µg/ml.

To conduct the test, 1 ml of DPPH solution was mixed with 1 ml of each concentration of T. claveryi extracts and VitC in test tubes and the reaction mixture was shaken vigorously using a Vortex electric mixer, and the tubes were left in the dark at laboratory temperature (28 ± 2 °C) for 30 minutes. The control was prepared as well. It contained the same volume of reaction solution except for the use of solvent (ethanol) instead of T. claveryi extracts. Then the absorbance of the DPPH radical was measured using a spectrophotometer at a wavelength of 517 nm.

The results were expressed as the percentage of DPPH radical inhibition. The method of (Sumathy et al., 2013) was adopted with some modifications according to the following equation:

Percentage of DPPH radical inhibition = absorbance of the control - absorbance of the sample/ absorbance of the control x100:

## 3. Results and discussion

**Sample collection**

The first survey trip was on November 20th - January 10th to the desert of the city of Rutba in Anbar Province and Badia to search for *T.claveryi* and 15 kg of *T. claveryi* were collected. It has fruiting bodies with a shape similar to the shape of a potato, spherical with an irregular body and often wrinkled, the surface of the body is smooth or tuberous with a unique and distinctive smell, and consists of two layers, the outer layer is light brown and spongy in an immature state, hardens with age and turns dark brown As for the inner layer, it is fleshy and fertile (Gleba) and appears in white to pink color and has white tissues interspersed with veins as in Figure (1).

The size of the fruiting body of the *T. claveryi* ranges from 3.7 to 6.5 cm. Its weight ranges from 9 to 148 g and its size can reach the size of an orange, and this is with As mentioned by Khlaif (2021) that explained *Terfezia claveryi* has a yellow-brown pericarp and Gleba with a spongy appearance and a pinkish-yellow color and the samples were of similar irregular spherical shape and size.

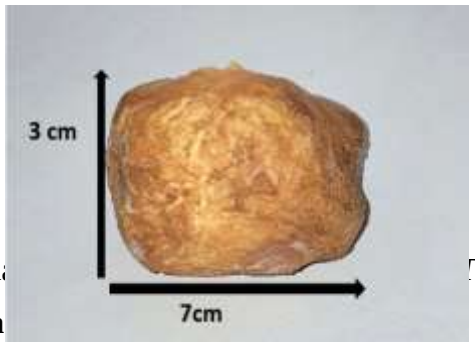


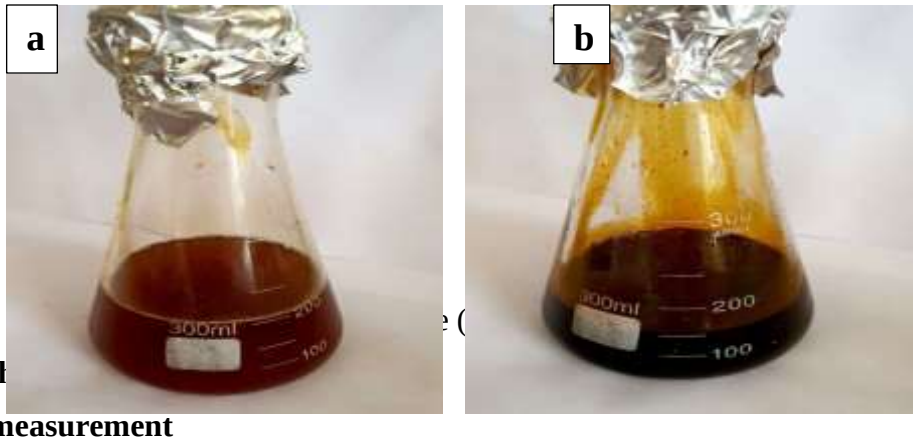

Figure (1) sh *Terfezia claveryi*

**Extracting the active compon**

The crude alcoholic extract of *T. claveryi* was prepared using the Soxhlet device. The extraction process was carried out using polar solvents, where 100 g of truffle fruiting body powder were weighed and ground by an electric grinder into a fine powder instantly. The powder was filled into a paper thimble tube. Then, it was placed in the extraction tube or the main chamber of the Soxhlet

device. The device was installed in a boiling flask containing alcohol (ethanol 70%) with a volume of 150 ml, accompanied by the installation of a condenser with a connection to a cold water source, to start the extraction process and continue for 3 hours.

