



Agricultural Waste *Annona squamosa* L. Peel and Seed Extract: Biosynthesis of Hand Sanitizer Gel against Skin Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Alcohol-based hand sanitizers are being recommended as an infection prevention measure for COVID-19. Washing hands is a simple matter to eliminate and minimize germs on the hands with water and by adding certain ingredients, the use of hand sanitizer becomes must in recent circumstances. We have developed reliable and eco-friendly process for the synthesis of hand sanitizer gel that is a really useful tool in the fight against SARS-CoV-2, the virus that causes coronavirus disease 2019 (COVID-19). Considering scanty literature available on the use of custard apple peel and seed for preparation of hand sanitizer gel, present study was undertaken to evaluate effect of hand sanitizer from custard apple peel and seed extract. The antimicrobial activity of the formulated gel was tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis subsp. spizizenii*, *Salmonella typhimurinum* and *Shigella sonnei* by The Quality Assurance and Testing Center 3 (QUATEST 3) in Ho Chi Minh city, Viet Nam.

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1. INTRODUCTION

The world is facing a medical crisis amid the COVID-19 pandemic and the role of adequate hygiene and hand sanitizer is inevitable in controlling the spread of infection in public places and healthcare institutions. The government and health sector' advocacy groups contributed tremendously to the spike in the use of various preventive methods such as city/town and national lockdown, self-isolation, quarantine, stay-at-home model, avoidance of crowd, social distancing, wearing of face-mask and hand gloves, periodic hand washing particularly with liquid soaps/detergents, use of alcohol-based hand sanitizers (ABHS). ABHS were recommended by the World Health Organization (WHO) as an alternative hand hygiene measure [1]. ABHS contains on average 60-95% by weight of one or more alcohols [2,3,4,5]. Hand sanitizers are hygienic products that are applied on the hands to prevent the spread of microorganisms like viruses, bacteria and fungi such as coronaviruses, rhinoviruses, influenza viruses, herpes simplex virus, ebola virus, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, traveler diarrhoea among others, especially where access to water is limited [6]. It has become a routine for many people to carry hand sanitizer with them at all times, especially during the recent disease outbreak. There has been a great surge in

demand for hand sanitization products leading to shortages in their supply. Furthermore, the continuous topical application of ethanol on the skin was reported to lower skin barrier functions, thereby rendering the membrane highly susceptible to harmful chemicals in soaps and cosmetics. The risk posed by an alcohol need to be addressed in particular by seeking an alternative which is a natural and safe antibacterial ingredients without side effects. Such green alternative will preserve the natural environment and the skin of people [7,8]. One of the plants that is potential as an antibacterial is custard apple (*Annona squamosa* L.). It has been well documented that custard apple peel, pulp, seeds, leaves, bark and roots are good sources of natural antioxidants and antimicrobial compounds [9,10,11,12].

Annona squamosa is rich sources of secondary metabolites like flavonoids, phenols, phenolic glycosides, aporphine alkaloids and saponins that were found in aqueous extract of peel and seed. It has been well documented that Custard apple (*Annona squamosa*) peel and seeds are good sources of natural antioxidants and antimicrobial compounds [10,11,12]. Essential oils and phenolic compounds from a variety of plants have been reported to have antiviral activities due to their power to solubilize the lipid membrane of enveloped viruses including human coronaviruses [8].



Fig. 1. *Annona squamosa* L. fruit (A), peel (B) and seed (C)

Fruit wastes are one of the main sources of municipal wastes. Due to the high consumption and industrial processing of fruit edible parts, fruits residues (principal peels and seeds) are generated in large quantities in large cities and become a severe environmental issue. Recently, many researchers have found that the high natural antioxidants contents were observed in the peel and seed of *Annona squamosa* [10,12,13,14]. Therefore, it would be beneficial if the fruit residues could be used to recover natural antioxidants especially phenolic compounds, making them fully used in the food, pharmaceutical, as well as cosmetic industry. This study purposed to know the *Annona squamosa* L. peel and seed extract as an active ingredient of hand sanitizer gel preparation.

2. MATERIALS AND METHODS

2.1 Plant materials

Fresh seeds and peels were purchased from factories at Tây Ninh which were collected, washed thoroughly under tap water and dried with tray dryer at temperature of 50°C constantly 48h. Dried seeds and peels were chopped with blender to produce powder samples. The powder samples was packed and stored at room temperature in the dark storage room before chemical analyses were done.

2.2 Chemicals

All chemicals used were of analytical grade and were purchased from Merck Company.

