Bioprocessing and Biomass Technology

Journal homepage: https://bioprocessing.utm.my

Research Article

Facile Synthesis of Antimicrobial Aloe Vera for Cosmetic Application

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ARTICLE INFO

Article History:
Received: 15th August 2022
Received in revised form: 30th October 2022
Accepted: 16th November 2022
Available online:

Keywords:
Aloe Vera, antimicrobial properties, active ingredients, inhibition zone

ABSTRACT

People have always been interested in cosmetic products especially when it uses only natural substances that could act as antibacterial resistance such as Aloe Vera (AV) gel that could be easily extracted from aloe vera leaves. Although pure AV gel alone provides many cosmetic applications and benefits including anti-inflammatory effect, the efficiency for antimicrobial purposes of the pure AV gel is less compared to AV gel with an addition of bioactive substances. Normally, the ingredients made for cosmetic applications consist of non-organics or chemicals that could harm the human's skin barrier. Thus, this study aims to analyse the antimicrobial properties of local AV and to optimize the antimicrobial properties of AV for the production of antimicrobial AV for cosmetic application. The AV leaves was harvested from a residential area in Skudai, Johor Bahru, Malaysia. Then, the antimicrobial properties of AV were analysed by characterization of AV complex using a Scanning UV-Vis Spectrophotometry followed by the culturing with microbial strains of *Staphylococcus aureus* and *Escherichia coli* used against AV mixture. AV was mixed with active ingredients such as Polyvinylpyrrolidone (PVP), Iodine (I_2) and Sodium Iodide (NaI). The determination of antimicrobial properties was done by using two approaches of disc diffusion and zone inhibition. An image of chromatogram for pure AV, AV-PVP-I_2, AV-PVP-NaI and AV-PVP-I_2-NaI mixture after characterization and the diameter of zone inhibition were measured. The biggest inhibition zone can be seen for AV-PVP-I_2 samples at a concentration of 50 μg/mL that was tested against *Staphylococcus aureus* gives a diameter of 21 mm. The smallest inhibition zone can be seen for AV-PVP-I_2-NaI samples at a concentration of 25 μg/mL that was tested against *Escherichia coli* gives a diameter of 5.6 mm. The future prospect of antimicrobial AV research will widen the use of AV, improving the extraction method of AV and enhancing environment safety for product applications.

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INTRODUCTION

Aloe vera (AV) is one of a type of Liliaceae family and is a succulent plant originated from northern Africa, also known as *Aloe Barbadensis Miller.* It is a cactus-like plant that can grow in both dry and cold climates. They can have up to 30 to 35 cm long and 10 cm wide leaves in pea green colour. According to Javed & Atta-Ur (2014), the leaves of AV consist of gel that is made up of 99% water and 1% of bioactive compounds. The bioactive compounds mainly composes of naphthoquinones, anthraquinones, simple and complex sugar, acemannan, aloin, aloe-emodin, aloesin, aloe-mannan, flavonoids, sterols, amino acids, vitamins, enzymes and minerals in which it provides anti-inflammatory, anti-oxidant, immune boosting, anti-cancer, healing, anti-ageing and anti-diabetic properties (Edis & Bloukh, 2020) that could be used as to treat inflammation, skin diseases, healing wounds and burns and even as an ointment for stomach ache.

According to the book of New Cosmetic Science in 1997, generally, cosmetics means any action such as rubbing, sprinkling or application to any parts of human body intended for cleaning, beautifying, enhancing attractiveness, changing the appearance of someone's body or face and for maintaining health of the skin hair. Clearly, the application of cosmetic has been necessary to people's lives for decades. Thus, the ingredients of cosmetics are improving day by day to suit the trend and adapt to modern technology. As for cosmetic purposes, AV gel was proven to consist of antifungal, antibacterial and antiviral activity that could cure skin infections such as acne, herpes and scabies (Bashir et al., 2011; Stanley et al., 2014). This is because all the bioactive substances can neutralize and binds with fibroblast growth factor (FGF-2) receptor or changing the pathways for FGF-2 by affecting the gap junction intercellular communications (GJIC) for exchange of low molecular weight molecules and proliferation of diabetic fibroblast. Besides the bioactive compounds, the molecular weight of the gel also contributes to beneficial effect on cosmetic application as AV gel contains high concentration of polysaccharides, lectin like proteins and prostaglandins. Although AV plants could grow in various climates, the environmental adaptation could affect the antimicrobial properties as AV gel may consist of different molecular weights of water-bioactive compounds (Edis & Bloukh, 2020).

Research has been conducted to prove that AV gel brings a lot of benefits to cosmetic applications as well as medical purposes. Based on Edis and Bloukh research on the synthesis of antimicrobial aloe vera in 2020, they have used smart Triiodide – Polyvinylpyrrolidone (PVP) biomaterials combining with extracted AV gel to produce new antimicrobial agents that could minimise the cost of medical to treat against surgical site infections (SSI) and act as a disinfecting agent. While based on Danish et al. (2020), research that have been done is to evaluate the antibacterial and antifungal activities of the whole AV plant that consist of root and leaves against different strains of bacteria and pathogenic fungal strains for medical purposes.

