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Antioxidant activities, cytotoxic properties and total phenolic content of *Syzygium malaccense* (L.) Merr. & L.M. Perry leaves extracts: A West Sumatera Indonesian plant

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Abstract: The purpose of this study was to determine the antioxidant activities and cytotoxic properties of the various extracts of *Syzygium malaccense* leaves and its correlation with total phenolic content. To determine the antioxidant properties 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide methods have been used while to determine cytotoxic properties brine shrimp lethality test method has been used. Furthermore, to determine total phenolic content the Folin-Ciocalteu method has been carried out. The results showed that all extracts have antioxidant activity both against DPPH and hydrogen peroxide. IC_{50} hexane, ethyl acetate and methanol extracts against DPPH were 181.26; 34.35; 36.87 mg/mL respectively and against hydrogen peroxide were 18.24; 12.03; and 13.04mg/mL, respectively. All extracts have also cytotoxicity properties on brine shrimp *A. Salina* where LC_{50} of hexane, ethyl acetate and methanol extracts were 204.69, 70.03 and 329.76µg/mL, respectively. Total phenolic content of hexane, ethyl acetate and methanol extracts were 587.17± 19.48, 1617.47±4.63 and 1319.49±9.26µg of gallic acid equivalents (GAE)/10 mg extracts, respectively. Antioxidant activity of these extracts are increased if their phenolic content is increased with the regression equations between IC_{50} and phenolic content is y= -0.1529x +263.75 with R^2= 0.9289 for DPPH method and y= 0.0062x + 21.745 with R^2= 0.9825 for hydrogen peroxide method.

Keywords: Guava fist, *Syzygium malaccense*, antioxidant, DPPH, phenolic, brine shrimp lethality test.

INTRODUCTION

An antioxidant is a substance either chemical or enzyme that can prevent or inhibit or reduce the oxidation or oxidative damage caused by oxygen or other chemicals. Oxidation process may produce one of them is free radical that is not stabile and has high reactivity and can either donate an electron to or accept an electron from other molecules. The most important free radicals in many disease is that containing oxygen such as hydroxyl radical, hydrogen peroxide, superoxide anion radical, oxygen singlet and others. These molecules are highly reactive species, and in the membranes of cells can damage biological molecules such as DNA, carbohydrates, proteins and lipids (Lobo *et al*., 2010).

Many substances that are derived from plant which called phytonutrients or phytochemicals are becoming more known because the compounds have antioxidant activity. Example of compound that has antioxidant activity is flavonoids and phenolic. In plants, function of flavonoids is as protectors to a wide variety of environmental stresses, meanwhile in humans, function of flavonoids is as modifiers of biological response. Flavonoids and flavonoids have been proved to have antioxidant Sen *et al*. (2013).

Based on their sources, antioxidant can divided into synthetic and natural antioxidants. butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), propyl gallate (PG) and tertiarybutyl hydroquinone (TBHQ) are examples of synthetic antioxidants. However, if these synthetic antioxidants used in food product, it has been correlated to health risk because these compounds have potential health hazards and their use under strict regulation (Lobo *et al*. 2010, Wettasinghe and Shahidi, 1999; Hettiarachchy *et al*., 1996). Therefore, this stimulated research for alternative natural antioxidant sources which will replace of synthetic antioxidants.

One of plant that has been used as traditional medicine is *Syzygium malaccense* which is under the family of Myrtaceae. It locally name is jambu bol (Indonesian, Malay), Malay apple (Malay), wax jambu, malay-apple, long fruited rose-apple (English). This plant is a tree with straight trunk to 20 m tall and 20-45 cm diameter, the branch often near the base with widely ovoid canopy. (Arumugam *et al*., 2014; Orwa *et al*., 2009). Application in traditional medicine has been used various parts of this plant. Leaves of this plant have been used for a wide variety of inflammatory conditions (Dunstan *et al*., 1997). Besides that, an infusion of the scraped bark or crushed leaves of this plant has been used to treat mouth infections of an infant. Other report expressed that extract of bark scrapings of this plant is administered to treat stomachache and throat infections. The use of this plant

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for treating mouth infections such as thrush is did in Indonesia (Whistler and Elivitch, 2006).