It appeared in a brown to reddish brown color (Figure 2). This result is consistent with Mohamed-Benkada (2024) who showed that the crude alcoholic extract should be brown in color. It turns into a very dark pink color. The process of separating the solvent (ethanol) from the extract begins using the rotary evaporator using the rotary vacuum evaporator (RVE) under low pressure and at a temperature not exceeding 40°C (Figure 2). If a brown to black extract with a viscous or gelatinous consistency is obtained, on the other hand, alcoholic extraction is preferred over aqueous extraction, as alcohol is considered an effective solvent that has the ability to extract many of the active components of the *T. claveryi* and preserve them.




**Biochemical Analysis**

**pH measurement**

The pH plays an important role in the role of the side effects of soap, and the quality of soap is good the closer the pH value is to the natural acidity of the skin as the measurement process was done using a pH meter. The results indicated that the pH level was 7.83, respectively, with three replicates, while the control soap had a pH value of 9.46 This result is similar with Hayati (2020) where they explained that the PH value of *Ganoderma lucidum* extract soap ranged from 9.1-9.5. The results are also close to Awang (2022) that showed the pH value of solid dishwashing soap with added SchizoComm mushroom extracts reached 10.34.

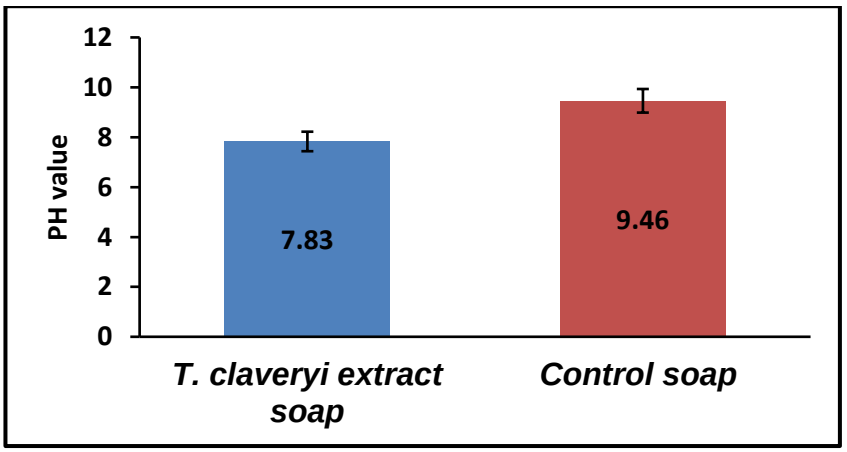



Figure (3) Measuring of the pH value

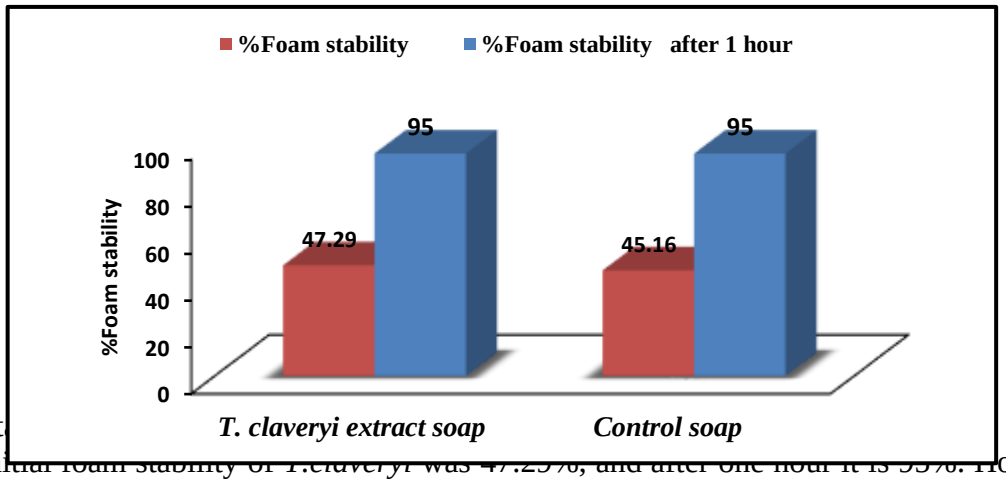
Supriadi & Cahyani (2022) reported that the pH value of soap preparation ranges 8-11, while soap with pH more than 11 will make the skin dry, if soap with pH less than 11 will cause skin irritation. The high pH value that appears in soap may be attributed to the presence of high amount of unspecified substances and due to incomplete alkali decomposition or incomplete reaction of alkali substances with fatty acids in the saponification process (Anindia, 2018). On the other hand, Tarun

(2014) found that the pH of most of the commercial soaps tested was between 9 and 10. The statistical analysis shows the pH measurements of *T. claveryi* extracted soap compared to the control soap (without extracts) (Figure 3).