In both of the research, the AV leaves is collected in different countries resulted from different climates. The AV gel extracted in Zehra and Samir research is from Arab, while Danish, Ali and Hafeez is from Pakistan. Climate change can affect the growth adaptability and the antimicrobial properties of the AV leaves. Ideally, the AV plant will grow very well in a hot dry climate or semi-desert conditions especially in Arabian Peninsula. In Pakistan, there are wide variations of weather changing at different locations, as in the research, the AV sample is taken from Burewela, Pakistan (Danish et al., 2020; Durga & Mary, 2012), in which the weather is mostly cloudy and very warm. Thus, the AV extracted gel sample taken for both of the research does not have the same climate weather and growth. Due to the contradiction of places of the AV sample taken, the antimicrobial activities may be different.

In order to prove that climate change plays an important role in antibacterial properties, in this research, AV plants was taken locally from Johor, Malaysia. On average throughout the year, Malaysia is categorized as quite humid compared to Arab and Pakistan. The method of the experiment will be conducted by using PVP, Iodine (I_2) and Sodium Iodide (NaI) with a supernatant AV gel extracted. The synthesis of this experiment is simple and cost-effective with faster reaction. This is because, only antimicrobial test is done against two selected microbial strains that usually can be found on skin's surface which is *Staphylococcus aureus* and *Escherichia coli* for much better generation of antimicrobial AV for cosmetic application. In the experiment, the parameters studied are as shown in **Table 1**.

Table 1 The manipulated, controlled, and measured variables for the experiments.

Manipulated Variable	1. The method of extraction by using distilled water and methanol. 2. The type of microbial strains by using *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive). 3. The concentrations of pure AV, AV-PVP, AV-PVP-I_2 and AV-PVP-I_2-NaI tested against microbial strains with 50 µg, 25 µg, 12.5 µg and 6.25 µg.
Controlled Variable	The temperature of pure AV, AV-PVP, AV-PVP-I_2 and AV-PVP-I_2-NaI store dark in refrigerator for 4 °C.
Measured Variable	The inhibition zone of pure AV, AV-PVP, AV-PVP-I_2 and AV-PVP-I_2-NaI samples against *Escherichia coli* and *Staphylococcus aureus.*

AV plants consist of highly bioactive compounds that could cure operation scars or even act as a disinfecting agent. AV plants also provide a lot of benefits not only in the medical purpose, but also cosmetic applications. Extracted gel from AV plants consist of naphthoquinones that can act as antiseptic or antimicrobial properties in which it can also be used to keep the skin hydrated and also provides anti-aging effect (Rastilantie et al., 2010). Normally, ingredients used for cosmetic application are based on chemicals and other non-organic materials that act as an antimicrobial agent such as benzalkonium chloride (BACs), alcohol, triclosan, glycerin and triclocarban. Benzalkonium chloride is widely used in treating acne as it contains in most of the acne products and antiseptic cuticle treatment (Id et al., 2020). Although BACs are extensively used for acne treatment, however it is not efficient as it has lots of side effects and disadvantages such as irritation to the skin to the extent that it could cause dermatitis (Choi et al., 2018). This is because BACs could penetrate through the human's skin. Thus, to encounter the problem, antimicrobial AV that

mainly contains organic and biomaterial compounds will be a potential substitution and far safer than the chemical ingredients. Due to most research mainly focusing on medical purposes, this study focuses on the facile or simple synthesis of antimicrobial AV for cosmetic purposes. The extraction of AV gel was extracted from local AV plants planted in Malaysia, which may affect the antimicrobial properties of the AV gel as the climate changes. The aims of this research are mainly to investigate the antibacterial properties of local AV with two objectives: (i) to analyse the antimicrobial properties of local AV in Malaysia and (ii) to enhance the antimicrobial properties of AV mixture for cosmetic applications.

METHODOLOGY

The research methodology is divided into seven parts; (1) extraction of AV gel from the leaves, (2) preparation of AV with addition of active ingredients, (3) bacterial strains and culturing, (4) determination of antimicrobial properties against bacterial strains, (5) characterization of AV complexes, (6) characterization and the statistical analysis.

Materials and Chemicals

The raw materials are AV leaves obtained from a residential area in Skudai, Johor, Malaysia. The biomaterial include Polyvinylpyrrolidone (PVP-K-30), sodium iodide (NaI) and Iodine, Mueller-Hinton Broth and methanol were purchased from Tricell Bioscience Resources from Skudai, Johor, Malaysia. Premium petri dish, centrifuge bottle and filter paper qualitative high speed 101 with diameter of 11 cm were purchased from the same supplier. The bacterial strains of *S. aureus* ATCC 6538, *E. coli* WDCM 00013 Vitroids prepared and obtained from Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Johor, Malaysia. The equipment used were blender, autoclave, oven dryer and laminar flow located in the IBD.

Preparation of AV Extract

The preparation of aloe vera extract was done by cutting and harvesting Aloe Vera leaves from a residential area in Skudai, Johor, Malaysia about 38 to 50 cm long around 8.30 to 9.00 am in the morning. Then, the AV leaves were brought into Institute of Bioproduct Development, IBD in Universiti Teknologi Malaysia, UTM to continue with the extraction process. The AV leaves were washed with distilled water to remove all the dirt or soil, then rinsed again with pure ethanol and again with distilled water. The AV leaves were dried in room temperature to remove the remaining water. After it has completely dried, the AV leaves were cut and sliced to expose the gel then the AV gel were scraped out with a spoon and placed inside the blender. The blender was switched on and the AV gel was blended for 3 minutes at maximum speed. The blended gel was transferred into a 1000 mL Erlenmeyer flask. Eighty grams of the colorless AV gel was transferred from the flask into a 250 mL Erlenmeyer flask and 80 mL of absolute methanol was added and mixed well. In another Erlenmeyer flask, 80 g of AV gel was added with 80 mL of distilled water (as the control). The mixtures were covered and stored it for 36 hours in room temperature. The light-yellow supernatant was kept in darkness at 4 °C for further uses.

