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Comparative Study on Hylocereus polyrhizus Peels Extract and Aloe barbadensis Leaves Extract as Potential Natural-based Sources in Antibacterial and Antioxidant Activities

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ABSTRACT

Aloe barbadensis leaves and Hylocereus polyrhizus are plants that are easily found in Malaysia. They are commonly studied for antibacterial activity and how they can benefit the human being. However it is not known about how the combination of both plants work to become the alternative way of remedies. The objectives of this study were to determine the antibacterial, antioxidant, and toxicity of Hylocereus polyrhizus peels and Aloe barbadensis leaves extracts. The antibacterial activities were evaluated towards the bacteria commonly associated with skin infections; which are Staphylococcus aureus, Propionibacterium acnes and Pseudomonas aeruginosa, using Kirby-Bauer Disc Diffusion method. The antioxidant activity was determined using DPPH assay. The toxicity activity was evaluated by exposing the extracts towards brine shrimp. As for the results, Aloe barbadensis extracts showed the highest antibacterial activity compared to Hylocereus polyrhizus and the combination of both extracts. For the antioxidant activity and the toxicity test, Hylocereus polyrhizus possessed the best result, where the IC_{50} obtained was 226.397 µg/ml and LC_{50} was 28588.76 µg/ml (non-toxic). In conclusion, Aloe barbadensis, Hylocereus polyrhizus and the combination of both have the potential to become alternative way as remedies instead of modern medicines.

Keywords: *Hylocereus polyrhizus, Aloe barbadensis, antibiotics, antioxidants, toxicity.*

INTRODUCTION

H. polyrhizus is one of genus *Hylocereus* in Cactaceae family (Aberoumand, 2011). The genus of *Hylocereus* is well known as pitaya or dragon fruit and *H. polyrhizus* is commonly called as red pitaya or red dragon fruit with regard to the colour of its flesh. This fruit originally comes from tropical, subtropical America and native to southern Mexico (Durán et.al., 2017). In the 19th century, dragon fruit is brought to Asia and in early 2000, it started to be known and certain countries such as Indonesia, Malaysia and Thailand cultivated and commercialized it (Hamidah et.al., 2016). According to Siow and Wong (2017), the fruit has red flesh with acceptable sweetness and flavour. *H. polyrhizus* has high content of betacyanin pigment, which was suitable for the fruit to become a natural food colourant (Naderi et.al., 2010) and pectin that can be used as natural gelling agent, thickener, stabilizer and emulsifier (Zaidel et.al., 2017). It was also found to have antibacterial activities (Nurmahani et.al., 2012) and antioxidant activities (Luo et.al., 2014).

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On the other hand, *A. barbadensis* or commonly known as *Aloe Vera* is a herb that is widely distributed throughout the world (Chatterjee et.al., 2013) and more than 200 species of the genus *Aloe* can be found nowadays (Joseph and Raj, 2010). The name *Aloe Vera* derives from the Arabic word "Alloeh", shining bitter substance, "Vera" from Latin that means true (Kumar and Yadav, 2014). It belongs to Asphodelaceae family, genus *Aloe*, originated from Africa and known as perennial, succulent xerophyte grown in temperate and subtropical part (Sharma et.al., 2014). For centuries, *A. barbadensis* is used for health, beauty and skin care properties because it contains many beneficial properties such as antiulcer, antidiabetic, antioxidative, antibacterial, antiviral and wound healing property (Bhuvana et.al., 2014). *A. barbadensis* gel consist of almost 99.5% water and 0.5% other ingredients and until now, there are over 150 nutritional benefits of *A. barbadensis* that have been discovered by scientists (Sunita and Ananya, 2013) and come in many forms of products (Datta et.al., 2012).
Though it is well-known that these plants have the antibacterial activities, there is no information towards the clinically important pathogen that cause the skin infections. There is also lack of information of what the result would be from the combination of both plants in antibacterial and antioxidant activities.