The leaves of *S. malaccense* is reported containing myricetin 3-alpha-L-arabinofuranoside, myricetin 3-O-L-rhamnoside (myricitrin) and myricetin 30-glucoside (Arumugam *et al*., 2014). Dunstan *et al*., (1997) reported that this plant contain ellagic acids and an anthocyanin, while Reynertson *et al*. (2008) reported t-cinnamic acid, quercetin and quercitrin are also found in *S. malaccense* The essential oil from fresh leaves of this plant that grown in Nigeria composed of (−)-β-pinene, (+)-α-pinene, α-terpineol, p-cymene and (−)-β-caryophyllene (Karioti *et al*., 2011).

The above data showed the potential use of *S. malaccense* in the management of many diseases. The present study is to determine the antioxidant and cytotoxicity properties of the leaves extracts of *S. malaccense* and their correlation with total phenolic contents of extracts. The results of this study are expected to provide probable the use of *S. malaccense* as an antioxidant and its ability to control oxidative stress-cytotoxic which can caused cancer.

MATERIALS AND METHODS

Chemicals and reagents

Methanol, hexane and ethyl acetate for maceration were obtained from their techniques quality with distillation process. DPPH, Folin-Ciocalteu, gallic acid were purchased from Sigma Chemical Company St. Louis MO, USA, while methanol p.a, sodium carbonate, buffer phosphate pH 7.4 and hydrogen peroxide were purchased from Merck KGaA Darmstadt Germany.

Instruments

To evaporate of filtrate from maceration was used rotary evaporator Heidolp WB 2000. In determination of antioxidant activity either DPPH free radical scavenging method or hydrogen peroxide scavenging method, to measure of test solution absorbance was used spectrometer UV/ VIS 1700 Series and in determination of toxicity properties, the growth medium of brine shrimp was prepared with sea water in a small tank that divided into two compartments, one of them is dark part and the other one is bright part.

Sample of Syzygium malaccense

S. malaccense leaves were collected from Lima Puluh Kota district, West Sumatera, Indonesia. The leaves were identified and the specimen was deposited in herbarium of Biology Department, Andalas University. The fresh leaves were washed and dried at room temperature and then milled into powder.

Extraction

Two hundred gram of the powdered leaves was macerated with 200 mL of hexane, ethyl acetate and methanol in separate flasks at room temperature for overnight. Then, the mixtures were filtered and the filtrates were dried using a vacuum evaporator and stored in freezer in airtight container. Schematic flow of this extraction is presented in fig. 1.

Antioxidant activities of extracts by DPPH free radical scavenging method

The experiment was performed as described by Itam *et al*. (2015) with some modifications. Ten mg of hexane, ethyl acetate and methanol extracts were dissolved in methanol which the total volume 10mL in separated volumetric flask (stock solution 1,000µg/mL). Various concentration of extracts were prepared from these stock solutions, i.e. 20, 30, 40, 50µg/mL in methanol. Furthermore, 0.1mL of each these extract solutions was pipetted into separated tube, and then to these solutions, 0.1mL of a solution of DPPH in methanol (0.1mM) was added. After incubation at room temperature for 30 minutes in the dark, the absorbance was recorded at 517nm using spectrometer UV-VIS 1700 Series. A control is containing 0.1mL of methanol and 0.1mL of 0.1mM DPPH. Ascorbic acid was used as references with various concentration 0.1, 0.5, 1, 1.5 and 2µg/mL. The free radical scavenging activity of the extracts was calculated based on the percentage of DPPH radical scavenged (I-FRSA %) using equation:

$$\text{I-FRSA (\%)} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Where A_{sample} is the sample solution absorbance, and A_{control} is the of the control solution absorbance. Inhibition concentration 50% (IC50) was estimated using regression equation from the calibration curve obtained from the graph of extract concentrations (X-axis) plotted against radical scavenging percentage (Y-axis).