Preparation of AV-PVP, AV-PVP-I_2, AV-PVP-NaI and AV-PVP-I_2-NaI

The preparation of Aloe Vera added with Polyvinylpyrrolidone was performed with 1 g of polyvinylpyrrolidone K-30 (PVP) dissolved in 10 mL of water. Then, 2 mL of PVP-water solution was mixed with 4 mL of methanol extracted AV and stirred at room temperature. The mixture was covered and stored for 36 hours in room temperature. The preparation of Aloe Vera, Polyvinylpyrrolidone added with Iodine were performed with 0.05 g of Iodine (I_2) dissolved in 3 mL of methanol. Then, 4 mL of the stock solution AV-PVP was mixed with I_2-methanol solution and stirred at room temperature. The preparation of Aloe Vera, Polyvinylpyrrolidone added with Sodium Iodide were done with 0.026 g of NaI dissolved in 3 mL of methanol. Then, 4 mL of the stock solution AV-PVP was mixed with NaI-methanol solution and stirred at room temperature. The preparation of Aloe Vera, Polyvinylpyrrolidone added with Iodine and Sodium Iodide were done with 0.05 g of I_2 and 0.026 g NaI dissolved in 3 mL of methanol. Then, 4 mL of the stock solution AV-PVP was mixed with I_2-NaI-methanol solution and stirred at room temperature.

Bacterial Strains and Culturing

The Mueller Hinton Broth (MHB) was used as a nutrient medium to grow the microbial strains of *Staphylococcus aureus* and *Escherichia coli.* Before that, microbial strains of *S. aureus* ATCC 25923 and *E. coli* WDCM 00013 Vitroids were stored at -20 °C in the refrigerator. MH agar were prepared by mixed Mueller Hinton with agar and autoclaved before plating it. Then, the two microbial strains were added onto the MHB for inoculation. The suspension was kept at 4 °C for further use.

Determination of Antimicrobial Properties of pure AV, AV-PVP, AV-PVP-I_2, AV-PVP-NaI and AV-PVP-I_2-NaI

The antimicrobial properties of the prepared samples were tested against two microbial strains of *Staphylococcus Aureus* (gram positive) and *Escherichia Coli* (gram negative). Procedure of zone of inhibition plate studies and disc diffusion method were used in determination of antimicrobial properties.

Procedure for Inhibition Zone Plate Studies

In determination of antimicrobial properties of the samples, bacterial strains were suspended into 10 mL MHB and incubated at 37 °C for 2 to 4 hours. After 4 hours of incubation process, the microbial culture of 100 µL following McFarland standard was seeded into the disposable, sterilized petri dishes with Mueller Hinton Agar (MHA) using sterile cotton swabs. The petri dishes were dried for 10 minutes and ready to be used for antimicrobial testing.

Disc Diffusion Method

The serial dilution done by from concentration of 50 µg/mL to 6.25 µg/mL. The disc diffusion method was done by soaking sterile filter paper disc into 2 mL of AV, AV-PVP-I_2, AV-PVP-NaI, AV-PVP-I_2-NaI with concentration of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL. Then, the sterile filter paper was dried for 24 hours. After 24 hours, the dried disc was put on the agar plates and incubated for 24

hours at 30 °C. The diameter of zone of inhibition (ZOI) was measured with a ruler to the nearest millimetre. The data was collected and marked.

Characterization of AV Complexes

The characterization of the samples was tested with ultraviolet-visible spectrophotometer to analyze and confirm the composition of the AV pure, AV-PVP-I_2, AV-PVP-NaI and AV-PVP-I_2-NaI. The absorbance spectrum of the compound and were observed and recorded. The spectrophotometer was set at the wavelength range from 250 to 800 nm.

RESULTS AND DISCUSSION

Antimicrobial Activities

The antimicrobial activities of the samples of pure AV, AV-PVP with addition of Iodine, AV-PVP with addition of Sodium Iodide and AV-PVP with addition of Iodine and Sodium Iodide were tested at different concentration of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL against *Staphylococcus aureus* that were swabbed onto the Mueller Hinton Agar plate. The inhibition zone for each of the samples at different concentration as in **Figure 1**.

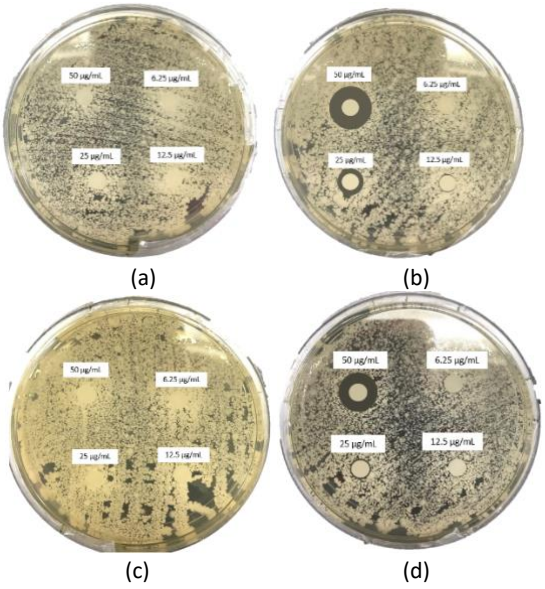

Figure 1 Inhibition zones of (a) pure AV, (b) AV-PVP-I_2, (c) AV-PVP-NaI and (d) AV-PVP-I_2-NaI at different concentration against *Staphylococcus aureus*