MATERIALS AND METHODS

Preparation of extracts

The *A. barbadensis* leaves and the *H. polyrhizus* peels which were collected or purchased in the residential area in Kangar and wet market around Kedah respectively, were cleaned with sterile tap water. Both *A. barbadensis* leaves and the *H. polyrhizus* peels were cut into smaller pieces and subjected to dry in the oven at 80^{o}C for 48 hours and 60^{o}C for 24 hours respectively. Both dried samples were grounded until powdery (Zaidel et.al., 2017). Then, both powders were soaked with 95% ethanol using ratio 1:10 in individual beakers, while agitated at 250 rpm in 25^{o}C for 48 hours (*A. barbadensis*) and two hours (*H. polyrhizus*). The process was continued by filtering the extract of *A. barbadensis* with filter paper Whatman No.1 and the *H. polyrhizus* with cellulose filter paper Whatman No. 4. The filtrates were poured into individual round bottom flask and were left to evaporate by using rotary evaporator until crude extract were obtained. Both of the crude extracts obtained were stored in individual dark bottles at 4^{o}C. Upon preparation for the desired concentrations, the crude extracts were diluted in the solvent (95% ethanol). The stock concentration of the extract was 10 mg/ml each. It was obtained by diluting 100 mg crude extract in 10 ml of solvent and mixed until homogenous solution was obtained.

The percentage yield of both extracts was determined gravimetrically by the following formula:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of crude extract}}{\text{Dry weight}} \text{ x } 100\%$$

Antibacterial susceptibility assay

Susceptibility of *S. aureus, P. acnes* and *P. aeruginosa* towards the extracted plants were conducted using the Kirby-bauer disc diffusion method. In brief, *S. aureus* and *P. aeruginosa* were grown overnight in nutrient broth and *P. acnes* were grown in brain heart infusion broth for 72 hours under anaerobic condition. Prior to the susceptibitlity assay, bacterial suspension were adjusted to 0.5 McFarland unit. Table 1 below shows the McFarland turbidity standard.

Table 1 : McFarland Turbidity Standard

McFarland scale	CFU (x 10^6/ml)	1% $BaCl_2$/1%H_2SO_4 (ml)
0.5	<300	0.05/9.95
1	300	0.1/9.9
2	600	0.2/9.8
3	900	0.3/9.7
4	1200	0.4/9.6
5	1500	0.5/9.5
6	1800	0.6/9.4
7	2100	0.7/9.3
8	2400	0.8/9.2
9	2700	0.9/9.1
10	3000	1.0/9.0

Source: Sutton (2011)

The subculture preparation method for *S. aureus* and *P. aeruginosa* was referred to Doughari and Manzara (2008). In order to prepare the subculture of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, one full loop of each bacteria from stock culture was inoculated into two distinct universal bottles containing nutrient broth. Then the broth was incubated at 37ºC for 18 hours. The different method was used in preparing the subculture for *Propionibacterium acnes* because it was a facultative anaerobic bacterium that was capable to grow under aerobic and aerobic condition. The method was in accordance to Naghdi and Ghane (2017) with slight modification. In order to prepare the subculture, one full loop of the bacteria was taken from the stock culture and was inoculated into a universal bottle containing brain heart infusion broth with 1% glucose. The broth was incubated in a candle jar (anaerobic condition) at 37ºC for 72 hours.

Kirby-bauer disc diffusion method

The method of disc diffusion referred to Singh et.al. (2015) was used to test the antibacterial activities with slight modification. By using spreaders, 10 µL bacteria suspension was spread onto the MHA agar. Then, the blank antibacterial susceptibility disc was dipped into the extract by using sterile forceps and the disc with 10 µL crude extracts was put on the agar. The Gentamicin antimicrobial susceptibility disc was used as positive control (Doughari and Manzara, 2008) and the extraction solvent, 95% ethanol was used as negative control. The plate's lid was covered and sealed with parafilm. The plates were stored in a 37ºC incubator for 24 hours (Nurmahani et.al. 2012). However, for the incubation of *P. acnes*, the plates were incubated in candle jar in incubator to retain the anaerobic condition at 37ºC. The test was done for the three pure crude extracts. The zone of inhibition was recorded after 24 hours of incubation periods. The experiment was performed in triplicate and in a sterile condition.

