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Original Article

Antioxidant, Skin Cleansing, Moistening and Foaming Characteristics of Botanical Surfactant Saponins Extracted from Pu'er Tea (Black Tea) Seeds

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ABSTRACT

Background: Natural and sustainable trends around the world are an inevitable choice for the cosmetic industry, thus various ingredients including surfactants derived from renewable and sustainable sources are technically innovative cornerstones, compared with synthetic ones. Natural products and natural product-based agents have been known to play crucial roles in many industries including the cosmetic industry. **Aim:** The primary aim of the present research is to extract botanical-derived saponins from Pu'er tea (black tea) seeds and investigate their characteristics, including surface tension reduction, foam power, hard water resistance, Skin/Eye safety and radical-scavenging ability. **Materials and Methods:** The safety and irritation potential of BTS Saponins were assessed using the Reconstructed Human Cornea-like Epithelium Model and the 3D Reconstructed Human Epidermis Model. This enabled the verification of BTS Saponins' safety, a necessary condition for their application as a constituent in cosmetics. Furthermore, the investigation also assessed the capacity of BTS saponins to counteract the DPPH· radical, thus substantially enhancing their prospective application in the field of cosmetics. **Results:** The results clearly demonstrate that BTS saponins possess an exceptional capacity to reduce surface tension and sustain foam of superior quality. It successfully completed the safety and irritation potential test, which is a prerequisite for its incorporation as a cosmetic ingredient. The antioxidant test results also demonstrated that BTS saponins can effectively counteract the DPPH· radical, thereby significantly increasing their suitability for cosmetic application. **Conclusion:** Based on these observations, it appears that BTS saponins have the potential to be an extremely efficient cleaning element. These findings point out the fact that botanical-based saponins can be potential alternatives to synthetic surfactants in the cosmetic industry.

Keywords: Botanical Surfactant, BTS Saponins, Surface tension, Foam, Skin/Eye Safety, Antioxidant.

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INTRODUCTION

A group of chemical substances called saponins are particularly abundant in a variety of plant species: *Sapindus mukorossi, Sapindus trifoliatus* (Saop nuts), *Medicago sativa*, *Cicer arietinum* (chickpeas), *Agrostemma githago, Saponaria officinalis* (soapwort), *Saponaria vaccaria, Gutierrezia sarothrae, Drymaria arenaroides, Aesculus hippocastanum, Asparagus officinalis, Bellis perennis, Q. saponaria* (soapbark tree), etc., widely used in fields of household, food additives, medicine, aquaculture, health additives and crop planting, etc.,[1,2] These compounds are called amphipathic glycosides. They are characterized by having a lipophilic triterpene derivative combined with one or more hydrophilic glycoside parts. When they are shaken in water, they produce a foaming effect similar to soap. The global production of this botanical surfactant increased from 13331.3 MT in 2011 to 14695.5 MT in 2016, with an average growth rate of 1.97%. This significant increase establishes it as one of the leading surfactants in terms of surfactivity. The global utilization rate of saponin capacity remained about at 54.26% in 2016. Based on the study worldwide saponin market insights and forecast study.[3] The global saponin market is projected to grow from US$ 953.9 million in 2019 to US$ 960.7 million by 2026, with a Compound Annual Growth Rate (CAGR) of 0.1% during the period from 2021 to 2026.

Surfactants, also referred to as surface-active agents, are crucial components in several industries, such as personal care and cosmetics.[4,5] Amphiphilic compounds possess both hydrophilic and hydrophobic areas, allowing them to decrease the surface

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tension between liquids and solids. Traditional surfactants are commonly derived from petrochemical sources, raising concerns about their possible impact on the environment and human health.[6,7] Consequently, both the industry and researchers are actively seeking environmentally acceptable alternatives, such as surfactants derived from botanical sources. Plant saponins are natural compounds that possess surfactant properties and can be found in a diverse range of plant species.[8,9]

In the cosmetics industry, there is a growing global trend towards finding natural, renewable and sustainable alternative ingredients. One particular class of surfactants, known as naturally botanical surfactants, is gaining popularity.[5,7] These surfactants are obtained directly from plants without any further chemical modification. It is important for these natural botanical surfactants to not only be effective in cleaning surfaces but also safe and environmentally friendly, especially when used in the cosmetic industry. However, there is limited research on the use and application of botanical saponins extracted from Pu'er tea seed (BTS Saponins, Figure 1) in cosmetic products.[10-13]

The main objective of this research is to isolate botanical-derived saponins from Pu'er tea (black tea) seeds and examine their properties, such as their ability to reduce surface tension, generate foam, resist the effects of hard water, ensure safety for the skin and eyes and scavenge radicals. In addition, we have conducted an examination of its safety and potential for causing irritation using the 3D Reconstructed Human Epidermis Model (Episkin®) and 3D Reconstructed Human Cornea-like Epithelium Model (BioOcullar®). For an ingredient to be used in cosmetics, safety is a necessary need. In addition to being one of the tea extracts, we have examined the skin-care benefits of BTS saponins by evaluating its ability to remove DPPH· radicals, excluding its qualities related to skin washing.