Table 2 Diameter of the inhibition zone (in mm) of samples at different concentrations against *Staphylococcus aureus*

Samples	Concentration of samples (µg/mL)			
	50	25	12.5	6.25
	Diameter of inhibition zone (mm)			
Pure AV	ND	ND	ND	ND
AV-PVP-Iodine	21.0	13.0	8.8	ND
AV-PVP-NaI	ND	ND	ND	ND
AV-PVP-I_2-NaI	16	8.6	ND	ND

*ND=not detected

The antimicrobial activities of the samples of pure AV, AV-PVP with addition of Iodine, AV-PVP with addition of Sodium Iodide and AV-PVP with addition of Iodine and Sodium Iodide were tested at different concentration of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL against *Escherichia coli* that were swabbed onto the Mueller Hinton Agar plate. The inhibition zone diameters were indicated by the hollow zone around the disc that were previously dipped in the samples. The inhibition zone for each of the samples at different concentration as in **Figure 2**. The quantitative value for the diameter of the inhibition zone (in mm) of samples at different concentrations against *Staphylococcus aureus* and *Escherichia coli* is summarized in **Table 2** and **Table 3**, respectively.

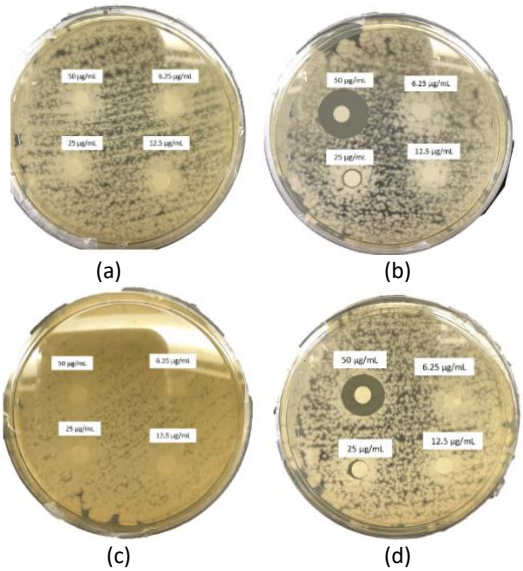

Figure 2 Inhibition zones of (a) pure AV, (b) AV-PVP-I_2, (c) AV-PVP-NaI and (d) AV-PVP-I_2-NaI at different concentration against *Escherichia coli*

Table 3 Diameter of the inhibition zone (in mm) of samples at different concentrations against *Escherichia coli*

Samples	Concentration of samples (µg/mL)			
	50	25	12.5	6.25
	Diameter of inhibition zone (mm)			
Pure AV	ND	ND	ND	ND
AV-PVP-Iodine	19.2	9.4	ND	ND
AV-PVP-NaI	ND	ND	ND	ND
AV-PVP-I_2-NaI	14.5	5.6	ND	ND

*ND=not detected

Characterization of AV Complexes

UV-vis spectroscopy is a low-cost, easy, versatile, non-destructive analytical approach that can be used to analyze a wide range of organic and inorganic chemicals. Spectrophotometers measure the absorbance or transmittance of light travelling through a medium as a function of wavelength and known as UV-vis spectrophotometers. Commonly, engineers use it for quantitative analysis, derive liquid phase reaction kinetics, and pinpoint the molecular mechanism (Rocha et al., 2018).

In this project, Ultraviolet-visible spectroscopy is used to characterize the samples of pure AV, AV-PVP with addition of Iodine, AV-PVP with addition of Sodium Iodide and AV-PVP with addition of Iodine and Sodium Iodide in liquid form. According to the manual of UV-Vis Spectrophotometers by Sharpe in 1984, the best wavelength to analyze a liquid sample is in between 190 nm

to 900 nm. Therefore, the wavelength was set from 250 nm to 800 nm to record the absorbance of the samples.

Pure AV antimicrobial properties and their characterization

No significant inhibition zone diameter could be measured and seen in this research using pure AV although several claims have reported the natural antifungal properties of pure AV. Olaleye and Bello (2005) studied on the comparative antimicrobial activities of AV gel tested on *Staphylococcus aureus* strains and resulted with 18 mm of inhibition zone diameter. Unfortunately, this research paper does not show any inhibition zone diameter for all concentrations of samples used.

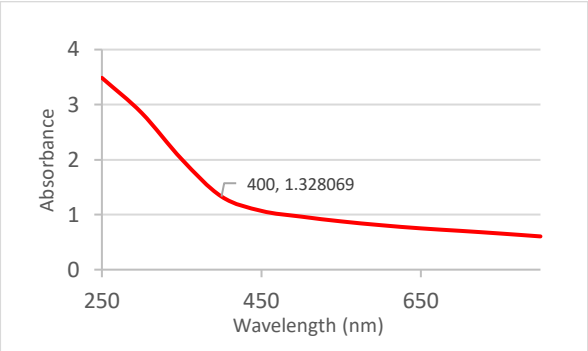

Figure 3 The graph of absorbance against wavelength for characterization of pure AV using UV-Vis Spectroscopy.