2.3 Extraction

Fresh sample of *Annona squamosa* peel and seed were collected, washed thoroughly with double distilled water and dried in shade and powdered. From 300 grams of peel and seed powder dissolved in 1 liter of distilled water at 80 – 90°C for 1 hour by hydrodistillation. The extract obtained was filtered through Whatman No.1 filter paper and the filtrate was collected in a separate flask. Each of the dried extracts of the fruit parts was stored in closed vial at 4°C.

2.4 Phytochemical screening of peel and seeds of *Annona squamosa*

The qualitative chemical test was used for detection of alkaloids, flavonoids, saponins, terpenoids, polyphenols and steroids [15,16,17].

2.4.1 Alkaloid test

Extract samples were dissolved in 2 mL of hydrochloric acid, heated for 5 minutes and filtered. The filtrate obtained was added by 2-3 drops of Dragendorff's reagent. The presence of the alkaloid compounds is indicated by an orange precipitate.

2.4.2 Flavonoid test

A total of 2 mL sample (\pm 0.05% w/v) were dissolved in 2 mL methanol, then added by Mg powder and 5 drops of concentrated HCl. The presence of flavonoid compound is indicated by the formation of red or orange colour.

2.4.3 Saponin test

A total of 2 mL sample (\pm 0.05% w/v) were dissolved in aquadest in a test tube then added by 10 drops of KOH and heated in 50°C for 5 minutes in the water bath, shaken for 15 minutes. If the foam is formed steady for 1cm and remained stable for 15 minutes, it indicates the presence of saponins.

2.4.4 Terpenoid test

A total of 2 mL sample (\pm 0.05% w/v) were added by 1 mL Lieberman-Burchard reagent. Terpenoid compounds are identified by the formation of a dark blue or blackish green.

2.4.5 Polyphenol test

A total of 2 mL sample (\pm 0.05% w/v) were dissolved in 10 mL aquadest, heated for 5 minutes and filtered. The filtrate formed was added by 4-5 drops of FeCl₃ 5% (w/v). The phenol is identified by the formation of a dark blue or blackish green.

2.4.6 Steroid test

A total of 2 mL sample (\pm 0.05% w/v) were added by 1 mL Lieberman-Burchard reagent. The formation of a dark blue or blackish green colour indicates the presence of steroid.

2.5 Formulation of Hand Sanitizer Gel

Annona sanitizer: Carbopol was developed in doubled distill water, then stirred by using a stirrer. In that add polysorbate 20, glycerol and *Annona squamosa* L. peel and seed extract. Then add alcoholic food 96% (final concentrations of ethanol \geq 70% v/v). Triethanol amine was added to avoid formation of possible

air bubbles in the product and pH 6.5 ± 0.2 while stirring until it forms a gel. Finally, perfume added and mixed with slowly stirring to obtain uniform product [4,5,6,18].

2.6 Determination of Total Phenolic Content

The Total Phenolic Content was determined using Folin- Ciocalteu method [19]. Appropriately diluted samples and standard gallic acid were made up to 3.5 ml using distilled water in a series of test tubes. Various samples and the standard were treated with 0.5 ml of Folin- Ciocalteu's reagent (2N) and incubated for 3 minutes in room temperature. The reaction was then neutralized with the addition of 1ml of 20% Na_2CO_3 . The reaction mixture was incubated at room temperature for 90 minutes and the absorbance of the blue colour developed was read at 760nm using a Spectrophotometer (HACH UV Vis - Spectrophotometer, model DR6000). The Total Phenolic Content was expressed as gallic acids equivalent in Microgram per ml of sample, using a standard curve generated with gallic acid.

3. RESULTS AND DISCUSSION

Annona squamosa is one of multipurpose tree with edible fruits and it has enormous medicinal and industrial value. Phytochemical screening results of the powdered sample of *Annona squamosa* peel and seed extracted in water showed the presence of all the constituents. Results in Table 1 showed that the aqueous extract of peel and seed of *Annona squamosa* was rich in polyphenols, saponins, flavanoids and steroids followed by fruit collected from Tây Ninh, Viet Nam.

Table 1. Phytochemical screening of peel and seed extract of *Annona squamosa*

Phytochemical analysis	Seed	Peel
Saponin	+	+
Alkaloid	-	+
Flavonoid	+	+
Terpenoid	-	+
Steroid	+	+
Polyphenol	+	+

