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Research Paper

Application of Tea Saponin in Hand Sanitizer

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ABSTRACT

This paper describes an experiment that uses the agricultural wastes, tea seed cake as raw material, purifies preliminary extraction of tea saponin using ultrasonic-assisted ethanol extraction and obtaining powdery products regarded as surfactants applied to make hand sanitizer with tea saponin. The experiment found that appearance of hand sanitizer with tea saponin has normal appearance. Various indexes conform to technical requirements in standards. Moreover, through detergency test and bacteriostasis experiment, it found that effects of hand sanitizer with tea saponin reach or are superior to alcohol-based sanitizer in the market.

Key words: Tea saponin, ultrasonic extraction, hand sanitizer, detergency, Bacteriostasis.

INTRODUCTION

Tea saponin is mainly extracted from agricultural wastes-tea seed cake, an all-purpose natural and non-ionic surfactant and has good activation (Shengfeng et al., 2003) including emulsification, decentralization and lubrication, etc. Meanwhile, it also has abundant pharmaceutical properties, mainly including the increase of immunoreaction, anti-inflammation, antibiosis and antioxidant properties, etc (Choon et al., 2014). At present, tea saponin has had relatively matured application in industries, mainly including hair shampoos, but has been less in household chemicals (Songpei et al., 2009). Regarding tea saponin as a cleaning and bacteriostatic agent to apply to hand sanitizer, the paper analyzes and compares the properties of products.

MATERIALS AND METHODS

Extracting and preparation

The following chemicals were used for the experiments: % Alcohol, 1 Chitosan acetum, 30% hydrogen peroxide and acetone. The recipe of hand sanitizer are tea saponin (self-made), Carbomer, polyethylene pyrrolidone, polyvinyl alcohol, 75% alcohol, glycerin, peppermint, essence and triethanolamine used for medical grade or food grade.

Detergency test

For the detergency test the following compoundes were used: lard, butter, vegetable oil and fatty acid.

Bacteriostatic effect test

For the bacteriostatic effect test, the following compounds were used: Strains of *Escherichia coli*, *Staphylococcus aureus* strains, nutrient agar, beef extract peptone and physiological saline.

Experimental apparatus

The experimental apparatus used are:

- XH-2008D Computer intelligent temperature control ultrasonic synthetic extraction apparatus at low

Table 1. Formulation.

Component	Effect	Grade	Proportion 1(%)	Proportion 2(%)
Carbomer	Thickening	Medical	—	1.00
Polyvinyl (PVP)	Thickening	Medical	1.80	—
Polyvinyl alcohol (PVA)	Thickening	Medical	8.60	—
75% ethanol	Solvent	Medical	40.00	52.00
Glycerol (glycerine)	Moisturizing	Medical	—	3.00
Tea saponin	Surfactant, antibacterial	Made in laboratory	1.50	1.50
Mint	Aroma	Food	0.20	0.10
Essence	Aroma	Food	0.90	0.80
Triethanolamine	Regulation of pH	Medical	0.35	0.55
Deionized water	Solvent	—	Allowance	Allowance
Total	—	—	100	100

temperature;
- FD-ID-80 lyophilizer, SH2-D(III) water recycled multi-use vacuum pump, electric mixer,autoclave;
- TDL-5-A low speed centrifuge and ultraviolet spectrophotometer;
- DK-S28 type electric-heated thermostatic water bath and water-jacket incubator.

The preparation of tea saponin production

30 g tea seed cake was pre-processed into three-necked flask and extracted for 120 min using 80% ethanol solution in the solid-to-liquid ratio of 1:8 with ultrasonic power 700 W. Ultrasonic pre-treatment time 15 min and extraction temperature of 70°C (Xiang et al., 2011; Yaogang et al., 2009). 20 ml of 1% chitosan acetum was added into tea saponin extracting solution filtrated when it is hot and heat reflux for 0.5 h (Wu and Man, 2013) then extract centrifugal filtrated supernate after static culture for 1 h (Hongjiang, 2012). 5% hydrogen peroxide was added into the supernate to heat reflux for 1.5 h (Changsheng et al., 2004) and freezed dried for powder after spinning steaming concentration. Soxhlet extraction using the powder in last step and acetone in the solid-to-liquid ratio of 1:30 proceeded to remove impurities dissolved in acetone with the processing time 5 h. Tea saponin production was then dried for standby application.