MATERIALS AND METHODS

Sample Preparation

Commercially available Saponins extracted from Pu'er Tea Seed (Tawny powder, active ≥ 95%, Spec-Chem Industry Inc.), hereafter referred to as BTS saponins. Pre-weighted saponins were dissolved into water to prepare an aqueous solution at various concentrations.

The surface tension of the BTS saponins aqueous solution was measured using an automatic tension meter (JK99M, Powereach Shanghai) at a temperature of 20±0.5°C.

Surface Tension

The surface tension of the BTS saponins aqueous solution was measured using an automatic tension meter (JK99M, Powereach Shanghai) at a temperature of 20±0.5°C. Prior to the measurements, all samples of the aqueous solution at different concentrations were kept at a temperature of 20°C. Each aqueous solution was measured at least three times and the results reported are the average values.

Foaming Power

To achieve a testing solution with a concentration of 2.5 μg/mL of BTS saponins, pre-weighed BTS saponins were introduced into deionized water. The stirring process was maintained in order to prevent excessive foam formation and to allow the solution to age at a temperature of 40±0.5°C for a total duration of 30 min using the circulated warm-water jacket. The walls of the receiver were rinsed thoroughly with distilled water. If water cascades down the walls in a continuous coating, it indicates that the system has been cleansed. The stopcock was closed at the bottom of the receiver once the aging period had elapsed. The stopcock was reversed to ensure that the solution level in the receiver is exactly at the 50-mL mark. Used a pipet to wash the walls of the receiver with 50 mL of the BTS saponins solution until all the contents have completely drained to the bottom of the receiver. Utilize suction conservatively to transfer the fluid into the pipet until it reaches the 200 mL mark. Promptly, the stopcock was opened and precisely adjusted it to the correct position at the headset. Commenced a timer subsequent to the complete utilization of the solution-filled pipet, ascertained the vertical extent of the foam and obtained additional measurements at intervals of three, five and subsequent time points. After the foam rim reaches its maximum average height, measure the quantity of foam generated at the top of the foam column to obtain the reading. The quantity of air remaining in the foam at this elevation is directly proportional to the initial quantity. (Please consult the Ross-Miles Method for further information).

In water of 150 ppm hardness

150 ppm hard water was prepared by adding pre-weighted Calcium Chloride ($CaCl_2$) and ($MgSO_4 \cdot 7H_2O$) and made sure the concentration of $CaCl_2$ and $MgSO_4 \cdot 7H_2O$ is 0.0999 μg/mL and 0.148 μg/mL, respectively. The pre-weighed BTS saponins added into prepared 150 ppm hard water to make sure the active content of BTS saponins in the testing solution is 2.5 μg/mL. The same steps were repeated as in deionized water by replacing deionized water with 150 ppm hard water.

Skin irritation (*in vitro*, 3D Reconstructed Human Epidermis Model)

The 12-well test plate was prepared and 200 μL of 37°C pre-warmed assay media was added to three holes in the first column. The models were transferred into the corresponding holes. Each plate contains three skin models that were incubated overnight at 37°C, 5% CO_2 and 95% humidity. The plates were removed and 2 mL of pre-warmed assay medium was added to three wells in the second column. The chemical being tested is BTS Saponins Powder. The negative control is Dulbecco's Phosphate Buffered Saline (DPBS) and the positive control is a 5% aqueous Sodium Dodecyl Sulphate

