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Total Phenolic Content, Total Flavonoid Content, Antioxidant and Antimicrobial Activities of Malaysian *Shorea*

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ABSTRACT

Five *Shorea* species which are endemic plants of Malaysia were used in this study. The methanol extract of *Shorea acuminata, Shorea macroptera, Shorea leprosula, Shorea bracteolata* and *Shorea resinosa* were investigated for total phenolic contents (TPC), total flavonoid contents, radical scavenging properties (DPPH), *in vitro* antioxidant and antimicrobial activities. For the antimicrobial activity, the extracts were tested against four bacteria; Gram negative bacteria *Escherichia coli*, Gram positive bacteria *Staphylococcus pyogenes, Staphylococcus aureus* and *Bacillus subtilis* and two fungi, *Candida albicans* and *Aspergillus niger*. The result of TPC analysis showed that *S. acuminata* displayed highest total phenolic content, 2731±0.09 mg/100 g, while in total flavonoid content, *S. resinosa* displayed the highest amount of flavonoid, 956.73±0.01 mg/100 g. However, in DPPH analysis, the samples of *S. resinosa*, *S. macroptera* and *S. acuminate* displayed better scavenging activity as compared to butylatedhydroxytoluene (BHT). The *in vitro* antioxidant activity indicated that *S. macroptera* showed the highest percent inhibition both in ferric thiocyanate method (FTC) (98.68%) and thiobarbituric acid method (TBA) analysis. In antimicrobial activity, *S. resinosa* displayed moderate inhibition against Gram negative bacteria *Escherichia coli*, Gram positive bacteria *Streptococcus pyogenes, Staphylococcus aureus* and *Bacillus subtilis*. Other plants were found to display selective inhibition against microbes.

Key words: *Shorea* sp., total phenolic content, radical scavenging, antioxidant, antimicrobial

INTRODUCTION

Plants contain phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities. Currently, about 25% of the active component was identified from plants that are used as prescribed medicines (Gill *et al*., 2011). Several plants have been screened for their antioxidant potential (Achuthan *et al*., 2003; Aniya *et al*., 2002; Jose and Kuttan, 1995; Lin *et al*., 1995; Nazri *et al*., 2011; Shylesh and Padikkala, 1999) because the most practical way to fight degenerative diseases is to increase antioxidant activity in our body and that could be achieved by consumption of vegetables, fruits or edible plants (Oboh and Akindahunsi, 2004; Oboh, 2005). Degenerative disease is caused by free radicals in our body that are able to damage living tissues and cause cell death (Yang *et al*., 2002). Several plant extracts and chemical

constituents have been found to show quite prominent antioxidant activity (Tripathi *et al*., 1996; Vani *et al*., 1997; Oseni and Akindahunsi, 2011). According to Chu *et al*. (2002), the majority of the antioxidant activity may come from active substances such as flavonoid, isoflavone, flavone, anthocyanin, catechin and iso-catechin rather than from vitamin C, E and β-carotene.

Most lowland rainforests in Malaysia are dominated by the Dipterocarpaceae family. About 67% of the total growing stock in Sabah and Sarawak is estimated to consist of *Shorea*, a common dipterocarp genus. *Shorea* is locally known as Meranti in Malaysia and for this study, five *Shorea* species namely *S. acuminata* (Meranti rambai daun), *S. bracteolata* (Meranti Pa'ang), *S. leprosula* (Meranti Tembaga), *S. resinosa* (Meranti Belang), *S. macroptera* (Meranti Melantai) were investigated for their potential as antioxidant and antimicrobial agent.

Our previous study on the chemical constituents of *Shorea* revealed the presence of oligostilbenoids as the main constituents (Norizan *et al*., 2011; Nazri *et al*., 2011; Zawawi and Ahmat, 2011). Oligostilbenoids found in *Shorea* species has been reported to possess important bioactivities such as antibacterial (Sotheeswaran *et al*., 1983) and 5α-reductase reactions (Hirano *et al*., 2001) while oligostilbenoids from other family also showed significant bioactivities such as antitumor (Ito *et al*., 2003), acetylchloinesterase (Sung *et al*., 2002), ecdysteroid antagonist (Sarker *et al*., 1999), anti HIV agent (Dai *et al*., 1998), anti-inflammation (Kitanaka *et al*., 1990), cytotoxic (Dai *et al*., 1998) and antioxidant activities (Tanaka *et al*., 2000). This paper describes the evaluations of total phenolic content (TPC), total flavonoid content, radical scavenging properties (DPPH), *in-vitro* antioxidant capacity using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods as well as *in-vitro* antimicrobial activity of five *Shorea* species which are *S. acuminata*, *S. macroptera*, *S. leprosula*, *S. bracteolata* and *S. resinosa*.