Preparations of hand sanitizer

Main components of its formula are thickener, humectant, cosolvent, pH modifier and essence, etc., raw materials. Hand sanitizer was made in proportions (Table 1). According to hand sanitizer made from two proportions in Table 1, it was found that polyvinyl alcohol, as thickener, not only has larger usage amount, but also higher viscidity for finished products. After usage, more residues remained on skin, so it is not applicable for modern people's requirements as hand sanitizer. After using products of Carbopol as thickener, no residues remained on skin, and skin was refreshed. Therefore, we ultimately selected Carbopol as thickener in the peoduction of hand sanitizer and the follow-up test in line with the 2 proportion was conducted.

Production methods

1) Mix parts of the deionized water and 75% ethyl alcohol into 3:1 for dissolving Carbopol; keep overnight by stewing, add triethanolamine after dissolving completely, and make to pH 7±0.5.
2) Use parts of the deionized water to dissolve tea saponin powder, utilize parts of 75% ethyl alcohol to glycerinum, mint and essence and stir to balance. Mix the aforementioned two solutions and stir to balance.
3) After stirring, add gel of step 1 Carbopol added into the solution configured in step 2; add the remaining deionized water and 75% ethyl alcohol to the mixed liquor; add margin triethanolamine and make to pH 7 so as to obtain hand sanitizer with tea saponin.

Determination of conventional index

- Measure appearance and smell of hand sanitizer: Fetch moderate samples to a beaker in line with requirements in GB19877.1-2005 to identify appearance and smell.
- Measure total active matter content: Measure total active matter content of hand sanitizer in accordance with the method in GB/T13173.2-2008. Because GB19811.1-2005 is not suitable for products, total active matter content here is only used for reference analysis.
- Measure pH: At room temperature 25°C, use newly-boiled deionized water to allocate sanitizer samples in line with

the proportion of mass ratio 1:10, for measuring.
- Measure some indexes, such as formaldehyde, methyl alcohol and heavy metal, etc: Measure the aforementioned matter content in line with the method and requirements in GB19877.1-2005.

Detergency test

Material preparations

a. Hand sanitizer samples: 1) Hand sanitizer with tea saponin; 2) D hand sanitizer; 3)W hand sanitizer.
b. Artificial greasy dirt: Allocated in the proportion of butter: lard oil: vegetable oil=1:1:2, add polyglycerol ester fatty acid with the total mass of 5% to obtain mixed artificial greasy dirt (preserve for about 6 months at -5°C).
c. Preparations of greasy dirt slide glass: Boil glass slide in deionized water for 15 min, place it in acidic washing liquor for 1 h after wiping, rinse with deionized water, after no acidic residues on glass slide, dry for using fetch glass slide from top to bottom to draw a line, and limit greasy oil under this line for reservation.

Smearing method

Weigh glass slide after preprocessing by using analytical balance precisely (correct to 0.001 g), mark as m0, use a clamp to fix up weighed glass slide along a 10 mm line to smear. After greasy dirt is maintained at certain temperature when indoor temperature is 20±0.5°C, oil temperature should be heated to 80°C. When indoor temperature is 25±0.5°C, oil temperature should be heated to 45°C. When indoor temperature is too high or too low, the experiment is not suitable for continuity. It is necessary to regulate indoor temperature to suitable temperature range); hand a clamp of fix glass slide gradually, immerse 10 mm line of glass slide into greasy dirt for the remaining 1 to 2 s, take it out slowly, place it 4 h or above at indoor temperature, use analytical balance to weigh (correct to 0.001 g), mark as m1 and maintain 0.5±0.05 g greasy dirt content in every glass slide.