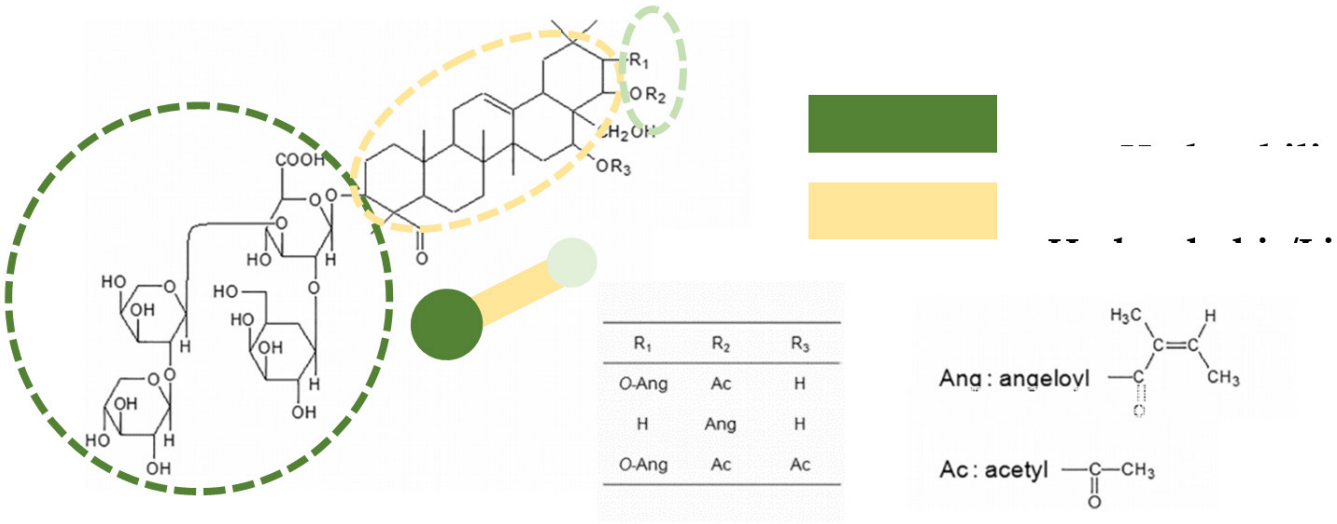

Figure 1: The structural schematic diagram of BTS Saponins.

(SDS) solution. There are three holes, each containing one of these substances. A 10 µL or 10 mg sample was collected and applied onto the skin surface for testing. Following 15 min exposures at room temperature, the inserts were removed and properly washed with sterile DPBS to completely eliminate all residues from the epidermal surface. The contents of the first column were moved to the second column using a pre-existing assay media. Afterwards, the sample was placed back in the incubator for a 42 hr post-treatment culture. 2 mL of pre-warmed MTT solution, including 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Thiazolyl blue; CAS number 298-93-1), was applied to three holes in the third column. The contents of the second column in each insert were moved to the third column and then incubated for duration of 3 hr. The epidermal tissue was incised using a punch tool and carefully detached from the collagen matrix. Subsequently, it was placed into a 1.5 mL EP tube. Isopropanol, an acidic compound, was introduced into the tube and well blended. The sample was kept in a dimly lit location overnight to fully extract the pigment at the ambient temperature. A 96-well plate reader is utilized to quantify the optical density at a wavelength of 570 nm subsequent to transferring 200 µL of the solution from each EP tube (Eppendorf Tubes/1.5 mL centrifuge Tubes) to a microtiter plate containing 96 wells.

Calculations

The average tissue viability of PC (SDS) ≤40% (SD≤18) and the tissue viability is calculated as below: Tissue viability (%)=Mean OD (sample)/Mean OD (NC)x100%. Tissue viability≤50% means irritating to the skin, while＞50% means non-irritating to the skin. (Refer to OECD 439)

Eye Irritation (3D Human Cornea-like Epithelium Model Reconstructed *in vitro*)

The pre-incubation was done according to the protocol given by the company that made and gave the RhCE tissue constructs. After being left to sit overnight, 20 µL of DPBS is used to wet the tissues. For 30 min and 2 min, the tissues are kept at 37°C, 5.1% CO_2 and 95% relative humidity. This is normal culture conditions. 42 µL of the positive control (methyl acetate) and negative control (ultrapure water) are put on the tissue right away to cover the top surface. 42 mg of the test substance was put on top of the tissue directly. Standard growth conditions (37±1°C, 5±1% CO_2, 95% RH) were kept up for 6±0.25 hr while the tissues were being incubated. The best and most recommended time is between one to 2 min. We exposed the tissues to the test substance for 6±0.25 hr and then rinsed them with clean DPBS. We made sure to fill and empty the tissue insert ten times to get rid of any test material that was still there. Following the rinsing of the tissue, it was immediately placed on a 24-well plate that had already been labeled and covered with 2 mL of Assay Medium that had been heated to room temperature. This was done for 25 min, giving the tissues time to soak. Any test material that was put into the tissue should be washed out during this time in the assay solution. Once the post-soak is done, each insert is taken out of the Assay Medium and blotted on absorbent paper. The insert is then put in the matching well of the 6-well plate that has already been labeled and has 0.9 mL of warm Assay Medium in it. The tissues were kept in a standard growth environment (37±1°C, 5±1% CO_2, 95% RH) for 18±0.25 hr. When the Post-treatment Incubation is over, each insert is carefully taken out of the 6-well plate and wiped on blotting paper. It was added to the 24-well plate, which already had 0.3 mL of MTT solution (1.0 mg/mL). All the tissues are put into the 24-well plate, which is then kept at 37±1°C with 5±1% CO_2 and 95% relative humidity for 180±5 min. Once the 180±10

min is up, each insert is taken out of the 24-well plate and its bottom is blotted with an absorbent material. It is then moved to a 24-well plate that has already been labeled and has 2.0 mL of isopropanol in each well so that the isopropanol flows into the insert on the tumor surface. The plates were wrapped in parafilm and put somewhere dark and cool (2 to 8°C) for the night. They can be taken out right away, though. It took 2-3 hr of shaking the plates at room temperature on an orbital plate shaker in order to get the MTT out of them.