Antioxidant activity

To measure the antioxidant activities of *H. polyrhizus* and *A. barbadensis* plants, radical scavenging assay were performed according to Zain and Nazeri (2016) with modification. In this experiment, 0.1 mM of DPPH solution was used. Concentration of 1000 µg/ml, 100 µg/ml, 10 µg/ml and

1 µg/ml were used for both extracts. The solution was placed in the test tube, was homogenized with a vortex mixer for about 20 seconds, and was incubated for 30 minutes in the dark at room temperature. The reduction of DPPH was measured by using a spectrophotometer at wavelength 517 nm. The DPPH solution without extract was used as the control, DPPH solvent as blank and L-ascorbic acid as standard or comparative compound. All concentration was done in triplicate and all the absorbance was recorded. The antioxidant activity was measured by using the following formula below.

$$\text{Percentage of Inhibition (\%)} = [1 - (\text{A sample} / \text{A control})] \times 100\%$$

A sample = Absorbance reading of sample

A control = Absorbance reading of control

Toxicity assessment of *H. polyrhizus* and *A. barbadensis* plants towards brine shrimp

Assessment of *H. polyrhizus* and *A. barbadensis* plants toxicity was performed towards brine shrimp according to Thu et.al. (2013), with modification. Briefly, 1.0 mg brine shrimp was hatched into nauplii in artificial seawater of 35 ppt (part per thousand) or 3.5% salinity (Libralato et.al., 2016) supplied with the oxygen content of 90% (Shaala et.al., 2015) and exposed to light for 24 to 48 hours.
The toxicity assessment was conducted for plant extracts of 1000 µg/ml, 100 µg/ml, 10 µg/ml and 1 µg/ml. Ten nauplii that were collected with the dropper were transferred to each vial with 1 ml of each concentration was added into 4 distinct clean glass vial containing 9 ml of seawater respectively. The dimethyl sulfoxide (DMSO) with concentration 99.7%, 30 µL, was mixed with seawater to serve as a negative control (Moshi et.al., 2010) and the artificial seawater without extract as the positive control. All concentration was done in triplicate. The vials were stored in a room environment with temperature 25 ± 1 oC for 24 hours. After the incubation period, the living nauplii were counted for LC50 (50% Lethal Concentrations) calculation. The toxicity class was determined by referring to Meyer's toxicity index. The formula used to calculate the percentage death of the nauplii was shown below.

$$\text{Percentage of Death (\%)} = \frac{\text{Initial number nauplii} - \text{Final number nauplii}}{\text{Initial number nauplii}} \times 100\%$$

Statistical analysis

The statistical analysis was analyzed by using one-way ANOVA with 95% confidence interval. The raw data was collected after each experiment. All calculations were analyzed by using SPSS Version 23 data analysis software.

RESULT AND DISCUSSION

Percentage yield of plant extraction

The percentage yields of the crude extract of both plants were presented in Table 2.

Table 2: The Percentage Yield of Crude Extracts

Samples	Mass of dried powdered (g)	Mass of crude extract (g)	Percentage yield (%)	p-value
Aloe barbadensis leaves	105.00 ± 0.01	15.03 ± 0.02	15.03	0.000*
Hylocereus polyrhizus peels	237.06 ± 0.01	2.57 ± 0.01	2.57	0.000*

The values were presented in mean ± SD, where n = 3
*The mean value different is significant at the p<0.05 by paired sample t-test

Table 2 shows the percentage yield of both plants; *A. barbadensis* whole leaves crude extract and *H. polyrhizus* peels crude extract. *A. barbadensis* whole leaves crude extract has a higher percentage yield compared to *H. polyrhizus* peels crude extract, which are 15.03% and 2.57% respectively. The mean difference or p-value between the percentage yield of both crude extracts was significant with p=0.000.

The result of extracted plant crude extract showed the differences in percentage yield through the maceration method. The highest percentage yield was shown by *A. barbadensis* crude extract, which was at 15.03%. According to Saritha et.al. (2010), the sample of *A. barbadensis* crude extract percentage yield was 15.41% by using 95% ethanol as solvent. The second crude extract was *H. polyrhizus* peels with a percentage yield of 2.57%. The peels of the *H. polyrhizus* was about 0.46 ± 0.07 cm, which was about 22% from the whole fruit with 92.65% moisture and the rest were made of protein, fat, ash and carbohydrate (Jamilah et.al., 2011). When both of the crude extracts were compared, *A. barbadensis* has a higher percentage yield than *H. polyrhizus*. However, the type of plant material, the temperature, and extraction time that were being used were the main influence of the amount of the crude extract produced (Sultana et.al. 2009).

