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Evaluation of Selected Malaysian Medicinal Plants for Treatment of Peptic Ulcer

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Abstract: An impressive number of medicinal plants contains high amount of flavonoids and antioxidant compound which are very useful as they promotes anti-inflammatory, anti-bacterial and various therapeutic values. Antibiotic resistance in human pathogens due to increasing abuse of drugs has reached an alarming stage. Gastric ulcer has become a major problem worldwide. Acid, peptic activity and collapse of mucosal defense mechanism has been proven to be the pathological process in the formation of gastric ulcer. Proton-pump inhibitors have been introduced as the treatment. However, long term use of these drugs has been proven to cause various adverse effects. Hence, there is a need to develop new antimicrobial and antiulcer drugs. The purpose of this study was to investigate the antimicrobial and anti-ulcer activities of selected Malaysian medicinal plants *in vitro*. The medicinal plant extracts were tested by *in vitro* antibacterial test against six bacterial species, both Gram positive and Gram negative organisms. Disc diffusion assay was employed for assessing antimicrobial activity. The ethanol-induced gastric ulcer method in laboratory animals was used to determine anti-ulcer activity of the plants. Screening for antioxidant levels has also been carried out on the plant extracts. Results showed that all plants have antibacterial and antiulcer activities within them except for *Swietenia macrophylla* with no antibacterial activity. As for the antioxidant screening, only two of the plants used were high in antioxidant level. It is concluded that these plant can provide significant protection from peptic ulcer.

Key words: Medicinal Plants · Antimicrobial · Antiulcer · Malaysia

INTRODUCTION

An impressive number of medicinal plants contains high amount of flavonoids and antioxidant compound which are very useful as they promotes anti-inflammatory, anti-bacterial and various therapeutic values [1]. Antibiotic resistance in human pathogens due to increasing abuse of drugs has reached an alarming stage [2, 3]. The options in combating multiple drug resistance organisms are decreasing [4, 5]. Besides that, gastric ulcer has become a major problem worldwide. Acid, peptic activity and collapse of mucosal defense mechanism has been proven to be the pathological process in the formation of gastric ulcer. Proton-pump inhibitors have been introduced as the treatment [6]. However, long term use of these drugs has been proven to cause various adverse effects. Hence, there is a need to develop new antimicrobial and antiulcer drugs. There are more than 35 000 plant species being used in the world that are useful for medicinal purposes. In peninsular Malaysia, 1200 species of higher plants and 2000 species in Sabah and Sarawak were reported to show medicinal properties and were also used for traditional health care [7, 8].

In this study, we chose the plants that we used based on its traditional usage, the availability in Malaysia and also based on previous researches information of its usage that has been proven together with their active chemical constituents that may help us to achieve our objectives. The plants that have been chosen were *Psidium guajava* Linn (leaf), *Illicium verum* Hk.f. (fruit) and *Swietenia macrophylla* (seed).

***Psidium guajava* Linn:** *Psidium guajava* Linn., from the family of Myrtaceae is a low evergreen tropical tree, with wide-spreading branches and square, downy twigs which is popular because of its edible fruits [9]. It is widely

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known as guava in common English, goyave in French, gurfa in Yoruba and gwaibwa in Ibo [10]. Traditionally, it has been used as a treatment of malaria, vomiting, diarrhea, dysentery, wound, ulcer, sore throat [11], vertigo and regulation of menstrual periods [12]. Previous research has proved that the plant exhibits hepatoprotective activity [13], anticough, antimicrobial [14, 15], anti-inflammatory, analgesic effects [16], antidiabetic [17, 18], antispasmodic [19], antioxidant, anti allergy, antigenotoxic, antiplasmodial, antinociceptive, antihyperglycemic and antihyperlipidemic activities [20, 21].