OD-value

Two × 200µL aliquots of the blue formazan solution were placed in each of the tissue's 96-well flat bottom microtiter plates. The isopropanol was taken as blank. Without the use of a reference filter, read the Optical Density (OD) in a 96-well plate spectrophotometer at a wavelength between 570 nm.

Calculations

Calculate the mean OD value of the blank control wells (OD_{Blk}) for each experiment. Calculations for Viability Tests only: Viability (%)=($OD_{Samples}$-OD_{Blk})/(OD_{NC}-OD_{Blk})×100%

Calculations for Viability plus Killed Control Tests Viability (%)=[$OD_{Samples}$-($OD_{Samples\_KC}$-OD_{NC_KC})]/(ODNC-OD_{Blk})×100%

Test articles are classified as non-irritants if their treated tissue viability is more than 60.0% when compared to the tissue viability of the negative control. Test articles are classified as irritants if their treated tissue viability is less than 60.0% when compared to the tissue viability of the negative control (See OECD 492).

Scavenging Ability of DPPH·Radical

The 1 mL DPPH solution and 0.5 mL testing samples of different concentrations was added in the test tube, respectively, mix well and put in the dark place for 60 min. After moving the mixture to a colorimetric plate, the absorbance value was calculated at 517 nm using absolute ethyl alcohol as the reference (zero setting), represented by the letter A1. Blank control: insert 1 mL absolute ethanol and 0.5 mL samples of different concentrations in the test tube, respectively, mixed well and put in the dark place for 60 min. The solution was transferred to a colorimetric dish. The absorbance value was measured at 517 nm with absolute ethyl alcohol as the reference (zero setting), denoted as A2. Control group. The 1 mL DPPH solution and 0.5 mL absolute ethyl alcohol was added in the test tube, mix well and leave in the dark place for 60 min. Moved the mixture to a colorimetric plate and absolute ethyl alcohol was used as the reference (zero setting), represented by the letter A0, to measure the absorbance value at 517 nm. Equation (K)=[1-(A1-A2)/A0]*100% was used to get the free radical scavenging rate. After doing the experiment thrice, the mean value was determined to be the ultimate outcome.

RESULTS AND DISCUSSION

The formation of glycosides is a characteristic of plant saponins, which are composed of a hydrophilic glycone moiety and a hydrophobic aglycone. Because of their one-of-a-kind structure, saponins are able to exhibit amphiphilic capabilities, which enables them to function as efficient surfactants.[14,15] The aggregation of saponins results in the formation of micelles. This occurs when the hydrophobic tails of the saponins bind together and their hydrophilic heads contact with water.[16] The production of micelles improves the ability to emulsify, disseminate and solubilize compounds, while concurrently lowering the surface tension of the substance. The wide variety of plant sources results in the formation of a wide range of saponin structures, which in turn determines the surfactant-like capabilities of the saponins.[17-19] Saponins derived from plants have the potential to be utilized in a variety of cosmetic applications, including but not limited to the following: as cleansers in shampoos; as foaming agents; as emulsifiers; as natural preservatives; as hair conditioners; as skin penetration enhancers; as anti-inflammatory and antioxidant agents.[20-24] Utilizing plant saponins as surfactants in cosmetics is a persuasive and environmentally conscientious method to fulfilling the growing need for natural and sustainable ingredients in personal care products. This desire is expected to continue to grow in the coming years.[25,26] Saponins are chemical substances that exist naturally and possess surfactant qualities. They can be found in a variety of plant sources, including soapberries (*Sapindus* spp.), yucca plants and quillaja trees, among others. Because of these characteristics, they are ideally suited for use in cosmetic formulations as agents that foam, emulsify and cleanse.[27-30]

Surfactivity

Since saponins are amphipathic, they can serve as surfactants and potentially lower the interfacial energy between hydrophobic and hydrophilic phases, as well as air and water, stabilizing the development of foam (Figure 2).

Figure 3 demonstrates that as the concentration of BTS saponins solution increases from 0.0 to 2 g/L, the interfacial tension energy decreases significantly from 55 mN/m to 27 mN/m. This indicates that BTS saponins possess remarkable surfactivity, making them suitable as effective botanical surfactants, detergents and foamers in rinse-off formulations.