Measure soil removal efficiency:

Fetch appropriate hand sanitizer samples to allocate sample solution with corresponding concentration, stir and heat at 30°C water bath until temperature is increased to 30°C by stirring; fix up glass slide with the clamp above the sample solution, ensure that 10 mm line above glass slide is immersed in the sample solution, maintain for 1 min; after fixing up glass slide, open magnetic stirring to set up rotation speed as 150 r/min for 5 min, put back glass slide and clamp after finishing; air glass slide for 3 h at indoor temperature until bone dry, and use analytical balance to weigh as m_2(correct to 0.001 g).

Samples in every group should be prepared for 3 glass slides. The same experiment should be conducted under the same indoor temperature, water temperature and rotation speed.

Calculation

The method of calculation used is given as:

$$C(\%) = \frac{m_1 - m_2}{m_1 - m_0} \times 100$$

Where:
C= Soil removal efficiency (%);
m_0= Mass of glass slide before smearing (g);
m_1= Mass of glass slide after smearing (g);
m_2= Mass of glass slide after processing sample solution (g).

Bacteriostasis experiment

Allocate 30 g/L trypticase soy broth for reservation after sterilization; connect *Colon bacillus* and *staphylococcus aureus* to 37°C to activate for 16 to 18 h to reservation, use normal saline to dilute activated bacterium solution 10 times, fetch 100 µl to inject into nutrient agar medium solid medium, use sterilized spreader to smear, filter paper with the diameter of 5 mm to immerse equal samples homogeneously, paste it on the surface of culture medium, after sterilizing three kinds of hand sanitizer in ultraviolet, and observe results after cultivating for 24 h at 37°C.

RESULTS AND DISCUSSION

Conventional index analysis of hand sanitizer

After putting self-made hand sanitizer with tea saponin to a bottle, test in line with stipulated requirements and methods after placing it for a while at indoor temperature and make a comparison with two brands of hand sanitizer sold in the market.

It can be observed from data in Table 2 that various values of hand sanitizer with tea saponin conform to the stipulated scope in standards, and its quality is qualified. Active matter content is slightly greater than other brands of hand sanitizer sold in the market, because mainstream hand sanitizer is an alcohol-based sanitizer. In the formula, it seldom adds some common anionic surfactants to prevent products from remaining on skin after usage. These residues will cause irritant damage to skin. It proves that tea saponin is gentle to skin under low concentration and has no toxic reaction (Li et al., 2011).

Table 2. Product performance index.

Items	Result			
	Requirements	Tea saponin sanitizer	D Sanitizer	W Sanitizer
Total active content (%)	For reference only	5.906	1.512	1.312
pH	4.0~10.0	6.27	6.71	6.70
Methanol content mg/kg	≤2000	＜2000	＜2000	＜2000
Formaldehyde content mg/kg	≤500	＜0.001	＜0.001	＜0.001
Arsenic content (As) mg/kg	≤10	＜10.0	＜10.0	＜10.0
Heavy metal content (Pb) mg/kg	≤40	＜40.0	＜40.0	＜40.0
Mercury content (Hg) mg/kg	≤1	＜0.01	＜0.01	＜0.01
Total number of colonies CFU/g	≤200	＜10	＜10	＜10

Table 3. Three kinds of hand sanitizer to oil pollution rate.

