ASM Sc. J., 11, Special Issue 3, 2018 for SANREM, 53-58

Determination of Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity of Various Organic Crude Extracts of *Licuala Spinosa* Leaves from Sabah, Malaysia

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In this study, the leaves of *Licuala spinosa* were used to determine the total phenolic and flavonoid content as well as antioxidant activity of different crude extracts. The samples were extracted successively with organic solvents such as hexane, chloroform and ethyl acetate respectively. The total phenolic content was determined by Folin-Ciocalteu's assay. Chloroform crude extract showed the highest total phenolic content (9.42± 0.06 mg GAE/g), followed by ethyl acetate crude extract (8.91± 0.06 mg GAE/g) and hexane crude extract (6.78±0.26 mg GAE/g). The total flavonoid content was determined by Aluminium chloride colometric assay and expressed as QE equivalent. Chloroform crude extract showed the highest total flavonoid content (8.96 ± 0.21 mg QE/g), followed by ethyl acetate crude extract (7.04 ± 0.02 mg QE/g) and hexane crude extract (3.05 ± 0.09 mg QE/g). The antioxidant activity of extracts were evaluated by 2,2-diphenyl-1-picyhydrazyl (DPPH) assay. In DPPH assay, IC_{50} values were used to determine the antioxidant potential of the sample. The lower the IC_{50} value, the higher the antioxidative property. Among all the extracts, chloroform extracts exhibited higher DPPH radical scavenging activity with IC_{50} value of 0.032 mg/mL. BHT used as the positive control showed IC_{50} value of 0.089 mg/mL

Keywords: *Licuala spinosa*, Total phenolics, Total flavonoids, DPPH activity, BHT

I. INTRODUCTION

Medicinal plant usage dates back practically to the existence of human civilization. From ancient records, the uses of medicinal plants by human have been traced such as the cinchona bark in Mesoamerica, opium poppy in Egypt and snakeroot plant in India (Tiwari *et al.* 2011). According to WHO (World Health Organization), 80% of people worldwide were estimated rely on herbal medicines for some aspect of their main health care needs (Ekor, 2014). Moreover, around 21,000 plants species have the possibility or potentials for being used as medicinal plants (Joy *et al.* 2001). Due to their pain-relieving and healing abilities, medicinal plants have been prized and relied on in about 75% of our medicines until today (Chevallier, 2012). Despite the advances and advantages of modern medicines,

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medicinal plants offer the benefits that pharmaceutical drugs often lack, aiding to support the body's effort to regain good health. In Malaysia around 2,000 plants species have therapeutic characteristics and can be used in traditional treatments (Rukayah *et al.* 2006). These medicinal plants are considered to contain rich resources of ingredients, which can be used in drug development and synthesis (Hassan, 2012)). The medicinal value of these plants lies in some chemical active substances that produce a definite psychological action on the human body and the most important bioactive constituents of plants are alkaloids, tannins, flavonoid and phenolic compounds (Rajendra *et al.* 2011). The *L. spinosa* belongs to family *aracaceae* (palmae) native to vast area of the Asiatic south-east, which includes Cambodia, India (Andaman and Nicobar Island), Indonesia (Java, Borneo and Sumatra), Malaysia, Myanmar, Philippines, Thailand and Vietnam. It grows mainly along the coasts and the banks of the river. The name of the species "spinosa" refers to the thorns present on the margins of the petiole (Henderson, 2009). L. spinosa is a densely clumping palm of medium height, with slender stems and heads of circular, divided fan leaves. The upright stems to 4 m tall and 8-10 cm in diameter with remnant fibers. The leaf is circular shaped with squared-off ends. The fruit and flowers are inflorescences (1-2.5 m long) are branched to two orders in long drooping spikes.The colour of the fruit is bright red with 0.5 inch in diameter. The seed is small, round and it can germinate in 6–8 weeks. *L. spinosa* grows well in shade or filtered light in acidic or neutral condition of pH soil (Riffle *et al.* 2003)). The present study was designed to investigate the total phenolic and flavonoid content of *L. spinosa* leaves by using some of significant methods and evaluate the antioxidant activity using DPPH scavenging assays.

Figure 1. *L. spinosa* plants and fruits in natural habit

II. MATERIALS AND METHOD

A. Plant Material and Sample Collection

The plant *L. spinosa* was used in this experiment. The fresh samples (leaves) were taken around Universiti Malaysia Sabah (UMS). The samples were washed with distilled water to remove dust and were dried under shade at room temperature for one week. About 1kg leaves were pulverized in a grinder for 3 min and stored in dark bags to protect from humidity and light, prior to analysis.

B. Preparation of Crude Extract

The dried leaves powder (200 g) was extracted with methanol using soxhlet extraction method at room temperature. The methanolic extract was recovered by evaporating the solvent by vacuum rotatory evaporator. This crude extract was further diluted with water and subsequently extracted with hexane, chloroform and ethyl acetate to get their respective residual fractions. These fractions were filtered using Whatmanns filter paper, and then evaporated under reduced pressure using rotary

evaporator in order to obtain the crude extracts. The extraction processes was repeated in triplicate. After solvent evaporation, all the crude extracts were weighed and kept for further usage in caped vials at 4 °C.

C. Determination of total phenolic contents

The total phenolic content was determined using spectroscopic method as described by Ainsworth *et al.* (2007). The reaction mixture was prepared by mixing 1 mL plant extracts (1mg/mL), 1 mL of 10% Folin-Ciocalteu's reagent dissolved in 13 mL of deionized water followed by the addition of 5 mL of 7% Na2CO3 solution. The mixture was mixed thoroughly and kept in the dark at room temperature for 2 h. The blank solution was also prepared. The absorbance was recorded using spectrometer at 760 nm. All the analysis was repeated three times and the mean value of absorbance was obtained. Total phenolic content was determined by extrapolating calibration line which was construed by gallic acid solution. The TPC was expressed as gallic acid equivalent (mg GAE) per gram of the dried sample.