MATERIALS AND METHODS

The plant samples were collected from Jengka Reserve Forest, Selangor, Malaysia in 2009 and voucher specimens were submitted to the Herbarium of Universiti Teknologi MARA, Pahang (UiTM), *S. bracteolata* (DO8/06/09), *S. macroptera* (DO7/06/09), *S. acuminate* (D11/06/09), *S. resinosa* (D10/06/09) and *S. leprosula* (DO9/06/09). The samples were cut into smaller pieces and ground into fine powder. The plants were macerated with methanol five times at room temperature, filtered, evaporated using rotary evaporator and the weight of the crude extracts recorded.

Antioxidant activity assay

Total phenolic content (TPC): The TPC was carried out according to Velioglu *et al*. (1998) method. The 0.01 g of crude extracts was appropriately diluted with 5 mL of methanol and 0.1 mL of the diluted sample was added with Folin-Ciocalteu phenol reagent and placed about 20 min in a dark place. 1.5 mL of 20% Na_2CO_3 solution were added to the sample and shaken vigorously. The solution was immediately marked up with distilled water and mixed thoroughly. The absorbance was measured at 760 nm using Perkin Elmer Lambda 35 UV-visible Spectrophotometer after incubation for 2 h in dark and at room temperature. A calibration curve was prepared, using the regression equation of the calibration curve of gallic acid ($y = 0.013x$, $r^2 = 0.998$) and contents were expressed as mg gallic acid equivalent (GAE)/100 g of sample.

Total flavonoid content (TFC): Two milliliter of 2% $AlCl_3$ in ethanol was added to 2 mL of the test sample and left in the dark at room temperature for 1 h. The UV absorption was measured

at 420 nm. Concentration of 0.1 mg mL^{-1} sample solutions were used while routine concentrations of 0.02-0.12 mg mL^{-1} were used to obtain a calibration curve of quercetin ($y = 2.519x+0.002$, $r^2 = 0.999$). Determinations were performed in triplicate.

Radical scavenging assay (DPPH): The DPPH radical-scavenging activity was determined by using the method proposed by Nazri *et al.* (2011) with some modification. The positive control was prepared by adding 4 mL of quercetin (0.05 mg mL^{-1}) to 1 mL of DPPH (0.4 mg mL^{-1}), whereas the negative control was prepared by each solvents used and 1 mL of DPPH solution. The radicals' stock solution was prepared fresh daily. The DPPH solution (1 mL) was added to 3 mL of polyphenol extracts with different concentrations (200, 400, 600, 800, 1000 and 1200 ppm). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm for 10 min. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. Radical scavenging activity is expressed as the inhibition percentage (IP) and was calculated as follows:

$$IP = \frac{(A\ control - A\ sample)}{A\ control} \times 100\%$$

The decolouration was plotted against the sample extract concentration and a logarithmic regression curve was established in order to calculate the IC_{50} (inhibitory concentration at 50%) which is the amount of sample extract (concentration) necessary to decrease by 50% the absorbance of DPPH.

Ferric thiocyanate method (FTC): Four milligrams of the dried solids from each fraction were dissolved in 4.0 mL of ethanol absolute and added with 4.1 mL of a 2.51% linoleic acid solution in EtOH and 8 mL of a 0.05 M phosphate buffer (pH 7.0). The mixture was incubated at 40°C in a dark screw-cap vial. During the incubation, 1.0 mL aliquot was taken from the mixture and added with 9.7 mL of 75% ethanol, followed by the addition of 0.1 mL of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid, the absorbance for the red color was measured at 500 nm .