Variable	C (Detergent rate) %			
	1	2	3	Average value
D. Sanitizer	57.5	52.9	55.0	55.1
W. Sanitizer	58.0	55.9	58.8	57.6
Tea saponin sanitizer	60.1	63.1	67.8	63.7

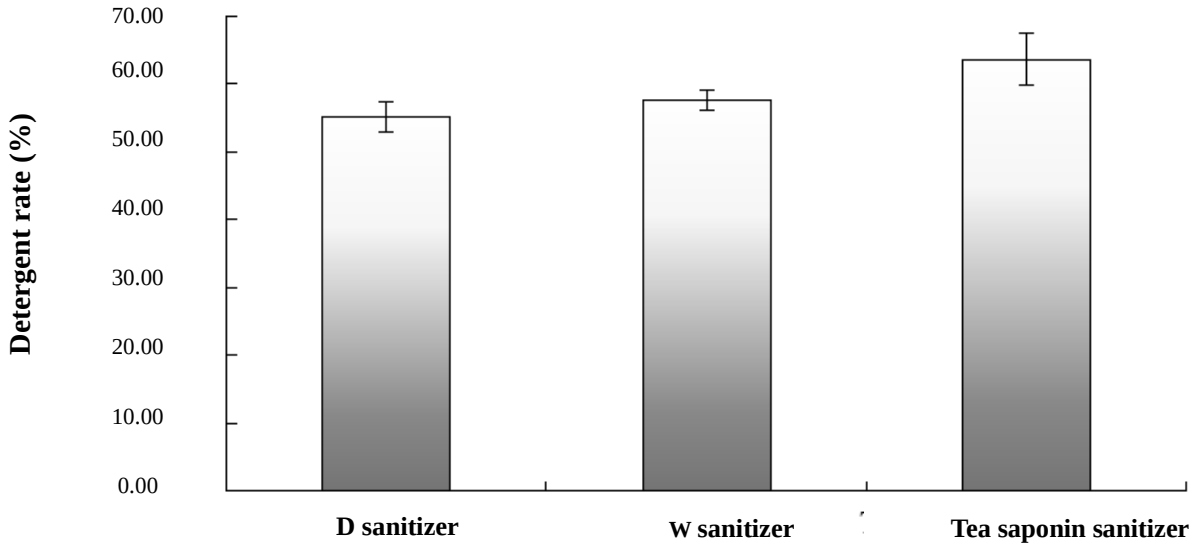

Figure 1. Three kinds of hand sanitizer to oil pollution rate.

It can be observed after observing and judging appearance and smell of hand sanitizer with tea saponin that this product is faint yellow and translucent jelly. The appearance is even and meticulous. It has comfortable feel. Generally speaking, the smell has slight ethyl alcohol. It is easily volatilized after being exposed. Furthermore, after this product is sealed and saved for 24 h at 45±0.5°C and -5±0.5°C, respectively, colloid is kept intact and has no hierarchy. The stability is good.

Analysis of greasy dirt removability

A comparison of greasy dirt removability was made among the different hand sanitizers. Allocate hand sanitizer with tea saponin, D hand sanitizer and W hand sanitizer into 5% of solution by using deionized water for standby application, and conduct a degreasing test under the conditions of the same indoor temperature of 21°C, water temperature of 30°C and rotate speed at 150 r/min.

Data in Table 3 showed that SPSS 19.0 was used to conduct the t- test analysis and comparison. If P value is less than 0.05, it means this data difference has statistical significance. It can be observed from Figure 1 and Table 3 that regarding tea saponin as the surfactant added into hand sanitizer to make a product has better degreasing effects than the traditional hand sanitizer sold in the market. Original greasy dirt on glass slide is almost washed

Table 4. Antibacterial circle diameter.

S/no.	Strain							
	***Staphylococcus aureus* (Diameter mm)**			**X ± s**	***Escherichia coli* (Diameter mm)**			**X ± s**
	a	**b**	**c**		**a**	**b**	**c**	
1	11.56	10.86	11.98	11.47±0.57	12.33	12.51	13.76	12.87±0.78
2	9.38	9.37	10.01	9.25±0.83	13.98	12.46	13.38	13.27±0.77
3	12.76	11.15	11.87	11.93±0.81	17.43	15.30	15.11	15.95±1.29