Many conditions influenced the percentage yield of the plants' extracts such as temperature, types of solvent and extraction time (Singh, 2008). The temperature used in this experiment for drying the *A. barbadensis* was 80 oC and *H. polyrhizus* was 60 oC. The different temperature was used because both gel and peels have different thickness. *A. barbadensis* leaves structure contains pulp and gel that contributed to higher moisture content. The pulp consist of approximately 98.5% of water content and the gel consist of approximately 99.5% water (Hamman, 2008) compared to *H. polyrhizus* peels with 92.65% moisture (Jamilah et.al. 2011). Next is the polarity of the solvent used. The higher the polarity of the solvent, the more compound of the plants and peels will be extracted out. In this experiment, 95% ethanol was used as the solvent because it was one of the higher polarity solvents. Other than that, the extraction time also played a vital role because the longer the extraction time, the more compound could be extracted out from the plants and peels.

Antibacterial activity of sample plants crude extract towards skin bacteria

Table 3 show the zone of bacterial inhibition obtained in the experiment.

Table 3: Zone of Bacterial Inhibition

Samples	Zone of inhibition (mm)		
	S. aureus	***P. aeruginosa***	***P. acnes***
Gentamicin (positive control)	20.83 ± 0.29	19.67 ± 2.08	20.33 ± 0.58
Ethanol 95% (negative control)	ND	ND	ND
Crude extract of *A. barbadensis*	17.33 ± 4.62	16.33 ± 4.04	13.00 ± 1.00
Crude extract of *H. polyrhizus*	10.33 ± 0.58	14.00 ± 3.61	8.00 ± 1.73
Combination of both crude extracts	15.00 ± 0.00	15.00 ± 0.00	13.00 ± 2.65

The values were presented in mean ± SD, where n=3, the mean different is significant at the $p<0.05$ by one-way ANOVA
Notes: ND = No diameter

Table 3 shows the zone of bacterial inhibition towards the plant extract. Among the samples tested, *a barbadensis* showed the highest antibacterial activity with a zone of inhibition 17.33 ± 4.62 mm and the lowest antibacterial activity with a zone of inhibition 8.00 ± 1.73 mm was shown by crude extract of *H. polyrhizus*. From the three types of bacteria, the zone of inhibition was higher in *S. aureus* plates and the lowest was in *P. acnes* plates. As stated in Table 3 above, generally between the three types of samples, the bacteria inhibition was lowest in *H. polyrhizus* sample. It can be said that the bacteria were less susceptible to *H. polyrhizus* compared to the other two samples. *H. polyrhizus* contain about 23.5% of betacyanin that contribute to the antimicrobial effect and it proves to have good antimicrobial spectrum towards Gram-positive and Gram-negative bacteria (Yong et.al. 2016). However, in *A. barbadensis* consist of various active compounds that contribute to the antimicrobial effect such as anthraquinones, aloin, aloe mannan, aloetic acid, aloe emodin, and saponin and the complementary action between the gel and the leaf enhance the effect of the plant towards the bacteria (Goudarzi et.al. 2015).

According to Thiruppathi et.al. (2010), anthraquinone and its derivatives, Barbaloin-IO-aloe-emodin-9-anthrone, Isobarbaloin, Anthrone-C-glycosides, and chromones were the compounds that gave powerful effect. The effect of these components causes the three types of skin bacteria were more susceptible to *A. barbadensis* compared to the other two samples. In a combination of both extracts, the result shown was intermediate between the *A. barbadensis* and *H. polyrhizus* because, when combined, the amount of both crude extracts was not as much as the single sample testing so, the result was intermediate. It seemed that the result was more influenced by the *A. barbadensis* compounds because, in single sample testing, *A. barbadensis* result was better than the *H. polyrhizus.* In order to express the ability of the bacteria towards the samples were susceptible, intermediate or resistant, international standard (ISO 20776-1) was followed and it defined the terms as follows; susceptible is when the bacteria inhibited in vitro by a concentration of a drug that associated with high chance of therapeutic success; intermediate is when the bacteria inhibited in vitro by a drug with uncertain therapeutic effect and resistant is when the bacteria inhibited in vitro by a drug with high chance of therapeutic failure (Rodloff et.al., 2008).