***Illicium verum* Hook.f.:** *Illicium verum* Hook.f, from the family of *Magnoliaceae* was originated from China and Vietnam, but can easily be found throughout Asian [22]. It is commonly known as star anise in English, bunga lawang in Malay and Indonesia, This plant is used traditionally in Chinese, Carribean and Latino populations as an infusion for the treatment of infant colic [23, 24]. Previous researches has showed that the plant possesses antifungal [25], antimicrobial [26, 24], antiseptic [27] and anti-HIV [28] activities. A study on the chemical constituents reveals that 76.93% of the component is anethole and the rest of 10.22% is P-allylanisole [25]. It is suggested that most of the medicinal properties that lies in the star anise may probably comes from the anethole present [24].

Swietenia macrophylla: *Swietenia macrophylla*, a huge plant, from the family of *Meliaceae* is characterized by evergreen large tree reaching about 30 to 40m with woody capsule fruits pointing towards the sky, hence the name, sky fruits. It was found in Brazil and South America, but is now cultivated widely in Asia, including Malaysia [29]. The seeds were described as woody spongy coat seeds with prominent thin wing and thick at the region that contains embryo [30]. It is commonly referred as sky fruit and big leaf mahogany in English, buah tunjuk langit in Malay. The seeds are used traditionally as a treatment of diarrhea [31], high blood pressure and even for skin allergies. Studies have proven that the plant has antidiabetic [31, 32], hypolipidemic [31], acaricidal [33], antidiarrheal [29], antimalarial [34], anti-inflammatory, anti-mutagenic and antitumor [35] activities resides within them. Screening also has been done and shows that the plants contains essential oils and active ingredients, tetranortriterpenoids and limonoids, such as swietenine, swietenolide, 8.30-epoxy-swietenine acetate, swietenolide diacetate, augustineolide and 3β, 6-dihydroxydihydrocarpain [36] mainly in the bark.

MATERIALS AND METHODS

Plant Extraction: Fresh plants were tap washed followed by washing with distilled water and shade-dried for 7-10 days. The dried plants then were finely powdered using electrical blender. 100g of the fine powder were soaked in 500ml of 95% ethanol in conical flask for 3 days. After 3 days, the mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1) and was distilled under reduced in an Eyela rotary evaporator (Sigma-Aldrich, USA).

Antimicrobial Activity

Extract Preparation: The extract was dissolved into absolute ethanol inside the sterile micro centrifuge tubes. Three concentrations of the extract solution used were 50mg/ml, 100 mg/ml and 150 mg/ml.

Disc Diffusion Method: The medicinal plant extracts were tested *in vitro* against six bacterial species (reference strain) in which three of them are Gram positive: *Staphylococcus epidermidis, Bacillus subtilis, MRSA* and the other three are Gram negative: *Klebsiella pneumonia, Escherichia coli* and *Pseudomonas aeruginosa* by the disc-diffusion assay [37]. The sterile Muller Hinton Agar medium was inoculated with the respective suspension of microbial cells and the organisms were spread evenly on the agar by using sterile cotton buds. Sterile filter paper discs of 6 mm diameter were impregnated with 20 µl of the extract solution of three concentrations (50mg/ml, 100mg/ml and 150 mg/ml). The paper discs were then allowed to evaporate and later placed on the surface of inoculated agar plates. Standard antibiotics were used as positive control whereas negative control was performed by using paper discs loaded with 20 µl of absolute ethanol. After that, the plates were incubated overnight (18h) at 37°C. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones by using transparent ruler. An inhibition zone of 14 mm or more was considered as high antibacterial activity. All tests were repeated three times and mean reading was taken to minimize test error.

Minimum Inhibitory Concentration (MIC)

Standardization of Bacterial Cell Suspension: Six colonies of test organisms were grown in sterile nutrient broth at 37°C overnight. The turbidity produced was then adjusted to a 0.5 McFarland with absorbance of 0.08-0.13 at wavelength of 625 nm. The bacterial solution was further adjusted to the ratio of 1 bacterium to 100ml of broth (1:100).