Micelles are generated as the concentration of the BTS saponin solution is raised and this phenomenon occurs for all additional surfactants introduced into the system beginning at a concentration of 2.5 g/L.[31] The concentration at which micelles form is referred to as the Critical Micelle Concentration (CMC) in the field of colloidal and surface chemistry. The critical mass of a surfactant is significant. After attaining the Critical Micelle Concentration (CMC), the surface tension either declines gradually or remains rather stable.[32,33] The estimated Critical

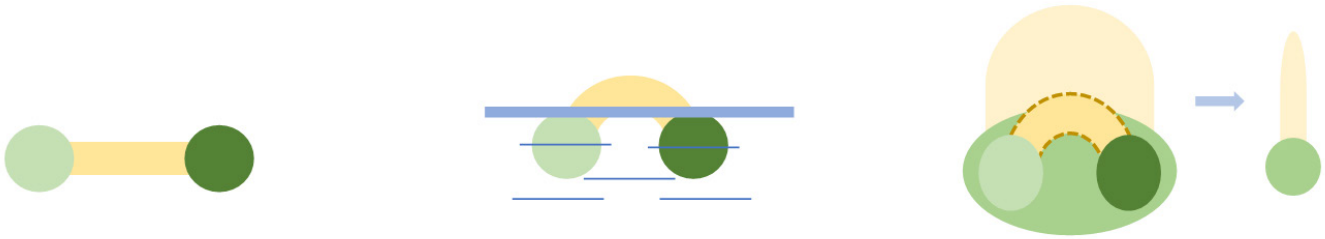

Figure 2: Stabilization of the foam formation using surfactants.

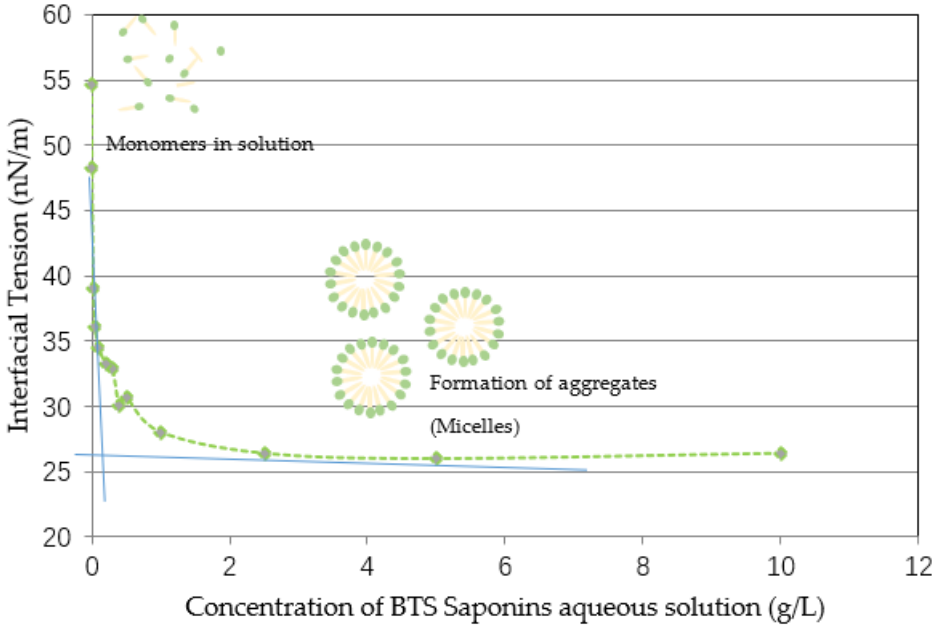

Figure 3: The surface tension of various concentrations of BTS Saponins.