Thiobarbituric acid method (TBA): Two milliliters of 0.67% thiobarbituric acid and 1.0 mL of 20% trichloroacetic acid solutions were added to 2.0 mL of the mixture solution containing linoleic acid which was prepared according to the FTC procedure. The mixture was then placed in a boiling water bath for 10 min. After cooling, the mixture was centrifuged at 3000 rpm for 20 min and the absorbance was measured at 532 nm.

Antimicrobial activity test

Microorganisms: The extracts were tested against four bacteria that are Gram negative bacteria, *Escherichia coli* and Gram positive bacteria, *Streptococcus pyogenes, Staphylococcus aureus* and *Bacillus subtilis* and yeast, *Candida albicans* and fungus, *Aspergillus niger*.

Preparation of antimicrobial disc: The micropipette was used to place 20 µL extract onto a 6 mm diameters of sterile antimicrobial disc. The disc was aseptically dried and placed on media inoculated with the tested microorganisms by using sterile forceps. A maximum of only four discs were placed on the media. The plates were incubated at 37°C for 24 h. The corresponding concentrations are expressed in terms of mg of extract per mL of solvent.

Disc diffusion method: Antimicrobial tests were carried out by disc diffusion method using the suspension containing 1×10^8 CFU mL^{-1} bacteria or 1×10^6 CFU mL^{-1} yeast on Nutrient Agar (NA) or Potato Dextrose Agar (PDA), respectively. The discs were impregnated with 20 μL of extracts and placed on the inoculated agar. The inoculated plates were incubated for 24 h at 37°C for bacteria strains, 48 h for yeast. A sterile cotton swab was dipped into 18-24 h broth culture of the test organism and carefully streaked over the surface of the sterile agar plate. The plate was then turned at right angles and the swab was streaked again over the entire surface to ensure a uniform film of the test microbes. 0.02 mg mL^{-1}. Streptomycin was used as a positive control for the bacteria whereas 100 mg mL^{-1} cycloheximide was used as positive control for the yeast. Antimicrobial activity was evaluated by measuring the inhibition zone against the test organisms. The assay in this experiment was repeated three times.

Statistical analysis: The experiments were done in triplicate. The result was given as the mean±SD. Analysis of Variance (ANOVA) was used for the analysis of data. Significance was accepted at $p = 0.05$.

RESULTS AND DISCUSSION

Five *Shorea* species, *S. acuminata*, *S. macroptera*, *S. leprosula*, *S. bracteolata* and *S. resinosa* which are endemic plants of Malaysia were investigated for their total phenolic content, total flavonoid content, *in vitro* antioxidant and antimicrobial activities.

The results showed that all *Shorea* species tested with TPC displayed high amount of phenolic with range of 2461-2731 mg/100 g GAE. Among the five *Shorea* samples, *S. acuminata* extracts displayed the highest phenolic content 2731.00±0.09 mg/100 g followed by *S. leprosula* extracts 2615.38±0.01 mg/100 g, *S. resinosa* extracts 2461.54±0.01 mg/100 g and *S. macroptera* extracts 2461.54±0.01 mg/100 g and the lowest total phenolic content is given by *S. bracteolata* extract with 2423.08±0.02 mg/100 g which is considered still very high. Most of the crude methanol extracts of these *Shorea* species gave total phenolic contents in the range of 2461.54±0.01 mg of GAEs to 2731.00±0.09 mg of GAEs and are presented in Table 1. Plant phenols are a major group of compounds acting as primary antioxidants or free radical scavengers (Kahkonen *et al*., 1999) due to their hydroxyl groups (Diplock, 1997) which contribute directly to the antioxidative action. Phenolic compounds are effective hydrogen donors, making them good antioxidants (Rice-Evans *et al*., 1997).

In the TFC analysis, all *Shorea* extracts tested displayed moderate flavonoid content with range of 535.93-956.73 mg g^{-1}. *S. resinosa* displayed highest amount of flavonoid content 956.73±0.01 mg g^{-1} followed by *S. leprosula* (873.36± 0.002), *S. acuminate* (851.48±0.001) and *S. bracteolata* (766.18± 0.001) while the lowest given by *S. macroptera* extracts 535.93±0.02 mg g^{-1}.

Table 1: Extraction yields, phenolic and flavonoid contents of Malaysian *Shorea* sp.