Serial Broth Dilution Method: Minimal inhibitory concentration (MIC) of the extracts was carried out by using broth dilution method by the recommendation of Sole I.O (2008) with slight modification. Stock solution of the extract was prepared by dissolving 300 mg of extract into 1ml of carboxymethylcellulose (CMC)giving the final concentration of 300mg/ml stock solution. The stock solution was then diluted using double fold serial dilution by transferring 500µl of stock solution into 500µl sterile nutrient broth to obtain 150 mg/ml concentration. The process is continued until 10 times dilution was done and several concentrations (75 mg/ml, 37.5 mg/ml, 18.75 mg/ml, 9.38 mg/ml, 4.69 mg/ml, 2.34mg/ml, 1.17 mg/ml, 0.59 mg/ml and 0.29 mg/ml) were obtained. Each concentration was inoculated with 500µl of standardized bacterial suspension and was incubated 24hours under the temperature of 37 °C. The lowest concentration which showed no turbidity in the tube was recorded as MIC. The procedure was repeated three times to minimize test error.

Minimum Bactericidal Concentration (MBC): The solutions from the MIC in serial dilution were sub cultured onto the media and were incubated at 37°C for 24 hours. The plate was observed after 24hour for colony growth. The MBC was determined by the lowest concentration of the extract with no colony growth.

Anti-Ulcer Activity

Gastric Ulcer-induction by Absolute Ethanol: The rats were fasted for 24 hours before the experiment, but were allowed free access to drinking water up until 2 hours before the experiment. Negative control group was orally administered with solvent (5% Tween 80, 5ml/kg). Positive control group received oral doses of 20 mg/kg Omeprazole in distilled water (5ml/kg). Experimental groups were orally administered with crude extract in solvent (5% Tween 80, 5ml/kg) at doses of 250 mg/kg and 500 mg/kg. One hour after this pre-treatment, all groups of rats were administered absolute ethanol (5ml/kg) in order to induce gastric ulcers [38]. The rats were sacrificed 1 hour later [39] under an overdose of xylazin and ketamine anesthesia and their stomachs were immediately excised.

Measurement of Mucus Production: Gastric mucus production was measured in the rats that were subjected to absolute ethanol-induced gastric lesions. The gastric mucosa of each rat was obtained by gentle scraping of the mucosa with a glass slide and the collected mucus were weighed by using a precision electronic balance [40, 41].

Measurement of Acid Content of Gastric Juice (pH): Samples of gastric contents were analyzed for hydrogen ion concentration by pH metric titration with 0.1 N NaOH solutions using digital pH meter [39].

Gross Gastric Lesions Evaluation: Ulcers of the gastric mucosa appear as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Gastric mucosa of each rat were examined for any damage. The ulcers were measured by a planimeter under a dissecting microscope (1.8x). The ulcerated area was measured by counting the sum of small squares (2mm x 2mm) which covers fully the length and width of each ulcer band. The ulcer area (UA) then was calculated by using the formula:

$$UA\ (mm^2) = \text{sum of all small squares} \times 4 \times 1.8$$

The inhibition percentage (I%) was calculated by using the formula under the recommendation of Wasman *et al*. [37].

$$(I\%) = [(UA_{control} - UA_{treated}) \div UA_{control}] \times 100\%$$

Histological Evaluation of Gastric Lesions: Specimens of the gastric walls of each rat were fixed in 10% buffered formalin and processed in a paraffin tissue processing machine. Sectioning of the stomachs was done at a thickness of 5 µm and was stained with hematoxylin and eosin for histological evaluation [40].

Antioxidant Assay

Ferric Reducing Ability of Plasma (FRAP) Assay: FRAP assay was carried out to measure the total antioxidants of the plants. The protocol were done following the recommendation of Tandon *et al.* [1].

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay: DPPH reagent was prepared by dissolving 2.5g of DPPH powder into 1 ml of dimethyl sulfoxide (DMSO). 100 µl of DMSO was pipette into 96-well microtitre plate. 100 µl of sample (1mg/ml) was pipette into the first row (A). After suspend, 100 µl of the solution from the first row were transferred into the second row and the same process was continued until the second last row (G), leaving the last row contains only DMSO. 10 µl of DPPH reagent was then pipette into each well and the work was done I dark room. Next, the plate was wrapped with aluminum foil and was incubated in room temperature for 20 minutes with continuous shaking. After 20 minutes, the absorbance was read at wavelength of 517nm. Each sample was tested as triplicate [1].