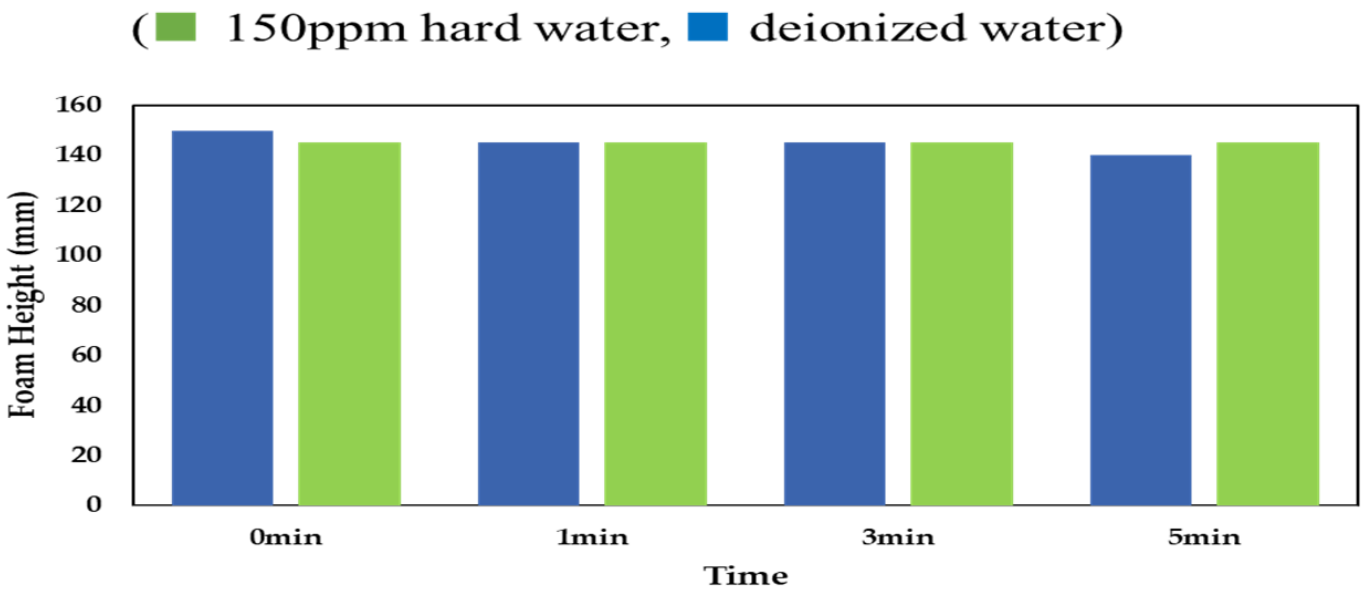

Figure 4: Foam height (mm) changes with time in water of various degrees of hardness.

Micelle Concentration (CMC) of BTS saponins is 2 to 3 g/L, as seen by the interfacial tension curve provided above.

Foam Power

The foam characteristics and resilience to hard water are crucial factors for both personal washing products and home cleaning solutions, including detergents.[34] As depicted in Figure 4, BTS Saponins has a form height over 100 mm, which suggests the presence of opulent and stable foam even at a low concentration of 2.5 µg/L. Furthermore, during a period of 5 min, the decrease in foam height is less than 10 mm. BTS Saponins exhibit exceptional resistance to hard water, meaning that the hardness of the water has a negligible impact on the foam power of BTS Saponins. The reason for this phenomenon is likely due to the ability of the polar groups of BTS saponins to form complexes with calcium and other ions typically present in hard water, while their nonionic properties remain unaffected.

Skin Irritation

During the testing conditions, the tissue survival rate of BTS Saponins is 72.07%, which exceeds the minimum requirement of 50%. The results of the OECD TG 439 chemicals *in vitro* skin irritation test using rebuilt human epidermis demonstrate that BTS Saponins do not induce skin irritation or corrosion. Hence, it is the optimal and calming choice for the ecologically aware brand. It is illustrated in Figure 5.

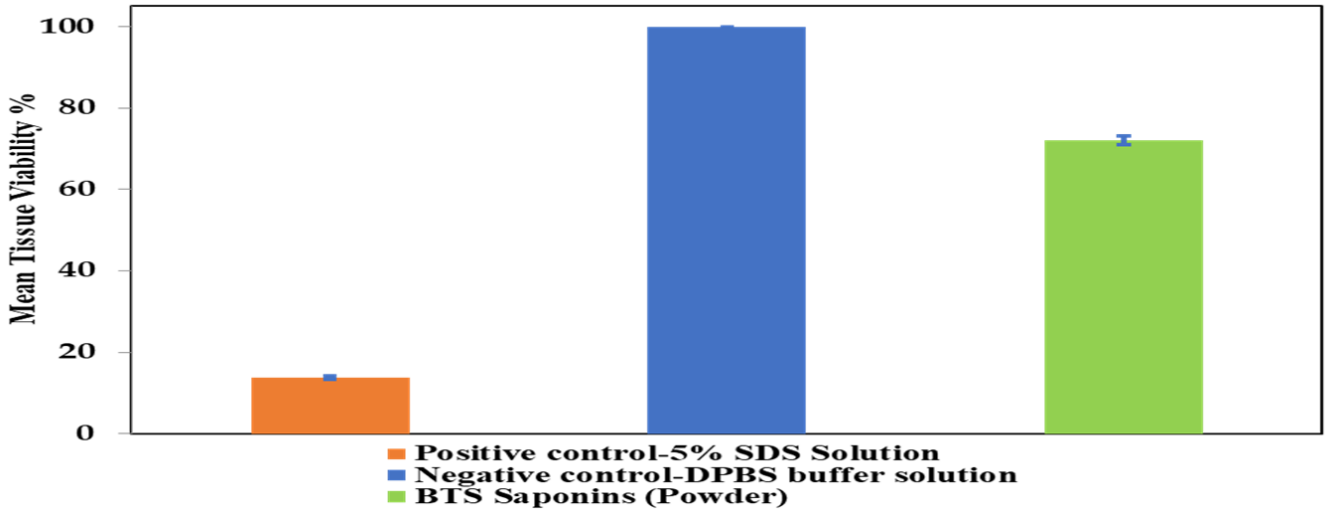

Figure 5: The mildness of BTS Saponins on skin tissue viability, Skin irritation indicated by Tissue Viability of Skin Model (The bigger the number is, the less irritation of the testing substance is, >50% means non-irritating to the skin).