D. Determination of total flavonoid contents

The total flavonoids content of the L. spinosa was determined by using aluminium chloride calorimetric method based on the methodology reported by Afify *et al.* (2012) with some modifications. 0.5 mL of sample (1mg/mL) was mixed with 1mL of 10% aluminium chloride, 1mL of potassium acetate (1M) and 2.5 mL of distilled water. Quercetin was used to make the calibration curve. The absorbance of the mixtures was measured at 415

nm by using UV-spectrophotometer. The total flavonoid content was expressed in terms of quercetin equivalent (mg QE/g of sample). All the analyses were repeated three times and the mean value of absorbance was obtained.

E. DPPH radical scavenging activity

The antioxidant activity of the extracts were quantitatively assessed on the basis of free radical scavenging activity of stable1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the reported method of Brand-Williams et al. with slight modification. 1mL of plant extract solution of various concentrations, ranging from 0.05-0.20 mg/mL was mixed with 1 mL of 0.5 mM DPPH solution in methanol. Incubation of the resulting solution was carried out for 30 min in dark room at 37 °C. BHT was used as positive control under the same assay condition. The absorbance was measured calorimetrically at 517 nm. The experiments were carried out in triplicate. The percentage inhibition was calculated using the following formula.

$$\text{DPPH Scavenging Activity (\%)} = [(Ao-As)/Ao)] \times 100$$

Here, Ao is the absorbance of the control (no sample, DPPH solution only) and As is the absorbance in the presence of the sample.

III. RESULTS AND DISCUSSION

In this study, the leaves of *L. spinosa* were extracted

with hexane, chloroform and ethyl acetate. Initial weight of the sample used was 300 g for leaves. The highest percentage yield of solid residue was obtained for hexane extract (Table 1).

Table 1. The percentage yield of extracts obtained from solid residue of plant material.

Solvents	Percentage Yield (%)
Hexane	1.057
Chloroform	0.677
Ethylacetate	0.397

The total phenolic content was determined by using Folin-Ciocalteu method (Annisworth *et al.* 2007). Gallic acid was used as standard calibration and total phenolic content in mg gallic acid equivalence (mg GAE/g). The total phenolic content of the crude extracts was solvent-dependent. The higher the polarity of solvent the more will be the total phenolic and flavonoid content in the extract. In this present study, the total phenolic content in fractions of different polarities varied, ranging from 6.78 ± 0.26 to 9.42 ± 0.06 mg of GAE/100 g powder weight. Chloroform extract exhibited the highest total phenolic content (9.42 ± 0.06 mg GAE/g), followed by ethyl acetate (8.912 ± 0.06 mg GAE/g) and hexane (6.778 ± 0.257 mg GAE/g) (Table 2).

Table 2. Total phenolic and total flavonoid contents in various plant extracts of *L. spinosa* red leaves

Solvents	Total phenolic contents (mg GA/gm)	Total flavonoid contents (mg QE/gm)
Hexane	6.78±0.26	3.05 ± 0.09
Chloroform	9.42± 0.06	8.96 ± 0.21
Ethylacetate	8.91± 0.06	7.04 ± 0.02

The total flavonoid content was determined by aluminium chloride colorimetric method as describe by Chang *et al.* (2002). Quercetin was used as standard calibration and total flavonoid content in mg quercetin equivalence (mg QE/g). The total content of flavonoids in different extracts of L. spinosa was determined from the regression equation of the calibration curve (y= 0.0072x + 0.0026, R2= 0.999) and expressed as milligrams of quercetin equivalents (QE). In this study, the chloroform extract exhibited the highest total flavonoid content (8.96 ± 0.21 mg QE/g), followed by ethyl acetate (7.04 ± 0.02 mg QE/g) and hexane (3.05 ± 0.09 mg QE/g).

Antioxidants are compound that having the ability to either delay or inhibit the oxidation processes (Pisoschi *et al.* 2011). This reaction takes place under the presence of atmospheric oxygen or reactive oxygen species (ROS). Antioxidant involved as a defensive mechanism of the organism from an attack of free radicals.

In this present study, the antioxidant activity of crude extracts from L. spinosa was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacity assay. The delocalization of the spare electron on the molecule makes DPPH as a stable free radical and does not dimerize like the other free radical. The appearance of a violet colour with an absorption band around 520 nm indicates the delocalization of DPPH molecule (Pisoschi *et al.* 2009). The leaves of L. spinosa in different organic solvents were tested for their free radical scavenging activity using DPPH assay. The IC_{50} value was calculated and found 1.181 mg/mL for hexane

extract, 0.088 mg/mL for chloroform extract and 0.121 mg/mL for ethyl acetate extract whereas IC_{50} value of BHT was found to be 0.032 mg/mL.

IV. SUMMARY

In conclusion, this study shows that higher total flavonoid content (8.96 ± 0.21 mg QE/g) and total phenolic content (9.42± 0.06 mg GA/g) is higher in chloroform extract followed by ethyl acetate. The lowest phenolic and flavonoid concentration was reported in the hexane extract i.e. 6.78±0.26 mg GA/g and 3.05 ± 0.09 mg QE/g, respectively.

V. ACKNOWLEDGMENT

The authors thank Faculty of Science and Natural Resources, Universiti Malaysia Sabah and School of Industrial Technology, Universiti Sains Malaysia for providing necessary research facilities to carry out the research work. Thanks are due to Malaysian Government and UMS for the providing the grant SBK0329-2017 to conduct this work.

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