Antioxidant activity (DPPH assay)

DPPH or 2, 2-Diphenyl-1-picrylhydrazyl used in the determination of antioxidant activities of *A. barbadensis* and *H. polyrhizus* extract. DPPH's role in the determination of antioxidant activities was as stable as free radicle scavengers or hydrogen donors. Its appearance was in purple powder form with maximum absorption at 517 nm. When antioxidant compounds in the extract react with the DPPH (violet

colour), the DPPH was reduced to DPPH-H and cause the decrease in absorbance, which resulted in decolourization of the DPPH solution to yellow colour (Shekhar and Anju, 2014). The amount of antioxidant component that was extracted out from the sample crude extract might cause the varying result from sample to sample and one of the contributing factors that affect the extraction of crude extract was the solvent used (Sultana et.al. 2009).

Table 5: Comparison Percentage of Inhibition between *A. barbadensis, H. polyrhizus*, Combination of Both Extracts and Ascorbic Acid

Concentration (µg/ml)	Percentage of inhibition (%)			
	A. barbadensis extract	***H. polyrhizus*** extract	**Combination of Both Extracts**	**Ascorbic Acid**
1000	61.45 ± 3.02	77.30 ± 3.06	63.07 ± 1.68	95.97 ± 0.00
100	39.41 ± 1.44	49.79 ± 10.26	42.89 ± 11.10	94.88 ± 0.00
10	37.34 ± 1.93	41.77 ± 4.21	35.08 ± 3.89	66.42 ± 0.00
1	34.00 ± 1.06	38.52 ± 3.18	36.10 ± 2.20	46.94 ± 0.00

The values were presented in mean ± SD ($n = 3$, $p < 0.05$)

The results in Table 5 showed that the highest percentage of inhibition of DPPH assay at concentration 1000 µg/ml was 77.30 %, at 100 µg/ml was 49.79%, at 10 µg/ml was 41.77% and at 1 µg/ml was 38.52%, where was all the highest reading came from the *H. polyrhizus* extract. Overall, the crude sample of *H. polyrhizus* peels crude extract has the highest antioxidant activity followed by the combination of both *A. barbadensis* and *H. polyrhizus* extract and lastly the sample of *A. barbadensis* whole plant crude extract but all the percentage inhibition were still lower than the ascorbic acid. When both extracts were combined, the percentage of inhibition become intermediate between *H. polyrhizus* and *A. barbadensis* extract, which was higher than *A. barbadensis* extract because the higher antioxidant activities in *H. polyrhizus* extract seems to have influenced the combination of both extracts antioxidant activity. Vitamin C was used as the standard in many antioxidant activities experiments because it was the potent water-soluble antioxidant in humans and also the free radical scavenger (Sirmali et.al. 2014). *H. polyrhizus* peels contained high betacyanins, constituents that had strong antioxidants activity (Rebecca et.al. 2010) and the compound also had contributed to the red pigment of the fruit (Sengkhamparn et.al. 2013). In *A. barbadensis* whole leaves, it contains about 66.06% of polyphenols that contributed to the antioxidant activity of the plant and this compound was able to act as reducing agent, singlet oxygen quencher also as hydrogen-donating antioxidant (Taukoorah and Mahomoodally, 2015). In this experiment, the percentage of inhibition of all crude extract dilution, combination of both extracts, and ascorbic acid showed the decreasing trend as the concentration decrease. In other words, the neutralizing power of the extract to neutralize the free radicals decreased. From the result obtained, the IC_{50} was calculated based on the formula in methodology section. Table 6 below showed the IC_{50} value of each extract and the combination of both extracts.

Table 6: IC_{50} of Each Sample

Sample	IC_{50} (µg/ml)
A. barbadensis extract	549.258
H. polyrhizus extract	226.397
Combination of both extracts	505.821

The IC50 or known as the half-maximal concentration was the concentration of the sample needed to inhibit 50% of free radicals, where the lower the IC50 value, the higher the antioxidant activity of the sample (Jadid et.al., 2015). From Table 6 above, *H. polyrhizus* extract obtained the lowest concentration of IC50, which was 226.397 µg/ml and the highest was *A. barbadensis* extract with concentration 549.258 µg/ml. From the data, it showed that the *H. polyrhizus* extract has the higher antioxidant activity compared to *A. barbadensis* extract and the comparison of both extracts, or in simpler words, the *H. polyrhizus* extract can inhibit more free radicle compared to the others extracts.