Statistical Analysis: All data except for antibacterial activity were expressed as mean ± standard error mean (SEM). The statistical significance of differences between groups was assessed using one-way ANOVA. A value of $p<0.05$ was considered significant.

RESULT

Psidium guajav and *Illicium verum* shows antibacterial activity towards all bacteria except for a few gram negative bacteria. However, *Swietenia macrophylla* extract does not show any effect towards the bacterial growth (Table 2). Gross evaluation of ethanol-induced gastric lesion model is shown in diagram 1. Results show that the rats pre-treated with the plant extracts has significant reduction of ulcer areas as compared to the rats pre-treated with only CMC. Flattening of the mucosa folds were also observed grossly in most of the rats pre-treated with the plant extracts. The reduction of the ulcer areas were comparable with the standard drug used for this experiment, Omeprazole.

Diagram 1 shows the gastric lesions for each group of rats namely negative control group, positive control, 250 and 500 mg/kg PG, 250 and 500 mg/kg IV, 250 and 500 mg/kg SM as well as 250 and 500 mg/kg DA. Ulcer area is characterized by hemorrhagic bands on the gastric mucosa. Negative control group shows extensive visible hemorrhagic bands on the mucosa indicating severe mucosal injury. With this, we ensure that the ethanol causes the formation of gastric ulcer. On the other hand, rats pre-treated with Omeprazole (positive control group) shows mild injuries to the gastric mucosa as compared with the negative control group. Generally, all groups that were pre-treated by plant extracts show the reduction of the ulcer areas. Higher concentration of extract (500 mg/kg) groups show more reduction of ulcer areas compared to the lower concentration of extract (250 mg/kg) groups of rats. The effects of the plant extracts were significant and dose-dependent. Histological evaluation has been done on all gastric tissues of all groups of treatment. The negative control group, which has been pre-treated with CMC only, showed extensive damage to the gastric mucosa, edema together with infiltration of leucocytes at the submucosal layer. Positive control group on the other hand, showed less damage, absence of edema and reduction in leucocyte infiltration to the submucosal layer. Rats which has been pre-treated by the extracts has also shown better protection as compared to the ones in negative control group (Diagram 2).

The graph shows that *Psidium guajava* ethanolic leaf extract extract has high inhibition percentage as compared to the controls (ascorbic acid and quercetin), suggestive of high antioxidant activity. Whereas, *Illicium verum* fruit extract has low inhibition percentage but higher than *Swietenia macrophylla* seed extract. Both the latter extracts were considered as having low antioxidant activity.

Table 1: Antibacterial results. Disc diffusion method (zone of inhibition)

		ZONE OF INHIBITION (mm)				
PLANT	ORGANISM	Positive control	Negative control	50mg/ml	100mg/ml	150mg/ml
Psidium guajava L.	*Klebsiella pneumonia*	23.0	-	-	-	-
	Pseudomonas aeruginosa	23.0	-	-	-	-
	Bacillus subtillis	17.0	-	6.3	7.8	8.5
	Escherichia coli	24.0	-	-	6.3	7.0
	Staphylococcus epidermidis	23.0	-	7.0	10.0	10.0
	MRSA	17.0	-	8.0	8.4	9.0
Illicium verum Hk.f.	*Kliebsella pneumonia*	23.0	-	-	-	-
	Pseudomonas aeruginosa	23.0	-	-	-	-
	Bacilus subtillis	17.0	-	7.5	8.5	9.5
	Escherichia coli	24.0	-	8.0	9.0	11.0
	Staphylococcus epidermidis	23.0	-	-	7.0	7.0
	MRSA	17.0	-	8.0	8.0	8.0
Swietenia macrophylla (seeds)	*Kliebsella pneumonia*	23.0	-	-	-	-
	Pseudomonas aeruginosa	23.0	-	-	-	-
	Bacillus subtillis	17.0	-	-	-	-
	Escherichia coli	24.0	-	-	-	-
	Staphylococcus epidermidis	23.0	-	-	-	-
	MRSA	17.0	-	-	-	-