Antioxidant activities of extracts by hydrogen peroxide scavenging method

The extracts ability to scavenge hydrogen peroxide was determined based on Sen *et al*. (2013) method with modification. A hydrogen peroxide solution (40mM) was prepared in phosphate buffer (pH 7), and solution of hexane, ethyl acetate and methanol extracts (100µg/mL) were prepared in methanol as stock solutions. Various concentration of these extracts were prepared from these stock solutions, i.e. 5, 10, 20, 30 and 40µg/mL in methanol. Then, two mL of each of these extract solutions were added to 3.4mL of phosphate buffer (pH 7) solution in tube. After that, 0.6mL of hydrogen peroxide 40 mM was added to these mixtures. After 10 minutes of incubation, absorbance of these solutions was measured at 230 nm using spectrometer UV/ VIS against phosphate buffer pH 7 as blank. A control is containing 2 mL of methanol and 3.4mL of hydrogen peroxide. Hydrogen peroxide scavenging activity (I-HPSA %) of extracts and reference compound was determined as following equation:

$$\text{I-HPSA (\%)} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

Where $A_{control}$ and A_{sample} are absorbance of control and sample, respectively. Inhibition concentration 50% (IC_{50}) was also calculated.

Cytotoxic activity by the brine shrimp lethality method

The brine shrimp lethality assay was performed following Meyer *et al*. (1982) and Vidotto, *et al*. (2013) methods with some modifications. The growth medium of larva was prepared in a small tank that divided into two compartments with sea water. The shrimp eggs of *A. salina* were added to the covered compartment (dark part), while above the open side of the tank was placed a lamp to attract hatched shrimps through perforations in the partition wall. After 48 h, the shrimps mature as nauplii and are ready for the assay. Four gram of each extract (hexane, ethyl acetate and methanol extracts) was dissolved in 4 mL of methanol of total volume, so that obtained 1,000 µg/mL of extract solution (3 kinds of extract stock solution). And then, 50, 250, 500, 750 and 1000 µL of each stock solution were transferred using a micropipette into separated vials corresponding to 10, 50, 100, 150 and 200 µg/mL, respectively. These solutions were allowed to dry at room temperature, and residue was dissolved in 50 µL of DMSO and 2 mL of sea water. Afterward, 10 of nauplii were introduced into each vial and the volume in each vial was made up to 5 mL with sea water. The control containing 50 µL of DMSO which was made up to 5 mL with sea water and 10 of nauplii. After 24 hours introducing the nauplii, the number of survival at each dosage was counted and recorded. LC_{50} values of extract were determined using probit value and regression equation.

Determination of total phenolic content

Total phenolic content in the leaves extracts were measured using the Folin-Ciocalteu reagent method that based on Kosar *et al*. (2005) and Sen *et al*. (2013) with modification. Ten mg of hexane, ethyl acetate and methanol extracts were dissolved in methanol in the different volumetric flask 10 mL. Then 0.5 mL of these each extract solutions was pipetted into test tubes containing 1.0 mL of Folin-Ciocalteu reagent. After five minutes, 2.0 mL of sodium carbonate (7%, *w*/*v*) was added to this mixtures, and then the volume was made up to 10 mL with water and the tubes were shaken thoroughly. After 2 hours of incubation at room temperature, absorbance was measured at 760 nm using spectrometer UV/VIS Shimadzu Pharma Spec UV-1700, Gallic acid was used as standard to obtain a calibration curve, and phenolic content of extracts were expressed as gallic acid equivalent per 10 mg dried extracts.

STATISTICAL ANALYSIS

The results of total phenolic contents were presented as mean ± standard deviation of triplicate experiments analyzed using Microsoft excel version 15.13.3.

RESULTS

The results of maceration to *S. malaccense* leaves using hexane, ethyl acetate and methanol solvents are shown in table 1. The components that can dissolve in these solvents are following order: methanol > ethyl acetate > hexane, but there is significant difference between methanol and ethyl acetate solvents.

Table 1: The results of maceration to *S. malaccense* leaves using hexane, ethyl acetate and methanol solvents

No	Extract	Percentage (%)
1.	Hexane	4.80
2.	Ethyl acetate	8.76
3.	Methanol	8.93

Table 2: IC_{50} values of hexane, ethyl acetate and methanol *S. malaccense* leaves extracts and ascorbic acid on DPPH

No	Extracts	IC_{50} (µg/mL)
1.	Hexane	181.26
2.	Ethyl acetate	34.35
3.	Methanol	36.87
4	Ascorbic acid	1.40

Table 3: IC_{50} values of hexane, ethyl acetate and methanol *S. malaccense* leaves extracts on hydrogen peroxide