Note: "+": present; "-": absent

In this study, the water extract of *Annona squamosa* peel and seed was formulated in hand gel sanitizer preparation. The results have provided promising evidence of utilizing the

crude extracts of *Annona squamosa* peel and seed as source for natural antibacterial. The result of antibacterial activity of annona sanitizer gel against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis subsp. spizizenii*, *Salmonella typhimurinum* and *Shigella sonnei* was found to be greater than 99,9999% with concentration 100% at 20°C in 5 minutes (Table 3). This result is similar to Al-Deen (2017) provided that among three different portions of *A. squamosa* fruit, peel showed the highest antimicrobial activity than pulp and seeds. In another study, Kadam et al (2018) reported similar results for peel extract of *A. squamosa* exhibited good antimicrobial activity against *P. putida*, *S. putrefaciens*, *E. coli* and *E. fecalis* with inhibition zones ranging from 10 – 21 mm in increasing trend with increasing concentration of *Annona squamosa* peel extract from 250 to 1250 mg/ml. In a recent study of Kadam et al (2020), total phenolic and ascorbic acid content of hydroethanolic (50%) extract of *Annona squamosa* seed were respectively $234.30 \pm 0.44 \mu\text{gGAE/mg}$ and $645.07 \pm 0.32 \mu\text{gAA/mg}$ of the extract as a result of which possessed significant antioxidant as well as antibacterial activity. According to the results, all different part of extracts obtained from *Annona squamosa* shown to be with antibacterial activity against tested microbial pathogens.

Table 2. Total phenolic content in *Annona* sanitizer gel

Time (day)	Concentration ($\mu\text{gGAE/g}$)
0	664.54 ± 1.11
30	652.66 ± 2.16
60	638.14 ± 5.21

The result of our analysis reveals that *annona* sanitizer gel has ethanolic content in the range recommended by regulatory agencies [2,3,4,5]. Health agencies discourage the use of all products other than those containing at least 60% (v/v) alcohol [4]. The ph test is a test for chemical stability. The ph test aims to analyze whether the gel has the appropriate ph value and suitable with the skin. Because the ph value of gel that does not conform with the skin's ph will cause irritation to the skin. Table 4 showed that range of heavy metals content in the regulatory standards for national technical regulation on the limits of heavy metals contamination in food and drinking water (Ministry of health in Vietnam) [20]. We have developed modern method by using agriculture waste to synthesize hand sanitizer gel by employing an aqueous peel and seed extract of *Annona squamosa*.

Table 3. Antibacterial activity of *annona* sanitizer gel

Characteristic	Concentration of sample ^a	Concentration of test organisms CFU/ml	Concentration of test organisms after being-contacted CFU/ml	Test Results ^c logR ^b
<i>Staphylococcus aureus</i>	100%	3.0 x 10 ⁸	<10	>6.48
<i>Pseudomonas aeruginosa</i>	100%	2.5 x 10 ⁸	<10	>6.40
<i>Escherichia coli</i>	100%	2.0 x 10 ⁸	<10	>6.31
<i>Bacillus subtilis subsp. spizizenii</i>	100%	2.5 x 10 ⁸	<10	>6.39
<i>Salmonella typhimurium</i>	100%	1.8 x 10 ⁸	<10	>6.25
<i>Shigella sonnei</i>	100%	1.6 x 10 ⁸	<10	>6.20

Notice: Test method: BS EN 1040:2005

(a) the concentration of a product test solution shall be 1.25 times the desired test concentration because it is diluted to 80% during the test and the method validation.

(b) According to BS EN 1040:2005, the bactericidal activity of chemical disinfectants and antiseptics is considered significant if log R_z ≥ 5 at 20°C in 5 minutes.

(c) Log R greater than 1, 2, 3, 4, 5 or 6 is corresponding to bactericidal activity of greater than 90%, 99%, 99.9%, 99.99%, 99.999% or 99.9999%.

Table 4. Determination of heavy metals in *Annona* sanitizer gel

Characteristic	Test method	Test result
pH of solution	TCVN 7764-1:2007	6,7
Ethanol % (v/v)	TCVN 11584:2016 (GC-FID)	74.2
Methanol % (v/v)	TCVN 6971:2001 (GC-FID)	< 0.1
Lead content (Pb)	ACM THA 05-(ICP-AES)*	Not detected
Arsenic content (As)	ACM THA 05-(HG-AES)*	< 0,013
Mercury content (Hg)	ACM THA 05-(CV-AES)*	Not detected

(*) Method was issued by Association of Southeast Asian Nations (ASEAN)

4. CONCLUSION

Annona sanitizer gel can be considered a strategic key useful in the containment of infections such as COVID-19 both at home and in communities because it can dramatically reduce the widespread outbreak of infections. Its composition is prepared according to delicateness of skin so that it cannot cause any type of irritation. It is concluded that from the result that the gel formulation is good in appearance, homogeneity. This preliminary in vitro study demonstrated that hand sanitizer gel using *Annona squamosa* extracts active against pathogenic bacteria. Thus, a new way can be found to provide safe and healthier living through germ-free hands. Although the removal is not 100% but a major number can be reduced. Furthermore, *Annona* sanitizer gel has been commercialized in Vietnam. *Annona* sanitizer gel

is mass-produced that is helps reduce environmental pollution.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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