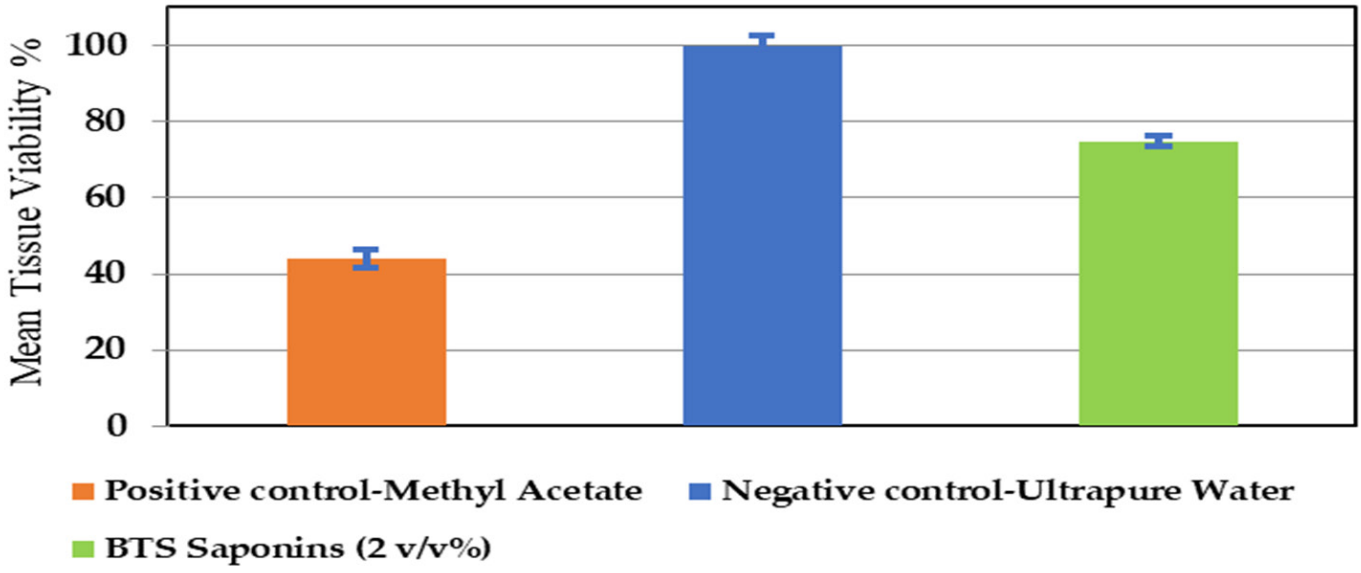

Figure 6: The mildness of SpecPure® BTS Saponins on eye tissue viability, Eye irritation indicated by tissue viability of Reconstructed Human Cornea-like Epithelium (RhCE) (The bigger the number is, the less irritation of the testing substance is, >60% means non-irritant).