The UV-vis spectra for pure AV samples at 25 µg/mL were shown in **Figure 3**. It can be seen that there is abundance of absorption signal in between 250 nm to 400 nm. There are several components consist in a pure AV such as Aloin, Aloe-Eodin, Aloesin, Aloenin, Rhein, Pyrogallol and Hesperidin (Edis and Bloukh, 2020). All these components having different UV-vis absorption signals that range from 208 nm up to 360 nm.

Previous studies have verified the possible spectrum wavelength numbers of each component consisting in the pure AV gel. Añibarro-Ortega et al. (2021) have optimized the extraction of aloesin from AV using green solvents and the results have shown positive antimicrobial effects against bacterial and fungal strains. The findings of the components are listed in Table 4 as below.

Table 4 The UV-Vis absorption signals of Pure AV by recent studies

AV components	UV-vis absorption signals (nm)	References
Aloin	353	(Logaranjan et al., 2016)
Aloe-Emodin	285	(Froldi et al., 2019)
Aloesin	360	(Zhao et al., 2016)
Aloenin	293	(Zhao et al., 2016)
Rhein	295	(Liu et al., 2018)
Pyrogallol	349	(Behboodi-Sadabad et al., 2017)
Hesperidin	329	(Kuntic et al., 2012)

From **Table 4**, it can be seen that the range of the spectra of all the components in the pure AV sample is in between 285 nm to 360 nm. Therefore, it can be said that the pure AV in this study consist of all these components as in previous research papers where the spectra are in between 250 nm to 400 nm. Although pure AV samples does not inhibit the microbial strains used, but the constituents of AV are presence.

AV-PVP-I_2 antimicrobial activities and their characterization

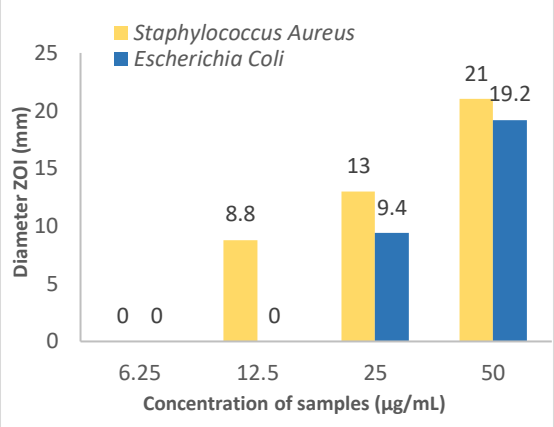

Figure 4 The graph of diameter of inhibition zone against different concentrations of AV-PVP-I_2 for *Staphylococcus aureus* and *Escherichia coli.*

From the **Figure 4**, the diameter of inhibition zone of AV-PVP-I_2 against *Staphylococcus aureus* strains is bigger compared to *Escherichia coli* strains with diameter of 21 mm and 19.2 mm respectively to both strains. In addition, highest concentration of AV-PVP-I_2 leads to bigger zone of inhibition diameter. **Figure 1** and **Figure 2** shows that there is no zone of inhibition at lower concentration of 6.25 µg/mL for both microbial strains. However, at 12.5 µg/mL concentration of samples, it shows no inhibition towards *Escherichia coli* strains while 8.8 mm against *Staphylococcus aureus* strains. At 25 µg/mL concentration of the samples, the inhibition zone diameter is 13 mm and 9.4 mm for *Staphylococcus aureus* and *Escherichia coli* strains respectively. According to Ericsson and co-worker (1960), the paper disc method utilizing serial dilutions were an accurate process of determining antimicrobial sensitivity. Therefore, the graph indicates that increasing the concentration of the samples increased the inhibition zone diameter against the microbial strains used and this is proven by previous studied on the antimicrobial activity of AV gel (Jothi, 2009). Higher resistance of *Escherichia coli* against antibiotics or drugs contributes to less ZOI compared to against *Staphylococcus aureus* (Wimmerstedt, 2008).

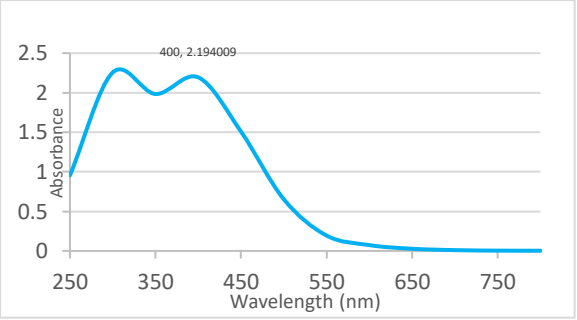

Figure 5 The graph of absorbance against wavelength for characterization of AV-PVP-I_2 using UV-Vis Spectroscopy.

The UV-Vis spectra of AV-PVP-I_2 are shown in **Figure 5**. The absorption bands were observed at 250 nm to 800 nm. Previous study has shown that the samples could be observed at 290 nm and 359 nm (Edis and Bloukh, 2020). The absorbance showing an inconsistency in between 250 nm till 400 nm, due to the presence of PVP that is verified by the absorption signal around 350 nm as mentioned by Sreekanth et al. (2019) about the carbonyl group of PVP electrolytes. The existence of all absorption signals linked to AV, PVP, and iodine in the UV-vis spectra confirms the composition of the complex biomolecules inside the samples.