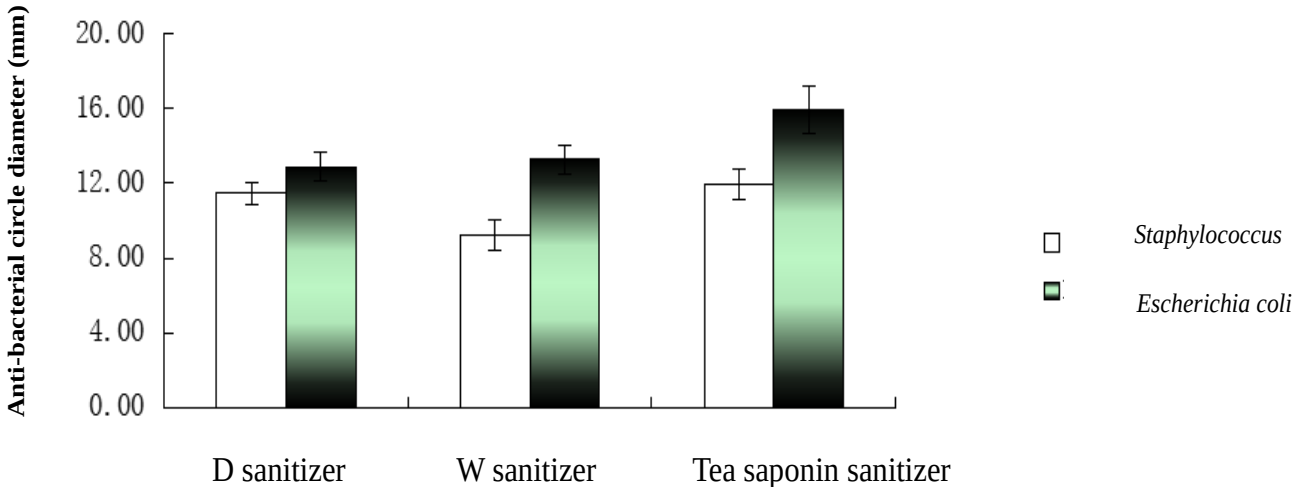

Figure 2. Comparison of antibacterial ability of 3 kinds of Sanitizer with 2 kinds of bacteria.

away after using hand sanitizer with tea saponin. The remaining greasy dirt is distributed evenly. On the other hand, parts of greasy dirt on the glass slide processed by other hand sanitizers are washed away, but greasy dirt with large area is concentrated together. The cleaning effect is ordinary.

Analysis of bacteriostatic ability

This experiment measures antibacterial effects of *Colon bacillus* and *Staphylococcus aureus* for hand sanitizer with tea saponin and other products sold in the market. In Table 4, bacteriostatic ability of hand sanitizer is expressed as x±s. Data conducted t- test analysis and comparison. P value is less than 0.05, indicating that this data difference has statistical significance. It can be observed from Figure 2 and Table 4 that when cultivating in the condition of more than $1*10^8$ CFU/L of two bacterium suspensions in culture medium, regarding tea saponin as the bacteriostatic agent to add into hand sanitizer has better antibacterial effect of *C. bacillus* than *S. aureus*. The general effect is better than common products sold in the market. Its main effective constituents include 60 to 70% ethanol, indicating tea saponin has better antibacterial effects on other bacteria than common low-concentration ethyl alcohol. Ethanol content in hand sanitizer with tea saponin is lower than common products, and it can reduce irritation on skin.

Conclusions

When considering tea saponin as a surfactant to apply to develop hand sanitizer, various indexes reach the standard stipulation. This product not only has better soil removability on artificial mixed greasy dirt than common products but also have bacteriostatic ability to restrain the growth of two typical pathogens. In the meantime, tea saponin has gentle nature and will not cause damage to the skin. Therefore, tea saponin extracted from agricultural wastes-tea seed cake applied to hand sanitizer has considerable economic benefit and is beneficial to environment protection.

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