No	Extract	IC_{50} (µg/mL)
1.	Hexane	18.24
2.	Ethyl acetate	12.03
3.	Methanol	13.04

Table 4: LC_{50} of hexane, ethyl acetate and methanol *S. malaccense* leaves extracts on *A. salina* larvae

No	Extract	LC_{50} (µg/mL)
1.	Hexane	204.69
2.	Ethyl acetate	70.03
3.	Methanol	329.76

Table 5: Total phenolic contents of various the *S. malaccense* extracts

No	Extract	Total phenolic content (µg GAE/10 mg ekstrak kering)
1.	Hexane	587.17 ± 19.48
2.	Ethyl acetate	1617.47 ± 4.63
3.	Methanol	1319.49 ± 9.26

Antioxidant activities of extracts by DPPH free radical scavenging method

FRSA of these extracts with various concentration are presented in fig. 2 meanwhile their inhibitory concentrations 50% (IC_{50}) are shown in table 2. The fig. 2 shows that all extracts have antioxidant activity, where

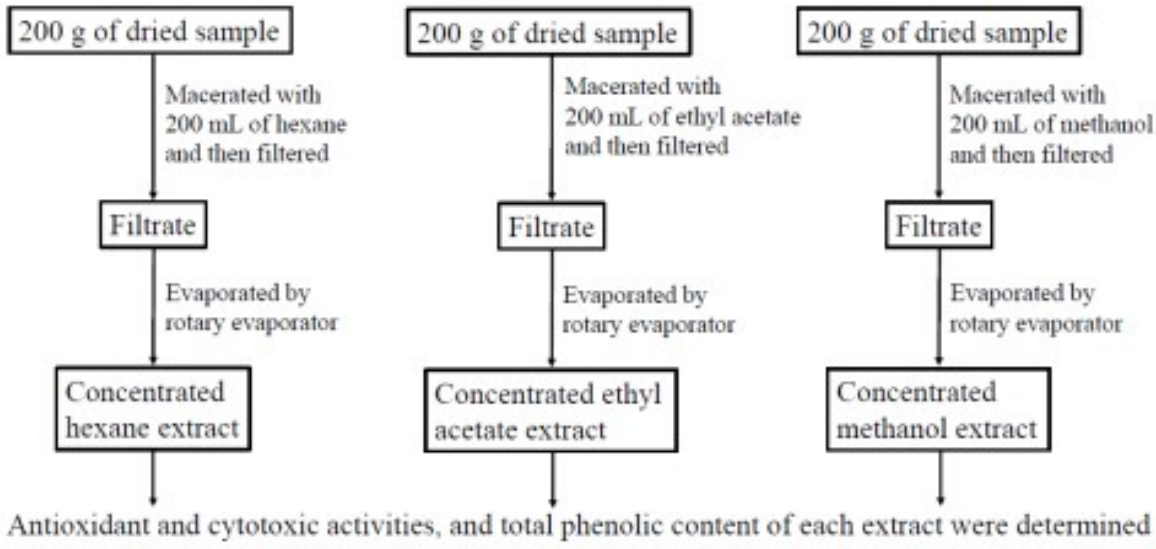

Fig. 1: Schematic flow of the extraction of leaves and stem bark of *S. malaccense*

antioxidant activity of extract increases with increasing of extract concentration. The order of antioxidant activity on DPPH is follow ethyl acetate > methanol > hexane extracts. Furthermore, table 2 shows the IC50 of each extracts and ascorbic acid as reference antioxidant compound.

Antioxidant activities of extracts by hydrogen peroxide scavenging method

Antioxidant activities on hydrogen peroxide of all extracts are presented in fig. 3 and table 3 displays IC_{50} of these extracts. Antioxidant activities increase if the extracts concentrations also increase, and ethyl acetate and methanol extracts possesses almost similar of antioxidant activity to hydrogen peroxide, meanwhile hexane extract is lower.