According to Wei et al. (2005), the presence of Iodine, Iodide ion and Triiodide ion in the sample should be in between the wavelength range of 200 nm to 460 nm. In this study the UV-vis spectrum of sample AV-PVP-I_2 have the maximum absorption signal at 400 nm with 2.19 absorbance unit (au), indicating that presence of Iodine ions in the samples

AV-PVP-NaI antimicrobial activities and their characterization

No antimicrobial activities can be seen for AV-PVP-NaI sample when tested against neither *Staphylococcus aureus* nor *Escherichia coli* strains. Previous studied proved that AV-PVP-NaI samples were not suitable to inhibit the activities of both microbial strains as it only consists of one iodide ions from Sodium Iodide compositions (Edis and Bloukh, 2020).

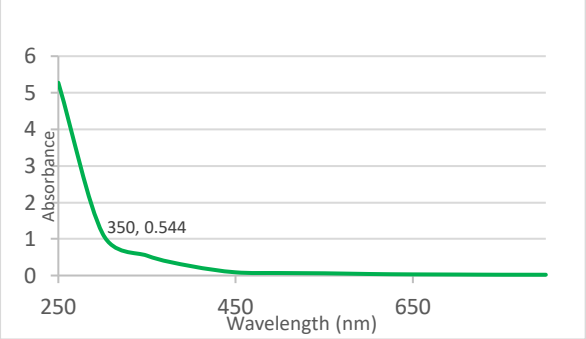

Figure 6 The graph of absorbance against wavelength for characterization of AV-PVP-NaI using UV-Vis Spectroscopy.

The UV-Vis spectrum of samples AV-PVP-NaI can be seen in **Figure 6** in between 250 nm to 350 nm. There is a small shoulder at 350 nm indicating the presence of iodide ions but there are no signals related to molecular iodide. According to Edis and Bloukh (2020), around 203 nm and 460 nm is the absorption signals for molecular iodide. Therefore, the successful encapsulation of AV biomolecules and triiodide ions into the PVP backbone in our bio complexes mixture was proven. Although AV-PVP-NaI does not inhibits both microbial strains used, but the mixture is confirmed to consist of PVP and NaI. The errors and precautions are discussed in the recommendations part regarding the zero-inhibition zone diameter of AV-PVP-NaI samples against microbial strains.

AV-PVP-I_2-NaI antimicrobial activities and their characterization

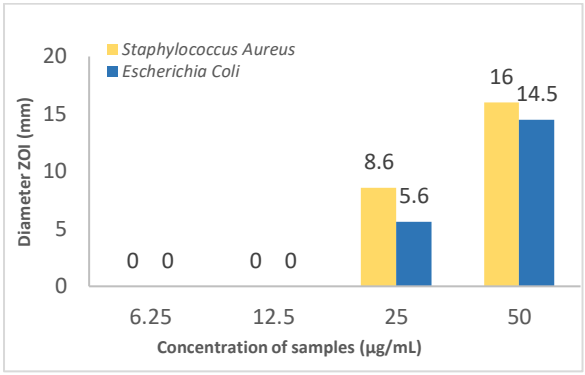

Figure 7 The graph of diameter of inhibition zone against concentration of AV-PVP-I_2-NaI for *Staphylococcus aureus* and *Escherichia coli*

Overall, the diameter of inhibition zone of AV-PVP-I_2 against *Staphylococcus aureus* strains is bigger compared to *Escherichia coli* strains based on **Figure 7** with diameter of 16 mm and 14.5 mm respectively. Not only that, highest concentration of AV-PVP-I_2 leads to bigger zone of inhibition diameter. **Figure 7** shows that there is no inhibition zone at lower concentration of 6.25 µg/mL and 12.5 µg/mL for both microbial strains. At 25 µg/mL concentration of the samples, the inhibition zone diameter are 8.6 mm and 5.6 mm for *Staphylococcus aureus* and *Escherichia coli* strains, respectively.

In addition, the ZOI for *S. aureus* (Gram +ve) is bigger compared to *E. coli* (Gram -ve) due to the type of gram positive and gram negative. Gram-positive bacteria have extensive coatings of peptidoglycan in their cell walls. Gram-negative bacteria have a cell wall made up of thin layers of peptidoglycan. Therefore, gram positive bacteria have higher susceptible to the samples compared to gram negative bacteria (Rohde, 2019).

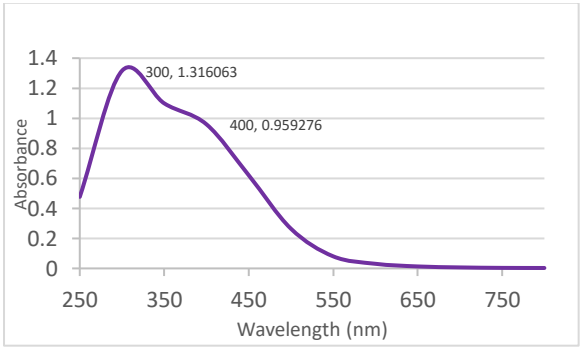

Figure 8 The graph of absorbance against wavelength for characterization of AV-PVP-I_2-NaI using UV-Vis Spectroscopy.