[1]Positive controls: (vancomycin: *MRSA, Staphylococcus epidermidis, Bacilus subtilis*); (imipenem: *Pseudomonas aeruginosa, Klebsiella pneumonia*); (ampicilin: *Escherichia coli)*

[2] Negative control: Absolute ethanol.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

PLANT	ORGANISM	MIC (mg/ml)	MBC (mg/ml)
Psidium guajava L.	*Klebsiella pneumonia*	-	-
	Pseudomonas aeruginosa	-	-
	Bacillus subtilis	37.50	75.00
	Escherichia coli	9.38	18.75
	Staphylococcus epidermidis	37.50	75.00
	MRSA	9.38	9.38
Illicium verum Hk.f	*Klebsiella pneumonia*	-	-
	Pseudomonas aeruginosa	-	-
	Bacillus subtilis	9.38	9.38
	Escherichia coli	9.38	9.38
	Staphylococcus epidermidis	9.38	9.38
	MRSA	4.69	4.69
Swietenia macrophylla (seeds)	*Klebsiella pneumonia*	-	-
	Pseudomonas aeruginosa	-	-
	Bacillus subtilis	-	-
	Escherichia coli	-	-
	Staphylococcus epidermidis	-	-
	MRSA	-	-

Table 3: Antiulcer activities. This table shows ulcer area, inhibition percentage, mucus weight and pH

Pretreatment (5mg/Kg)	Mucus production (g)	pH of gastric content	Ulcer area (mm^2) (mean ±SEM)	I (%)
Carboxymethyl cellulose (CMC) (Ulcer Control)	0.30±0.02	3.89±0.06	850.00±12.65	-
Omeprazole (20 mg/kg)	0.52±0.02	6.84±0.09	148.80±1.43	82.48
Psidium guajava L.(250 mg/kg)	0.56±0.01	4.84±0.05	34.56±6.19	95.92
Psidium guajava L(500 mg/kg)	0.73±0.03	5.63±0.03	10.08±4.88	98.80
Illicium verum Hk.f.(250 mg/kg)	0.44±0.02	4.39±0.01	7.2±4.55	99.14
Illicium verum Hk.f.(500 mg/kg)	0.73±0.04	4.63±0.04	0.00±0.00	100.00
Swietenia macrophylla(250 mg/kg)	0.81±0.03	4.90±0.03	0.00±0.00	100.00
Swietenia macrophylla(500mg/kg)	0.72±0.01	3.26±0.04	0.00±0.00	100.00

Table 4: Antioxidant assay. FRAP assay. Table showing total antioxidants of the plants *Psidium guajava, Illicium verum* Hk.f. and *Swietenia macrophylla* seeds with ascorbic acid and quercetin as controls.

	Rep 1	Rep 2	Rep 3	Frap value
Ascorbic acid	0.9965	0.8833	0.9109	180.3261
Quercetin	1.3227	0.9569	1.013	216.6957
Psidium guajava L	1.8577	2.0995	2.0332	412.1884
Illicium verum	0.4399	0.4602	0.4718	77.51449
Swietenia macrophylla seeds	0.2001	0.2015	0.1939	21.25362

DISCUSSION

We chose to use ethanol as our solvent based on previous study that shown that ethanolic extracts are more effective as compared to water extract [41]. Ethanol had high volatility thus it has the ability to extract more active compounds from the sample than water. From the antioxidant screening, *Psidium guajava* showed high antioxidant activity. However, *Swietenia macrophylla* and *Illicium verum* was tested to be low in both antioxidant level and activities. Antioxidants works as a neutralizer towards oxygen free radical agent in order to delay, reduce and prevent damage that produced by them [42]. Naturally, there were wide varieties of antioxidants which were different in physical, chemical properties and their mechanism of actions. Their main categories include enzymes, high and low molecular weight compounds, minerals and vitamins [42]. Flavonoids, saponins and tannins are some of the major antioxidants that can be found in the plants. Previous studies had proved that flavonoids possess pharmacological properties as antitumour, antimicrobial and anti inflammatory [12]. Saponins on the other hand work by changing the cell wall permeability resulting toxicity to the cell. Tannins works by blocking the key enzymes of the cell metabolism thus, leading to a decrease of bacterial cell proliferation. Medicinal plants produce their effects not by single chemical working alone. However, they usually consist of