Samples	Yield (%)	Total phenolic contents (GAE, mg/100 g)	Total flavonoid contents (mg g^{-1})	IC_{50} (μg mL^{-1})
Shorea acuminata	10.34	2731.00±0.09[a]	851.48±0.001[a]	0.125[a]
Shorea bracteolata	10.00	2423.08±0.02[b]	766.18±0.001[b]	na
Shorea leprosula	11.15	2615.38±0.01[a]	873.36±0.002[a]	na
Shorea resinosa	10.61	2461.54±0.01[b]	956.73±0.01[a]	0.125[a]
Shorea macroptera	10.16	2461.54±0.01[b]	535.93±0.02[c]	0.140[a]

Values are means of triplicate, Different superscript in column indicate significant different at $p<0.05$, na: Not available

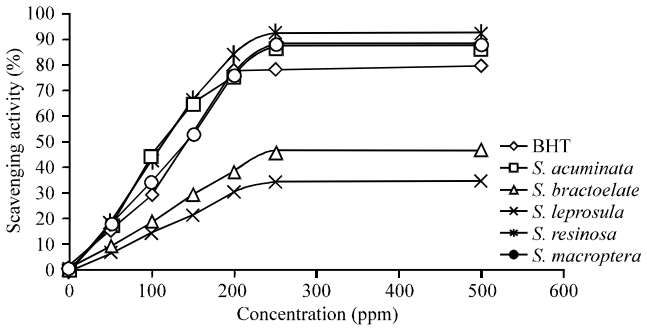

Fig. 1: Scavenging effect of methanolic extract on DPPH radicals, compared with BHT

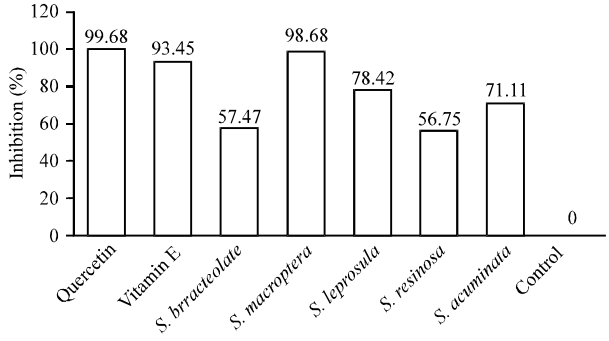

Fig. 2: Percent inhibitions of *Shorea* species by FTC method (day 6, 1 ppm)

The total flavonoid contents findings for these *Shorea* extracts were summarized in Table 1. The analysis of TFC indicates that *Shorea* contain considerable amount of flavonoids.

In DPPH radical scavenging activity, *S. resinosa*, *S. macroptera* and *S. acuminata* displayed better scavenging activity as compared to the standard BHT with % scavenging of 92.50, 88.23 and 87.23, respectively (Fig. 1). Percent scavenger for BHT is 79.7%. *S. bracteolata* and *S. leprosula* showed lower % of scavenging activity (46.55 and 34.55%, respectively) when compared to BHT. The highest scavenger is shown by *S. resinosa*, while the lowest scavenger is *S. leprosula*. *S. resinosa*. *S. macroptera* and *S. acuminata* demonstrated significant free radical scavenging ability by giving IC_{50} values of 0.125, 0.140 and 0.125 μg mL^{-1}, respectively as shown in Table 1 and are considered as excellent scavenger and even better than the standard BHT. The methanol extracts of *S. bracteolata* and *S. leprosula* did not give any values of IC_{50}. The IC_{50} value is defined as the concentration that causes a decrease in the initial amount of DPPH radicals by 50% (Huang *et al*., 2005). It is the concentration where the active crude extract will exhibit 50% of antioxidant activity (Chiang *et al*., 2003) and crude extracts exhibit 50% of inhibition at concentration less than 20 μg mL^{-1}. These concentrations are considered positive for antioxidant activity (Geran *et al*., 1972).

