Short Research Article

Agricultural waste *Annona Squamosa* L. peel and seed extract: biosynthesis of hand sanitizer gel against skin pathogens

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 Quality Assurance and Testing Center 3 (QUATEST 3), Viet Nam.

Key words: *Annona squamosa*, COVID-19, annona sanitizer gel, polyphenol, antimicrobial activity.

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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 rending 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].

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

Annona squamosa is rich sources of secondary metabolites like flavonoids, phenols, phenolic glycosides, aporphine alkaloids and saponins were extracted adequate amount withaqueous seed and peel extract. 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].

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. peed extract as an active ingredient of hand sanitizer gel preparation.

2.MATERIAL AND METHODS

2.1 Plant materials

Fresh seeds and peels were purcharged form factories at Tây Ninh which were collected, washed throughly under tap water and dried with tray dryer at temperature of 50^{o}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.

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2.2 Chemicals

All chemicals used were of analytical grade and were purchased from Merch Company,

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2.3 Extraction

Fresh sample of *A. squamosa* peel and seed were collected, washed throughly 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^{o}C for 1 hour by hydro distillation. 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^{o}C.

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

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

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

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2.4.3 Saponin test

A total of 2 mL sample (± 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 (± 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 (± 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 (± 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 ≥ 70% v/v). Triethanol amine was added to avoid formation of posible air bubbles in the product and pH 6.5 ± 0.2 while stiring until it form a gel. Finally, perfume added and mixed with slowly stirring to obtain uniform product [4, 5, 6].

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3. RESULTS AND DISCUSSION

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

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

Phytochemical analysis	Seed	Peel
Saponin	+	+
Alkaloid	-	+
Flavanoid	+	+
Terpeniod	-	+
Steroid	+	+
Polyphenol	+	+

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

Table 2. Total phenolic content in annona sanitizer gel

Time (day)	Concentration (µgGAE/g)
0	664.54 ± 1.11
30	652.66±2.16
60	638.14±5.21

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]
Staphylococcus aureus	100%	3.0×10^8	<10	>6.48
Pseudomonas aeruginosa	100%	2.5×10^8	<10	>6.40
Escherichia coli	100%	2.0×10^8	<10	>6.31
Bacillus subtilis subsp. spizizenii	100%	2.5×10^8	<10	>6.39
Salmonella typhimurium	100%	1.8×10^8	<10	>6.25
Shigella sonnei	100%	1.6×10^8	<10	>6.20

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

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(b) According to BS EN 1040:2005, the bactericidal activity of chemical disinfectants and antiseptics is considered significant if log R≥ 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%.

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 *A. 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^{o}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 *A. 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 *A. squamosa* seed were respectively 234.30 ± 0.44 µgGAE/mg and 645.07 ± 0.32 µgAA/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 *A. squamosa* shown to be with antibacterial activity against tested microbial pathogens.

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)

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. 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*.

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.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

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