The results show significant differences between the two groups, with *T.claveryi* soap recording a pH value of 7.83, while the control soap recorded a pH of 9.46. An independent t-test was used to analyze these differences. The statistical analysis value T was 2.943 with P value = 0.018*, indicating a significant difference between the two groups at a statistical significance level (p value < 0.05). These results highlight the effect of *T.claveryi* extract in modifying the pH of soap which enhances our understanding of the properties that can affect the different uses of soap in different industries.

**Foam Stability**

The foam stability test is performed by measuring the decrease in foam volume by adding about 1 g of soap sample to a 50 ml measuring cylinder containing 10 ml of distilled water with three repetitions and the average value is calculated. The mixture is then shaken and left for an hour. Based on the measurement results, it was noted that adding *T.claveryi* extracts to transparent soap did not significantly affect foam stability. The results were close compared to Anuroop (2023) as they showed that Polyherbal soap has the highest foam height and foam retention time.




The foam st the control soap. The initial foam stability of *T.claveryi* was 47.29%, and after one hour it is 95%. However, the initial stability of the control soap is 45.16%, and after one hour was 95%. A t-test was used to compare the two groups, where the T value for *T. claveryi* was 2.944 with *P value = 0.0231, and for the control soap was 4.006 with P value= 0.013*.

These values indicate that there is a significant difference between the two soaps in foam stability. Both P values are less than 0.05. Thus, it concludes that *T. claveryi* soap has better foam stability compared to control soap, which enhances the possibility of using *T. claveryi* extracts to improve the foam properties of transparent solid soap.

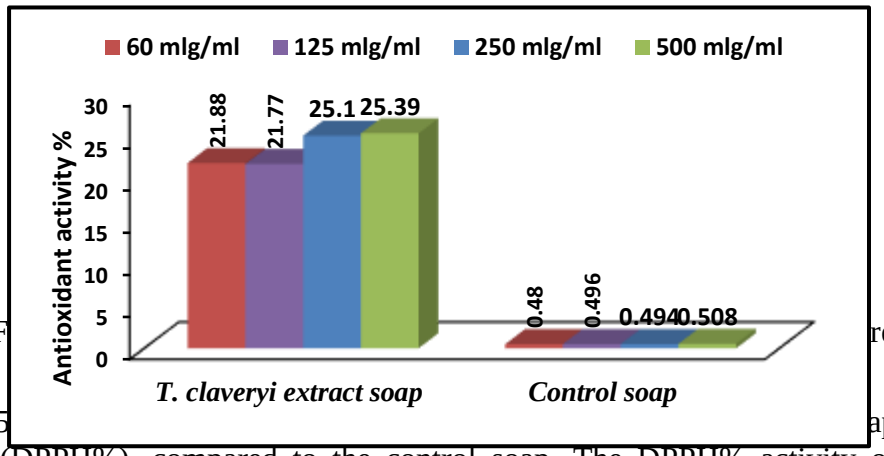
The foam properties of soap were affected by the foam stability, surfactants, active ingredients of fatty acid structure used, and the amount of coconut added. Lauric acid and myristic acid can produce soft foam. They can generate stable foam because they are good raw materials for solid soap and when used, they will produce foam soap with high cleaning degree (Cavitch, 2001). Anindia (2018) stated that the formulation of each soap should contain fatty acid so that the resulting foam stability is good and sufficient to prove it in the foam stability data during the first five minutes.