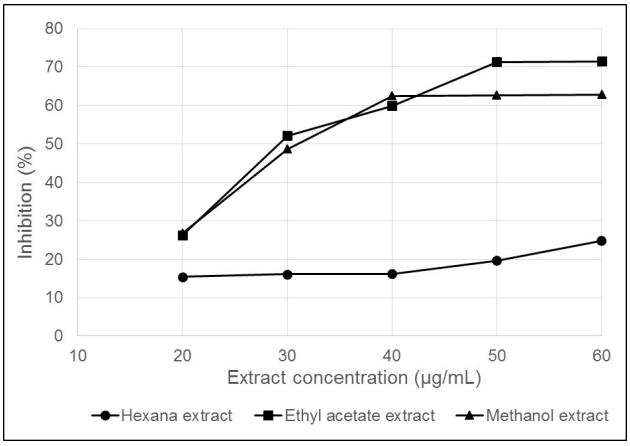

Fig. 2: Antioxidant activity of various concentration of *S. malaccense* leaves extracts on DPPH radical

Cytotoxic activity by the brine shrimp lethality method

The results of cytotoxic activity of hexane, ethyl acetate and methanol extracts are presented in fig. 4 and lethal concentration 50% (LC_{50}) of extracts are shown in table 4. fig. 4 showed that increasing extracts concentration the mortality of brine shrimp (*A. salina*) is also increases. Among these extracts, ethyl acetate extract is the extract that having highest cytotoxic activity compared hexane and methanol extracts.

Determination of total phenolic content

Total phenolic content were determined from the calibration curves of gallic acid ($Y = 0.006x - 0.018$ ($R^2 = 0.956$) and the results are presented in table 5. The results showed that ethyl acetate extract possessed the highest phenolic [(1617.47 ± 4.63) µg GAE/10 mg of dry extract material], followed by the methanol and hexane extracts.

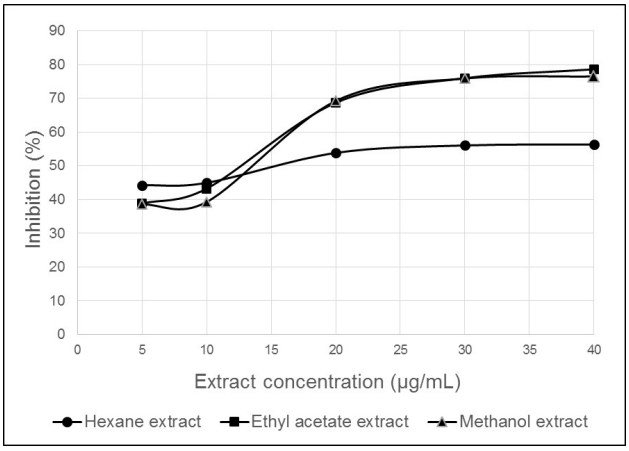

Fig. 3: Antioxidant activity of various concentration of *S. malaccense* leaves extracts on hydrogen peroxide

DISCUSSION

This plant was macerated with three of different solvents and then the solvents were evaporated. The yields of maceration using ethyl acetate and methanol solvents are

almost equal in percentage but higher than the yields of maceration using hexane solvent as shown in table 1. This mean the components in *S. malaccense* easier dissolve in ethyl acetate and methanol, and it is also relative semipolar to polar. Phenolic and flavonoids which is one of components in this plant is a compound that easily dissolve in ethyl acetate and methanol, and it is also categorized the relative semipolar compound (Al-matani *et al*., 2015 and Aguda *et al*., 2016).

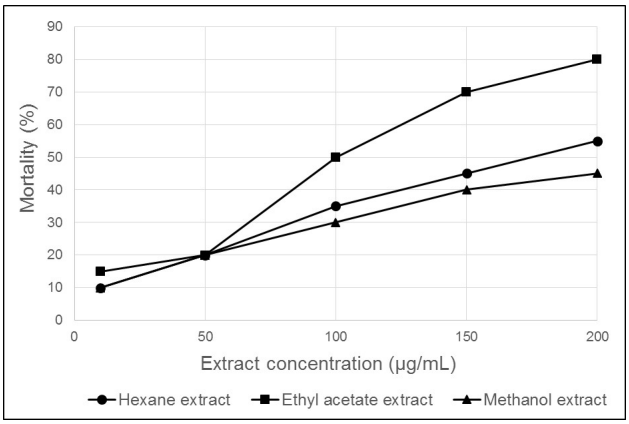

Fig. 4: Cytotoxic activity of various concentration of *S. malaccense* leaves extracts on *A. salina* larvae