The UV-Vis spectra of AV-PVP-I_2-NaI are shown in **Figure 8**. The absorption bands were observed at 250 nm to 800 nm. According to Sreekanth et al. (2019), the presence of PVP can be verified when the absorption signal around 350 nm. From Figure 8, the absorbance showing an inconsistency in between 300 nm till 400 nm proves that PVP is presence in the samples. The existence of all absorption signals linked to AV, PVP, and iodine in the UV-vis spectra confirms the composition of complex biomolecules inside the samples.

The concentration of iodide is increasing with addition of Sodium Iodide (NaI) in which will leads to abundance of triiodide ions inside the samples, reducing the molecular iodine concentration and interrupt the chemical structure of encapsulated AV on the PVP-backbone (Edis and Bloukh, 2020). The presence of Sodium Iodide in the sample should be in between the wavelength range of 290 nm to 359 nm. In this project the UV-vis spectrum of AV-PVP-I_2-NaI sample having absorption signal maximum at 400 nm with 0.95 absorbance unit (au), indicates that there is presence of triiodide, iodide and iodine in the PVP-polymer backbone.

We can see that from **Figure 1** and **Figure 2**, the zone inhibition diameter of samples AV-PVP-I_2 and AV-PVP-I_2-NaI were bigger when tested against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) strains.

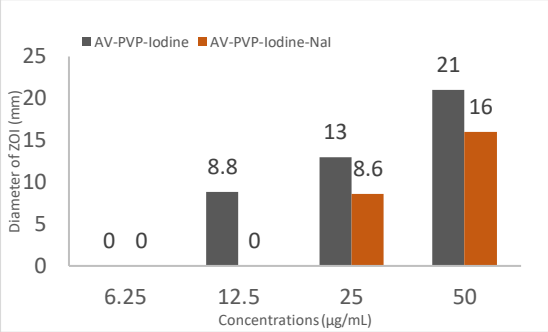

Figure 9 The graph of absorbance against wavelength for characterization of AV-PVP-I_2-NaI using UV-Vis Spectroscopy.

As shown in **Figure 9**, higher zone of inhibition can be seen for AV-PVP-I_2 sample compared when there is presence of Sodium Iodide. The main reason AV-PVP-I_2-NaI sample was less efficient is due to the addition of Sodium Iodide that interrupt the formation of the mixture. NaI are hydrophilic type of ionic compound that causes additional disturbance by repulsion, increasing steric crowding and steric hindrance and resulted in the desorption of different phenolic compounds and triiodides (Haj Bloukh et al., 2020). Due to the disruption of the compound structure, the absorption intensities for AV-PVP-I_2-NaI sample are lower than AV-PVP-I_2 sample.

The results obtained from this study is that different source of raw material used significantly affects the antimicrobial efficacy of the AV. The local Malaysian AV show a slightly lesser antimicrobial efficacy compared to the one conducted by Edis & Bloukh (2020). This is due to the weather and the surrounding ecosystems supporting the development of bioactive compounds in the raw material.

CONCLUSIONS

Antimicrobial AV for cosmetic application with simple or facile procedures was analyzed and enhanced. The result obtained from this study for the best AV mixture is AV-PVP with addition of Iodine by using methanol as solvent for extraction process. This study has widened the potential use of AV other than medical purposes, improving the process of extracting the AV using selected solvent and enhancing the environment safety by developing a safe antimicrobial product especially for cosmetic application.

Acknowledgement

This work was supported by UTM Fundamental Research Grant (References Grant Q.J130000.2551.20H88) and Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia.

References

Añibarro-Ortega, M., Pinela, J., Ćirić, A., Lopes, E., Molina, A. K., Calhelha, R. C., ... & Barros, L. 2021. Extraction of aloesin from Aloe vera rind using alternative green solvents: Process optimization and biological activity assessment. *Biology*. 10(10): 951.

Bashir, A., Saeed, B., Mujahid, T. Y., & Jehan, N. 2011. Comparative study of antimicrobial activities of Aloe vera extracts and antibiotics against isolates from skin infections. *African Journal of Biotechnology*, *10*(19): 3835–3840. https://doi.org/10.4314/ajb.v10i19.

Behboodi-Sadabad, F., Zhang, H., Trouillet, V., Welle, A., Plumeré, N., Levkin, P.A. 2017. UV-Triggered Polymerization, Deposition, and Patterning of Plant Phenolic Compounds. *Advanced Functional Materials.* 27: 1700127.

Choi, S. M., Roh, T. H., Lim, D. S., Kacew, S., Kim, H. S., & Lee, B. M. 2018. Risk assessment of benzalkonium chloride in cosmetic products. *Journal of Toxicology and Environmental Health - Part B: Critical Reviews*, *21*(1): 8–23. https://doi.org/10.1080/10937404.2017.1408552

Danish, P., Ali, Q., Hafeez, M. M., & Malik, A. 2020. Antifungal and antibacterial activity of aloe vera plant extract. *Biological and Clinical Sciences Research Journal*. (1): e004-e004.

Durga, A., & Mary, R. R. 2012. Analysis of Phytochemical Constituents And Antimicrobial Activities of Wedelia Chinensis Against Pathogens. *International Journal of Scientific Research*. *3*(8): 484–486. https://doi.org/10.15373/22778179/august2014/156

Edis, Z., & Bloukh, S. H. 2020. Facile synthesis of antimicrobial aloe vera-"smart" triiodide-PVP biomaterials. *Biomimetics*. *5*(3): 1–30. https://doi.org/10.3390/BIOMIMETICS5030045

Ericsson, B. H., Tunevall, G., & Wickman, K. 1960. The paper disc method for determination of bacterial sensitivity to antibiotics: Relationship between the diameter of the zone of inhibition and the minimum inhibitory concentration. *Scandinavian Journal of Clinical and Laboratory Investigation*. *12*(4): 414-422.