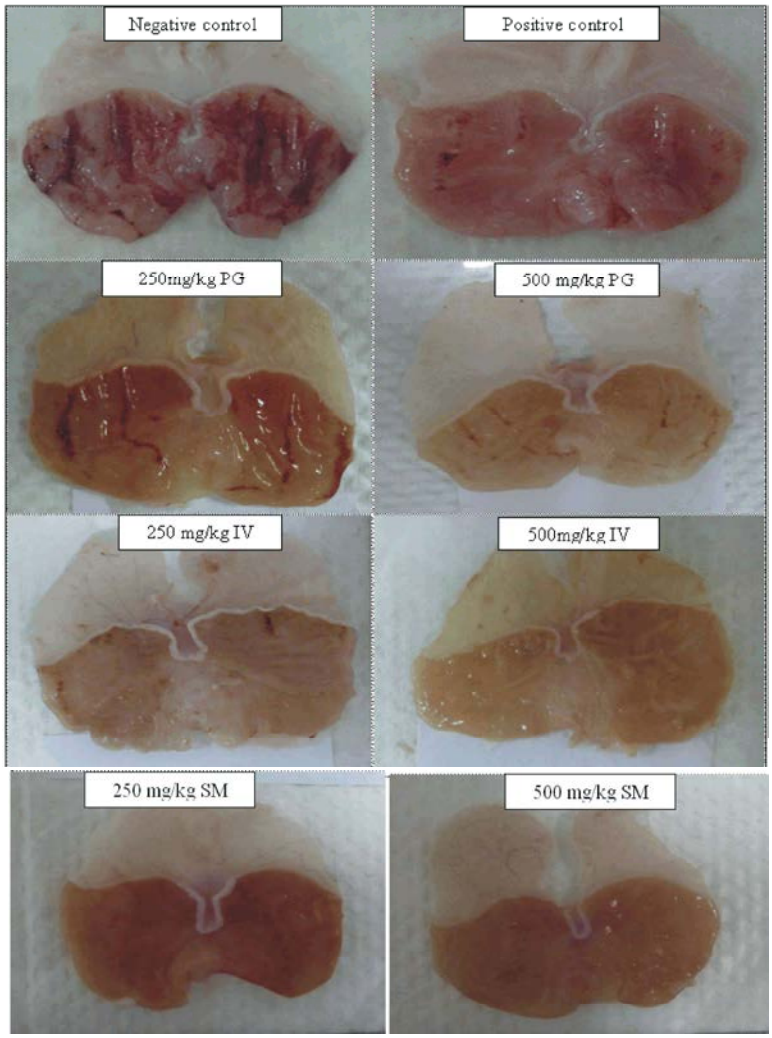

Diagram 1: Gross evaluation of ethanol-induced gastric lesion model. PG: *Psidium guajava* L. IV: *Illicium verum* Hk.f, SM: *Swietenia macrophylla*

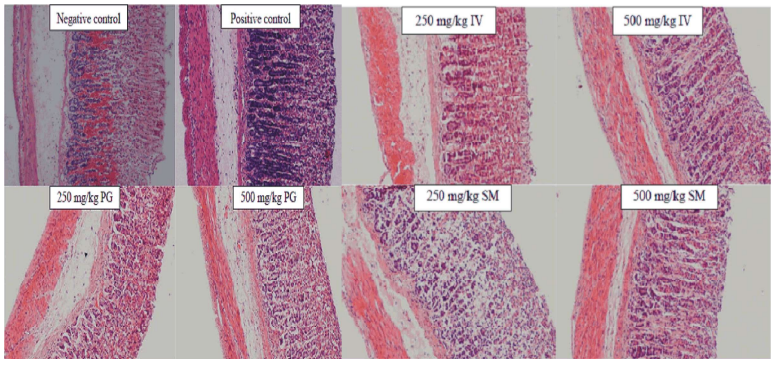

Diagram 2: Histological evaluation of gastric tissues. PG: *Psidium guajava* Linn., IV: *Illicium verum* Hk.f., SM: *Swietenia macrophylla*)