In FTC analysis, three of the *Shorea* species namely *S. resinosa*, *S. leprosula* and *S. macroptera*, exhibited strong antioxidant potential with percent inhibition between 71.11-98.68% while Quercetin and vitamin E showed percent inhibition of 99.68% and 93.45%, respectively (Fig. 2). The extract of *S. Macroptera* (98.68%) showed the highest percent inhibition and as good as the standard as its % inhibition is better than vitamin E. The other two of the *Shorea* extracts demonstrated moderate antioxidant activity at 56.75% for *S. bracteolata* and 57.47% for

Table 2: Percent inhibition of *Shorea* sp. between FTC and TBA method (day 6,1 ppm)

Samples	FTC (% inhibition)	TBA, (% inhibition)
Shorea acuminata	71.77[a]	81.40[a]
Shorea bracteolata	57.47[b]	52.09[b]
Shorea leprosula	78.42[a]	77.87[a]
Shorea resinosa	56.72[b]	48.57[b]
Shorea macroptera	98.68[a]	95.16[a]
Quercetin	99.68[a]	92.98[a]
Vitamin E	93.45[a]	94.53[a]

Values are means of triplicate, Different superscript in column indicate significant different at $p<0.05$, when compared five with standard, FTC: Ferric thiocyanate, TBA: Thiobarbituric acid

S. acuminata, respectively. The total antioxidant property of the methanol extracts of *Shorea* species is given as follows in descending order: *S. macroptera*>*S. leprosula*>*S. resinosa*> *S. acuminate*> *S. bracteolata* at 98.68, 78.42, 71.11, 57.47 and 56.75%, respectively.

The findings of this study showed that FTC results of the five *Shorea* species do not correlate with TPC and TFC results. The extracts of *S. macroptera* displayed highest antioxidant activity, 98.68% among the five *Shorea* species but its TPC values was the lowest amongst the five *Shorea* species 2461.54±0.01 mg g^{-1} and its TFC values was also the lowest (535.93±0.02). On the other hand, the extracts of *S. acuminata* displayed lowest antioxidant activity 54.79% but recorded the highest of TPC values, 2731.00±0.09 mg g^{-1} and moderate amount of flavonoid content (851.48±0.001) as shown in Table 1.

The plant extracts tested showed low absorbance values, indicating a high level of antioxidant activity. None of the plant extracts showed absorbance values greater than the negative controls (without plant extracts) at the end point of both methods, indicating the presence of antioxidant activity. However, the plant extracts of *S. macroptera* exhibited strong antioxidant activity as determined by both the FTC and TBA methods, surpassing the activity of the standard antioxidant, vitamin E. The extract of *S. macroptera* indicated the highest antioxidant as its absorbance was the lowest and located between both standards as shown in Table 2.

FTC test is related to the peroxide formation in the initial stage of lipid oxidation, while TBA test measures the amount of malondialdehyde (MDA) at later stage. The results in FTC showed higher inhibition than in TBA. This may indicate that the amount of peroxide in the initial stage of lipid peroxidation is much greater than the amount of peroxide in the secondary stage. This is due to the MDA, produced on the final day of the incubation period (1 day after the control reached maximum) is a very unstable compound and turn into alcohol and acid which cannot be detected by spectrophotometer (Ottolenghi, 1959) which might cause mutagenic and cytotoxic events (Zin *et al*., 2002). At a low pH and high temperature (100°C), MDA binds TBA to form a red complex that can be measured at 532 nm after incubation for 24 h. By determining the amount of malondialdehyde (MDA), the correlation between both FTC and TBA methods can be verified. These results correlated well with those obtained previously, using the FTC method. A comparison between FTC and TBA measured is shown in Fig. 3.

For the analysis of antimicrobial activity, the methanol extracts of *Shorea* species were tested against four microbes: *Escherichia coli, Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtilis* and yeast including *Candida albicans* and *Aspergillus niger*. *S. acuminata* extract (25-100 mg mL^{-1}) inhibited the growth of *S. pyogenes* (7.0-10.0 mm) and *S. aureus* (6.0-12.0 mm) moderately at higher concentration, 100 mg mL^{-1} and weakly at a lower