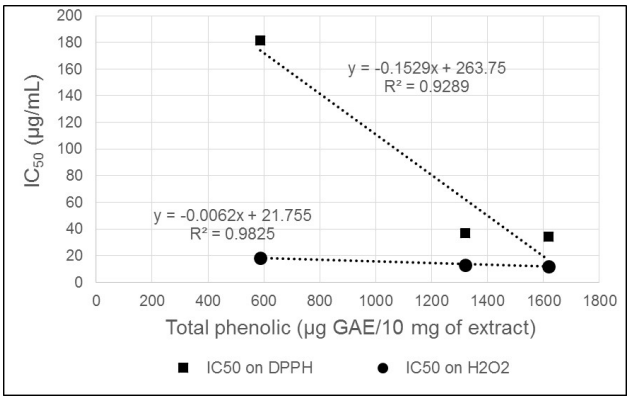

Fig. 5: Correlation curve between total phenolic contents of *S. malaccense* and their antioxidant activity (IC_{50})

Hexane, ethyl acetate and methanol extracts were able to reduce the stable radical DPPH to yellow-coloured 1,1-diphenyl-2-picrylhydrazyl. This indicate that all extracts have antioxidant activity. The highest antioxidant activity on the DPPH radical is ethyl acetate extract. Thus, in the ethyl acetate extract contains the components with highest antioxidant activity than methanol and hexane extracts. Ethyl acetate is a solvent which can dissolve the relative semi-polar organic component, while methanol is a solvent which can dissolve either the relatively polar organic components. One of compound that can dissolve in ethyl acetate and methanol that has activity as antioxidant is phenolic or flavonoids (Al-matani, *et al*., 2015). Alamprese *et al.* (2005), Djeridane *et al.* (2006), Lo and Cheung (2005), Sun and Ho (2005) and Al-matani, *et al*., (2015) reported that there was a positive correlation between phenolic contents and its antioxidant activity. Huang and Mau (2006) also reported that the positive correlation between polyphenolic contents of *Agaricus blazei* and its antioxidant activity, meanwhile Shaidi *et al.* (1994) reported that the polyphenolic compounds with one or more other components present in the extracts may contribute to the overall observed antioxidant activity (Ordonez *et al.*, 2006). *S. malaccense* was also reported containing phenolic and flavonoids. Furthermore, table 2 shows that ethyl acetate and methanol extracts have strong antioxidant activity where IC_{50} of ethyl acetate and methanol extracts is 34,35 and 36,87 µg/mL, respectively, meanwhile hexane extract has moderate antioxidant activity, the IC_{50} 181,26 µg/mL (Jun *et al*,. 2003; Mustarichie *et al*., 2017). However, antioxidant activity of *S. malaccense* leaves extracts is lower than ascorbic acid as reference where IC_{50} of ascorbic acid is 1.40µg/mL.

In determination of antioxidant activities to hydrogen peroxide, all extracts have ability to neutralize hydrogen peroxide where increasing of extracts concentration, the antioxidant activities was also increase. Antioxidant activity of ethyl acetate and methanol extracts are almost equal meanwhile antioxidant activity of hexane extract is lower than both ethyl acetate and methanol extracts as shown in fig. 3. Similar with antioxidant activities of extracts on DPPH, antioxidant activities on hydrogen peroxide is also affected by their phenolic content which can dissolve in ethyl acetate and methanol. According to Jun *et al*., 2003 and Mustarichie *et al*., 2017 antioxidant activity of these extracts is categorized as a very powerful, because IC_{50} of extracts less than 50 µg/mL as expressed in table 3.

Ethyl acetate extract is an extract that has highest cytotoxic properties than that of hexane and methanol extracts. This is expressed with the lethal concentration 50% (LC_{50}) of extract, where ethyl acetate extract has lowest LC_{50} than that of others as shown in table 4. LC_{50} is a concentration of a substance that can kill fifty percent the experimental animal. In this case is brine shrimp *A. salina*. Cytotoxic activity of a natural products or drug materials was categorized based on LC_{50} which divided into four categories i.e. strong, moderate, weak and non-toxic for LC_{50} 0-100, 100-500, 500-1000 and greater than 1000 µg/ml, respectively (Nguta *et. al*., 2012; Nguta and Mbaria 2013). Based on these categories, ethyl acetate extract was categorized as having strong cytotoxic activity while hexane and methanol extracts were categorized as having moderate cytotoxicity.