many chemical components that worked together as a single moiety [12]. Thus, the inhibition of the bacteria by the plant extracts in this study may due to the presence of some active chemical compound in the extract. These compounds may be acting on their own or most probably work together with others to inhibit the bacterial growth.

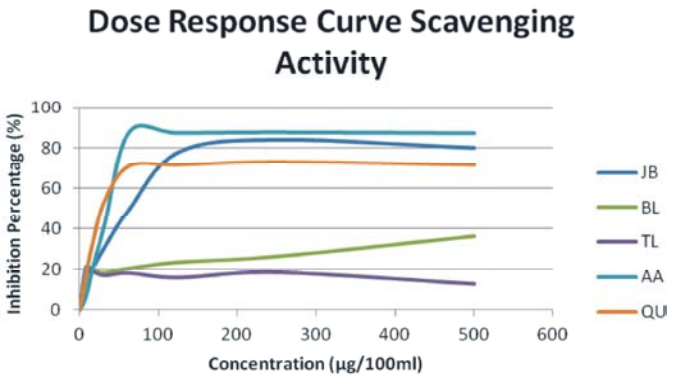

Diagram 3: Dose response curve scavenging activity. AA: ascorbic acid; QU: quercetin; JB: *Psidium guajava* L.; BL: *Illicium verum* Hk.f.; TL: *Swietenia macrophylla* seeds

Gastric ulcer is caused by the imbalance between aggressive and protective mechanism of the gastric mucosa. The aggressive mechanism includes the endogenous and exogenous factors such as acid, pepsin, alcohol, tobacco, non-steroidal anti-inflammatory drugs and also by certain infection [43]. Besides that, reactive oxygen species has been proven to play an important role in gastric ulcer formation [44]. The reactive oxygen species causes oxidative stresses which in turn will increase the release of histamine and pepsin thus reduces the levels of DNA, RNA and protein in the tissue resulting in tissue damage. A study done by Moraes De Carvalho *et al.* [43], shows that ethanol can cause gastric lesions in multiple ways either by disrupting the mucus-bicarbonate barrier or causing cell rupture at the wall of blood vessels. The damage produced by the ethanol will increases in dose-dependent way [39]. In this study, the plant extract play it roles as a protective agents for ethanol-induced gastric mucosa injury by reducing the gastric ulcer area, increasing the gastric mucous production as well as decreasing the acidity of gastric content. Besides that, the reduction in neutrophil infiltration had also been demonstrated. Plant extracts with high levels of antioxidants showed to have high antiulcer activities. However, *Swietenia macrophylla* which has very low level of antioxidant had also showed high antiulcer activities. From this study, the usage of antioxidants alone to prevent gastric ulcer is still in doubt. However, antioxidants may helps in the prevention of gastric ulcer together with other mechanisms available.

CONCLUSION

In conclusion, *Psidium guajava* and *Illicium verum* exhibit some degree of anti bacterial activities against *Staphylococcus epidermidis, E.coli, Bacillus subtilis* and *MRSA.* However, none of them exhibit antibacterial activities against *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. *Swietenia macrophylla* on the other hand did not exhibit any antibacterial activities against all bacteria tested. All four plants protect the gastric mucosa by increase gastric mucous production, decreasing the acidity of gastric content, reducing the ulcer areas and inhibiting the infiltration of leucocytes to the submucosal layers. However, the exact mechanism of action for antibacterials and antiulcer properties of these plants are still unknown. Deeper studies should be done to determine the exact active principles that contribute to these properties should be done. The effectiveness of using antioxidant alone as antibacterial and antiulcer agent should also be tested. A lot of work and further study need to be done to consider these plants as antimicrobial or antiulcer agent.

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