**Measure of the biological antioxidant activity of active compounds**

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable magnetic molecule, and when a DPPH solution is mixed with an antioxidant, its dark color becomes lighter. The degree of color change indicates the inhibition strength of antioxidant compounds in

terms of the ability to donate hydrogen or electron. By reviewing the results provided, it was found that they achieved the desired results in oxidizing free radicals after 30 minutes of neutralization with *T. claveryi* extract soap.

The antioxidant activity was proceeding at an ascending pace with increasing concentration used, as the lowest concentration used 60 mg/ml, gave a slight and simple change to DPPH from violet or purple to pale yellow, and the intensity of the yellow color increased with increasing concentration when measuring the absorbance with a spectrophotometer at a wavelength of 517 nm. This result is agreed with Hayati (2020) as found that the transparent Ganderma soap provided antioxidant activity through the oxidation of free radicals and changing the purple color to a light color.




F rent types on antioxidant

Figure (5 ap extract on antioxidant activity (DPPH%), compared to the control soap. The DPPH% activity of *T. claveryi* soap was measured at concentrations of 60, 125, 250, and 500 mg/ml. The values were recorded as follows: 21.88%, 21.77%, 25.1%, and 25.39%, respectively. The F test was used to compare between groups, where the F test value was 3.920 with P value = 0.021*, indicating a significant difference between the concentrations in DPPH% activity. The DPPH% activity was measured and the values were recorded as follows: 0.48%, 0.496%, 0.494%, and 0.508%, respectively. The t-test was used to compare groups. It was noted that the P value was less than 0.05 at all studied concentrations, which were 0.019*, 0.029*, 0.014*, and 0.017*, respectively, indicating that there was a significant difference between *T.claveryi* soap and control soap at all concentrations.

The results also showed that *T.claveryi* soap has significantly higher antioxidant activity compared to the control soap, which supports the effectiveness of using *T.claveryi* extracts in skin care products and transparent solid soaps in particular, while the control soap has no antioxidant activity. Wahiba et al. (2016) indicated that the antioxidant activity of *T.claveryi* is due to its high content of antioxidant chemicals such as phenolic, flavonoids, β-carotene, and tannin. Phenolics are the most abundant in the *T. claveryi*, and it also contains other compounds such as ascorbic acid.

## 4. Conclusion and future scope

The study concludes that solid coconut soap was formulated and prepared, and 0.5% concentration of T.claveryi extracts was added. Effect of T.claveryi extract in modifying the pH of the soap, which enhances our understanding of the properties that can affect the different uses of soap in different industries.The mushroom extract, also showed higher initial foam stability compared to the control soap. However, after one hour, the foam stability was 95%. This indicates that the addition of T. claveryi extract enhances the initial foam stability of the soap, but does not affect the long-term foam stability. Furthermore, the results showed that T. claveryi soap had significantly higher antioxidant activity compared to the control soap, which supports the effectiveness of using T. claveryi extracts in skin care products and transparent solid soaps in particular.

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