Toxicity test

Toxicity test was run by using brine shrimps or scientifically known as *an Artemia salina* which came from the taxon of *Artemia* spp. According to Libralato et.al., (2016), there were some advantages using the brine shrimp to test the toxicity, which was cost-effectiveness, rapid result because it only needed 24 to 48 hours to hatch and 24 hours to observe the result. The other advantage was the cysts (eggs) of the brine shrimp was sold commercially, easy to manipulate, and maintain because the small size of the shrimp and the brine shrimp had high adaptability to a various testing condition that makes it become an indicator for the presence of cytotoxic properties in the sample.

The reason nauplii at instar II and III were used for toxicity test because, at stage instar I, the brine shrimp was still less sensitive because it only consumed the yolk in the eggs. However, at stage instar II and III, they did not have any food supply, so, they consume anything available in the environment as their food supply, so, and that causes them to become more sensitive and suitable for the toxicity test (Libralato et.al., 2016). Nauplii were considered dead when they did not exhibit any external body movement (Ullah et.al., 2013).

Table 7: Result of LC_{50} value of Brine Shrimp Lethality Assay

Sample	Concentration (µg/ml)	Log_{10} concentration	Percentage of mortality (%)	**LC_{50}	*Toxicity class
A. barbadensis	1000	3.00	50	1119.78 µg/ml	Non-toxic
	100	2.00	30		
	10	1.00	30		
	1	0.00	20		
H. polyrhizus	1000	3.00	30	28588.76 µg/ml	Non-toxic
	100	2.00	30		
	10	1.00	10		
	1	0.00	10		
Combination	1000	3.00	30	28588.76 µg/ml	Non-toxic
	100	2.00	30		
	10	1.00	10		
	1	0.00	10		

**Classification according to Meyer's toxicity index for BSLA (Hamidi et.al., 2014);
**LC_{50} determination by using Finney probit analysis

According to Table 7 above, the highest percentage of mortality of the brine shrimp nauplii was 50% at 1000 µg/ml sample dilution of *A. barbadensis* whilst the lowest was 10% for the sample dilution of 10 µg/ml and 1 µg/ml for both *H. polyrhizus* and combination of both extracts. From the data obtained, the LC50 was estimated by using the probit regression analysis by using SPSS Version 23 data analysis software. According to Onzago et.al., (2014), in Meyer's toxicity index, the sample considered as toxic if LC50 < 1000 µg/ml and if LC50 > 1000 µg/ml it was considered as non-toxic. Overall, for the samples tested, all of the samples were non-toxic because the LC50 obtained was more than 1000 µg/ml. In conclusion for the toxicity test of all the samples, the solvent chooses during the extraction process play an important role because different solvent has different extraction potential as the component extracted has influence towards the brine shrimp toxicity assay (Hamidi et.al., 2014).

CONCLUSION

This study was conducted to discover the alternative sources of antibacterial agent that can help to combat bacteria and at the same time safe to use and have good effects on the human. The *A. barbadensis* extract showed the highest percentage yield of crude extracts, which was 15.03%, compared to the *H. polyrhizus* extract, which was 2.57%. The difference between the percentage yields might be due to the time of extraction used. For the antibacterial testing, *A. barbadensis* showed a potential therapeutic success and it could become a good antibacterial product because of the susceptibility of the bacteria towards the extract. Besides, the solvent used also played an important role because it can extract out the important compounds responsible for antibacterial activities. For the antioxidant activity, IC50 of *H. polyrhizus* was the highest followed by the combination of both extracts and lastly the *A. barbadensis* extract, which means, the *H. polyrhizus* had the highest antioxidant activities because of the lower the IC50, the higher the antioxidant activities of the extract. This result showed that *H. polyrhizus* peels have the potential to become a good antioxidant agent to combat the free radical. Lastly, for the toxicity test brine shrimp (*Artemia salina*) was used to test the toxicity of the samples with four different concentrations of each sample. From the analysis of the result by using Meyer's toxicity index, all three samples were non-toxic. Overall, the result suggested that the *A. barbadensis* suitable for the antibacterial usage which means only applied on the skin because the toxicity test of the *A. barbadensis* showed the lowest LC50, which is near to the toxic class. For *H. polyrhizus*, it is more suitable for the antioxidant purpose, where the antioxidant agent usually was being consumed by the consumer because the toxicity index of the *H. polyrhizus* has a huge difference with *A. barbadensis* and its LC50 are at a higher rate. The combination of *A. barbadensis* and *H. polyrhizus* extracts showed intermediate results for all the tests, which may be influenced by the ability of each extract because both extracts have different responses towards each test.

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