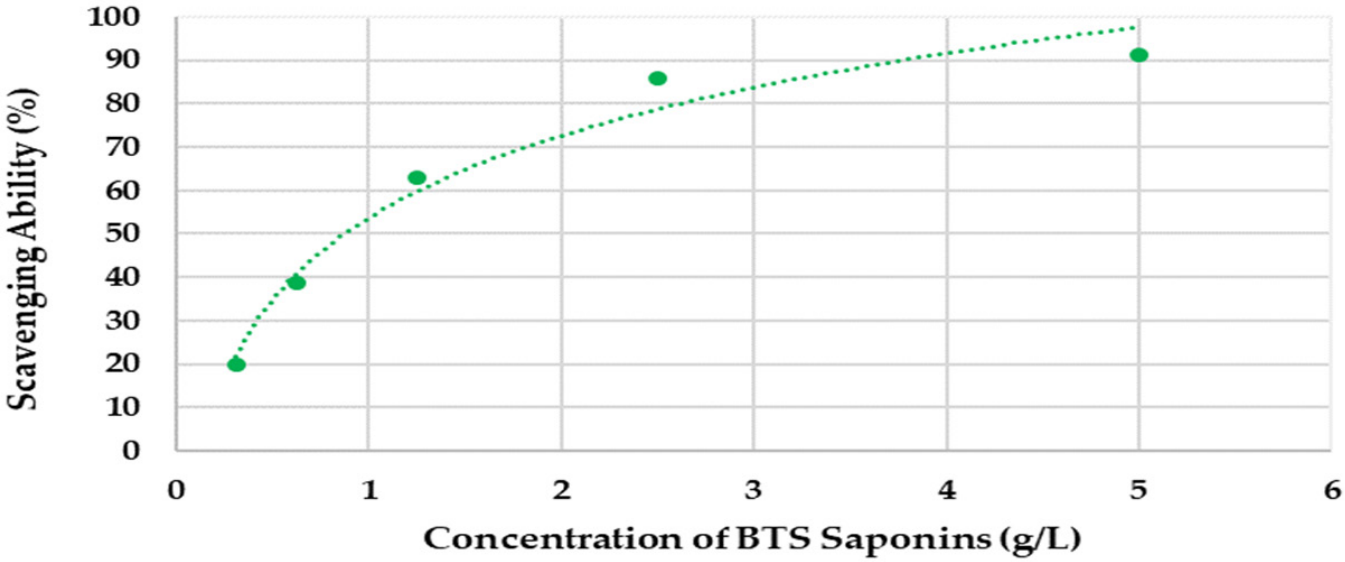

Figure 7: The scavenging capacity of BTS saponins on DPPH free radical.

Eye irritation

The *in vitro* test conducted on a reconstructed human Cornea-like Epithelium skin model has determined that 2% (v/v) of SpecPure® BTS Saponins is not irritating to the eyes. The test results indicate that the tissue viability of BTS Saponins is greater than 60% when compared to the negative control-treated tissue viability. Therefore, the concentration is 2% (volume/volume). SpecPure® BTS Saponins are suitable for use in rinse-off products, including shampoo, body wash, infant cleanser, make-up remover, facial cleanser and more (Figure 6).

Skin-care benefits: Antioxidant

Based on the Brand-Williams method, our findings demonstrate that a concentration of 5 g/L of BTS Saponins can effectively eliminate 91.4% of DPPH radicals. Additionally, the half maximum Effective Concentration (EC_{50}) of BTS Saponins on DPPH is measured to be 0.87 g/L. These results indicate that BTS saponins possess exceptional antioxidant properties, making them a great choice as a green cleansing component. Antioxidants play a crucial role in combating oxidative stress, a process that can lead to skin damage and premature aging. Free radicals are unstable chemicals that can cause damage to collagen and skin cells. Antioxidants counteract the harmful effects of free radicals. Antioxidants have the ability to prevent the breakdown of collagen and enhance the production of collagen[35-37] As a result, they might potentially reduce the visibility of fine lines, wrinkles and other signs of aging (Figure 7).

CONCLUSION

This study examined the surfactivity and foam performance of BTS saponins, including foam height, stability and resistance to hard water. The results demonstrated that BTS saponins have a remarkable ability to reduce surface tension and stabilize high-quality foam. These findings suggest that BTS saponins have the potential to be a highly effective cleaning ingredient. A study was done to assess the safety and irritation potential of BTS Saponins using the 3D Reconstructed Human Epidermis Model and Reconstructed Human Cornea-like Epithelium Model. This enabled the verification of the safety of BTS Saponins, which is a necessary condition for its inclusion as an ingredient in cosmetics. In addition, the study also investigated the capacity of BTS saponins to counteract the DPPH· radical, thereby greatly enhancing its suitability for application in cosmetics. Therefore, BTS saponins, being a type of green botanical surfactants, are highly suitable as cleansing ingredients due to their beneficial effects on the skin.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CAGR: Compound Annual Growth Rate; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **BTS:** botanical saponins; **DPBS:** Dulbecco's phosphate buffered saline; **SDS:** Sodium dodecyl sulphate; **MTT:** Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **CMC:** Critical micelle concentration; **RhCE:** Reconstructed Human Cornea-like Epithelium.

AUTHOR CONTRIBUTIONS

Conceptualization, T.W.; methodology, T.W. and Y.Y.Z; validation, J.S.Z. and L.T.; formal analysis, T.W. and J.S.Z; investigation, T.W. and J.S.Z.; writing-original draft preparation, T.W.; writing-review and editing, T.W. All authors have read and agreed.

SUMMARY

This study extracts botanical-derived saponins from Pu'er tea (black tea) seeds and examines their surface tension reduction, foam power, hard water resistance, skin/eye safety and radical-scavenging properties. BTS Saponins were tested for safety and irritation using the Reconstructed Human Cornea-like Epithelium Model and 3D Reconstructed Human Epidermis Model. This confirmed BTS Saponins' safety, a prerequisite for cosmetic use. Additionally, the study evaluated BTS saponins' ability to combat the DPPH· radical, improving their potential in cosmetics. The results show that BTS saponins minimize surface tension and sustain high-quality foam. It passed the safety and irritation potential test for cosmetic ingredient use. The antioxidant test findings show that BTS saponins successfully combat the DPPH· radical, making them more suitable for cosmetic use. Based on these findings, BTS saponins may be an effective cleaning agent. These findings suggest that botanical-based saponins could replace manufactured cosmetic surfactants.

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