Froldi, G.; Baronchelli, F.; Marin, E. 2019. Grison, M. Antiglycation Activity and HT-29 Cellular Uptake of Aloe-Emodin, Aloin, and Aloe arborescens Leaf Extracts. *Molecules*. 24: 2128.

Haj Bloukh, S.; Edis, Z.; Ibrahim, M.R.; Abu Sara, H. 2020. "Smart" antimicrobial nanocomplexes with potential to decrease surgical site infections (SSI). Pharmaceutics. 12: 361.

Id, Z. B., Boucherit, K., Rodrigues, E., & Ferreira, I. C. F. R. 2020. *Cosmetics Preservation : A Review on Present Strategies*. 1–41. https://doi.org/10.3390/molecules23071571

Javed, S., & Atta-Ur, R. 2014. Aloe vera gel in food, health products, and cosmetics industry. In *Studies in Natural Products Chemistry* (1st ed., Vol. 41). Elsevier B.V. https://doi.org/10.1016/B978-0-444-63294-4.00009-7

Jothi, D. 2009. Experimental study on antimicrobial activity of cotton fabric treated with aloe gel extract from Aloe vera plant for controlling the Staphylococcus aureus (bacterium). *African Journal of Microbiology Research*, *3*(5): 228-232.

Kuntic, V., Pejic, N., Mi´ci´c, S. 2012. Direct Spectrophotometric Determination of Hesperidin in Pharmaceutical Preparations. *Acta Chimica Slovenica*. 59: 436–441.

Liu,W., Cai, A., Carley, R., Rocchio, R., Petrovas, Z.M., Chartier, C.A., Meng, X., Su, J., Cho, B.P., Dain, J.A., et al. 2018. Bioactive anthraquinones found in plant foods interact with human serum albumin and inhibit the formation of advanced glycation endproducts. *Journal of Food Bioactives*. 4: 130–138.

Logaranjan, K., Raiza, A.J., Gopinath, S.C., Chen, Y., Pandian, K. 2016. Shape- and Size-Controlled Synthesis of Silver Nanoparticles Using Aloe vera Plant Extract and Their Antimicrobial Activity. *Nanoscale Research Letters.* 11: 520.

Makarova, O., Johnston, P., Walther, B., Rolff, J., & Roesler, U. 2017. Complete genome sequence of the disinfectant susceptibility testing reference strain Staphylococcus aureus subsp. aureus ATCC 6538. *Genome Announcements*, *5*(19): e00293-17.

Nejatzadeh-Barandozi, F. 2013. Antibacterial activities and antioxidant capacity of Aloe vera. *Organic and Medicinal Chemistry Letters*, *3*(1): 5. https://doi.org/10.1186/2191-2858-3-5

Olaleye, M. T., & Bello-Michael, C. O. 2005. Comparative antimicrobial activities of Aloe vera gel and leaf. *African Journal of Biotechnology*, *4*(12).

Rastilantie, M.-, Christaki, E. V, & Florou-Paneri, P. C. 2010. Www.World-Food. *Agriculture & Environment*, 8(2): 245–249. http://en.wikipedia.org/wiki/Aloe-Vera,

Rocha, F. S., Gomes, A. J., Lunardi, C. N., Kaliaguine, S., & Patience, G. S. 2018. Experimental methods in chemical engineering: Ultraviolet visible spectroscopy—UV-Vis. *The Canadian Journal of Chemical Engineering*, 96(12): 2512-2517.

Rohde, M. (2019). The Gram-positive bacterial cell wall. *Microbiology Spectrum*, 7(3), 7-3.

Sharpe, M. R. 1984. Stray light in UV-VIS spectrophotometers. *Analytical Chemistry*, 56(2): 339A-356A.

Sreekanth, K., Siddaiah, T., Gopal, N. O., Kumar, Y. M., & Ramu, C. 2019. Optical and electrical conductivity studies of VO2+ doped polyvinyl pyrrolidone (PVP) polymer electrolytes. *Journal of Science: Advanced Materials and Devices*. 4(2), 230-236.

Stanley, M. C., Ifeanyi, O. E., & Eziokwu, O. G. 2014. Antimicrobial effects of Aloe vera on some human pathogens. *International Journal of Current Microbiology and Applied Sciences*. 3(3): 1022-1028.

Wei, Y. J., Liu, C. G., & Mo, L. P. 2005. Ultraviolet absorption spectra of iodine, iodide ion and triiodide ion. *Guang pu xue yu guang pu fen xi= Guang pu*. 25(1): 86-88.

Wimmerstedt, A., & Kahlmeter, G. 2008. Associated antimicrobial resistance in Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae and Streptococcus pyogenes. *Clinical Microbiology and Infection*. 14(4): 315-321.

Zhao, Y.; Sun, Y.N.; Lee, M.J.; Kim, Y.H.; Lee, W.; Kim, K.H.; Kim, K.T.; Kang, J.S. 2016. Identification and discrimination of three common Aloe species by high performance liquid chromatography-tandem mass spectrometry coupled with multivariate analysis. *Journal of Chromatography B*. 1031: 163–171.