Phenolic content of *S. malaccense* leaves extract showed a concentration-response relationship on antioxidant activity either to DPPH scavenging activity or hydrogen peroxide scavenging activity. Increase the phenolic content of extract, antioxidant activity is also increase. In this case, antioxidant activity is expressed with IC_{50} (table

2 for DPPH scavenging activity and table 3 for hydrogen peroxide scavenging activity), where IC_{50} is a concentration of a substance that can inhibit fifty percent the radical activity. The highest total phenolic content is ethyl acetate extract, followed by methanol and hexane extracts. It is appropriate with antioxidant activity of extracts where the highest of antioxidant activity is also ethyl acetate extract, and also followed by methanol and hexane extracts either to DPPH scavenging activity or hydrogen peroxide scavenging activity. Correlation between total phenolic content and antioxidant activity of these extracts are presented in fig. 5 which showed that regression equation between total phenolic content and antioxidant activity to DPPH and hydrogen peroxide are $y = -0.1529x + 263.75$ ($R^2 = 0.9289$) and $y = -0.0062x + 21.755$ ($R^2 = 0.982$), respectively. This mean phenolic content of extracts affect antioxidant activity either to DPPH or to hydrogen peroxide scavenging activities. Previously, Sen S. *et al*. (2013) also reported that there is correlation between phenolic content of extract with antioxidant activity in DPPH scavenging activity. An increase in concentration is also an increase in scavenging capacity.

CONCLUSION

Various leaves extracts of *S. malaccense* displayed free-radical-scavenging properties on DPPH radical and hydrogen peroxide, and cytotoxic activity on *A. salina* larvae. Ethyl acetate and methanol extracts have strong antioxidant activity, meanwhile hexane extract has moderate antioxidant activity on DPPH and all these extracts have very powerful antioxidant properties on hydrogen peroxide. Cytotoxic activity of ethyl acetate extract was categorized as having strong cytotoxic activity while hexane and methanol extracts were categorized as having moderate cytotoxicity. All antioxidant activities either on DPPH or on hydrogen peroxide have positive correlation with their total phenolic contents, increase in total phenolic concentration is also increase in antioxidant activity. Therefore *S. malaccense* extracts having potential as traditional medicine to treatment of diseases which has been caused by oxidation process such as cancer and diabetes mellitus etc. Further studies in our laboratory is to isolate the components from these extract.

REFERENCES

Aguda R and Chen CC (2016). Solubility of Nutraceutical Compounds in Generally Recognized as Safe Solvents at 298 K, *Int. J. of Chem. Eng. and App.,* **7**(5): 289-294.

Alamprese C, Pompei C and Scaramuzzi F (2005). Characterization and antioxidant activity of nocino liqueur. *Food Chem.,* **90**: 495-502.

Al-matani SK, Al-Wahaibi RNS and Hossain MA (2015). In vitro evaluation of the total phenolic and flavonoid contents and the antimicrobial and cytotoxicity activities of crude fruit extracts with different polarities from *Ficus sycomorus*, *Pac. Sci. Rev. A: Nat. Sci. and Eng.,* **17**: 103-108.

Arumugam B, Manaharan T, Heng CK, Kuppusamy UR and Palanisamy UD (2014). Antioxidant and antiglycemic potentials of a standardized extract of *Syzygium malaccense*. *LWT-Food Sci. Technol.*, **59**: 707-710.

Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P and Vidal N (2006). Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chem.*, **97**: 654-660.

Dunstan CA, Noreen Y, Serrano G, Cox PA, Perera P and Bohlin L (1997). Evaluation of some Samoan and Peruvian medicinal plants by prostaglandin biosynthesis and rat ear oedema assays. *J. Ethnopharmacol*., **57**(1): 35-56.

Hettiarachchy NS, Glenn KC, Gnanasambandam R and Johnson MG (1996). Natural antioxidant extract from Fenugreek (*Trigonella foenumgraecum*) for ground beef patties. *J. Food Sci.*, **61**(3): 516-519.

Huang SJ and Mau JL (2006). Antioxidant Properties of Methanolic Extracts from *Agaricus blazei* with Various Doses of g-irradiation. *L W T*, **39**: 707-716.

Itam A, Abdul Majid AMS and Ismail Z (2015). Antioxidant and antiangiogenic properties, and gas chrmoatographic-time of flight analysis of *Sonchus arvensis* Leaves Extracts, *J. Chem. Soc. Pakistan*, **37**(06): 1250-1259.

Jun M, Fu HY, Hong J, Wan X, Yang CS and Ho CT (2003). Comparison of antioxidant activities of isoflavones from kudzu root (*Pueraria labata* Ohwi). *J. Food Sci. Technol.,* **68**(6): 2117-2122.

Karioti A, Skaltsa H and Gbolade AA (2011). Analysis of the leaf oil of *Syzygium malaccense* Merr. et Perry from Nigeria. *J. Essent. Oil Res.,* 313-315.

Kosar M, Dorman HJD and Hiltunen R (2005). Effect of an acid treatment on the phytochemical and antioxidant, characteristics of extracts from selected Lamiaceae species. *Food Chem.*, **91**: 525-533.

Lo KM and Cheung PCK (2005). Antioxidant activity of extracts from the fruiting bodies of *agrocybe aegerita* var. alba. *Food Chem.,* **89**: 533-539.

Lobo V, Patil A, and Chandra A (2010). Free radicals, antioxidants and Functional Foods: Impact on human health, *Pharmacogn Rev*., **4**(8): 118-126.

Meyer BN, Ferrigni NR, Putnam JE, Jacobson LB, Nichols DE and McLaughlin (1982). Brine shrimp; a convenient general bioassay for active plant constituents. *J. Med. Plant Res., Planta Med.*, **45**: 31-34.

Mustarichie R, Runadi D and Ramdhani D (2017). The antioxidant activity and phytochemical screening of ethanol extract, fractions of water, ethyl acetate and n-

hexane from mistletoe tea *(Scurrula atropurpurea* BL. Dans). *Asian J. Pharm. Clin. Res.,* **10**(2): 343-347.

Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kabasa JD and Kiama SG (2012). Evaluation of acute toxicity of crude plant extracts from Kenyan biodiversity using brine shrimp, *Artemia salina* L. (Artemiidae). *The Open Conf. Proc. J.,* **3**: 30-34.

Nguta JM and Mbaria JM (2013). Brine shrimp toxicity and antimalarial activity of some plants traditionally used in treatment of malaria in Msambweni district of Kenya. *J. Ethnopharmacol.,* **148**(3): 988-992.

Ordonez AAL, Gomez JD, Vattuone MA and Isla MI (2006). Antioxidant activity of *sechium edule* (jacq) swartz extracts. *Food Chem.*, **97**: 452-458.

Orwa C, Mutua A, Kindt, R, Jamnadass R, Anthony S (2009). *Agroforestree Database: a tree reference and selection guide version 4.0*. World Agroforestry Centre, Kenya.

Reynertson KA, Yang H, Jiang B, Basile MJ and Kennelly EJ (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chem*., **109**(4): 883-890.

Sen S, De B, Devanna N and Chakraborty R (2013). Total phenolic, total flavonoid content and antioxidant capacity of the leaves of *Meyna spinosa* Roxb., an Indian medicinal plant. *Chin. J. Nat. Medicines*, **11**(2): 0149-0157.

Shaidi F, Wanasunadar UN and Amarpwics R (1994). Natural antioxidant from low-pungency mustard flour. *Food Res. Int.*, **27**: 489-493.

Sun T and Ho CT (2005). Antioxidant activity of buckwheat extracts. *Food Chem.*, **90**: 743-749.

Vidotto C, Silva DB, Patussi R, Brandão LFG, Tibúrcio JD, Alves SN and Siqueira JM (2013). Brine Shrimp Lethality Test As A Biological Model For Preliminary Selection Of Pediculicidal Components From Natural Source, *Biosci. J*., **29**(1): 255-263.

Wettasinghe M and Shahidi F (1999). Antioxidant and free radical-scavenging properties of ethanolic extracts of defatted borage (Borago officinalis L.) seeds. *Food Chem.,* **67**: 399-414.

Whistler WA and Elivitch CR (2006). *Syzygium Malaccense* (Malaya Apple), Spesies Profiles for Pasific Island Agroforesty. *Ver.,* **2**(1): 1-13.