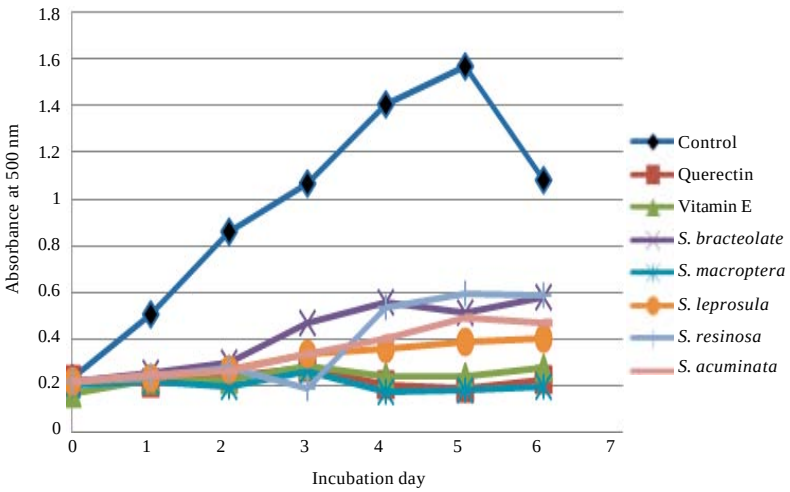

Fig. 3: Lipid oxidation profile of methanol extract of *Shorea* sp. in linoleic acid buffer model system

concentration, 25 mg mL^{-1}. The inhibition zone was increased by 1 mm with the increment of 25 mg mL^{-1} dose of *S. acuminata* methanol extract. *B. subtilis, E. coli, C. albicans* and *A. niger* were found resistant against this methanol extract.

S. macroptera, S. leprosula, S. bracteolata and S. resinosa were able to inhibit *S. aureus* and *B. subtilis* in a dose dependant manner with the highest inhibition was shown by *S. Leprosula* extract (25-100 mg mL^{-1}) against *B. Subtilis* (11-15 mm) and *S. resinosa* extract (25-100 mg mL^{-1}) against *S. pyogenes* (9-15 mm) both at 100 mg mL^{-1} while the lowest inhibition were displayed by *S. acuminata* against *S. aureus* (6 mm) at 25 mg mL^{-1}. All species were found able to inhibit *S. aureus* in a dose dependant manner. It is interesting to note that only *S. resinosa* was able to inhibit the growth of *E. coli*, a microbe commonly found in the lower intestine of warm-blooded organisms. The best result was given by *S. resinosa* which was able to inhibit the growth of four gram negative and gram positive microbes that are *S. pyogenes, S. aureus, B. subtilis* and *E. coli*. *S. macroptera* and *S. bracteolata* were able to inhibit the growth of three microbes: *S. pyogenes, S. aureus* and *B. subtilis* while *S. acuminate* and *S. Leprosula* only inhibited two microbes which are *S. pyogenes and S. aureus* and *S. aureus and B. subtilis*, respectively also in dose dependant manner. It is clearly shown from the results tabulated in Table 3 that none of the *Shorea* species was able to inhibit the growth of the two tested yeast *C. albicans and A. niger*.

The result indicated that most of the *Shorea* extracts was able to inhibit gram positive bacteria (*S. pyogenes, S. aureus, B. subtilis*) as compared to gram negative bacteria (*E. coli*). This is further confirmed by the previous studies by Kelmanson *et al.* (2000), Masika and Afolayane (2002), Masoodi *et al.* (2008), Karaman *et al.* (2003), Khanahmadi *et al.* (2010) and Zongo *et al.* (2010) that describe the high sensitivity of gram positive bacteria towards plant extracts and their component.

According to Zakaria (1991), *E. coli* was the bacteria strains not susceptible to most plant extracts which could be observed from the result of this study. These could be due to several possible reasons, in which the distinctive feature of gram-negative bacteria is the presence of a double membrane surrounding each bacterial cell. Although, all bacteria have an inner cell membrane, gram-negative bacteria have a unique outer membrane. This outer membrane excludes

Table 3: Zone of inhibition of the methanol extracts of *Shorea* species

Microorganisms	Dosage (mg mL^{-1})	Inhibition zone (mm)				
		S. acuminata	*S. macroptera*	*S. leprosula*	*S. bracteolata*	*S. resinosa*
S. pyogenes	100	10±0.578^{b}	14±0.578^{a}	-	11±0.578^{b}	15±0.578^{a}
	75	9±0.578^{b}	13±2.000^{a}	-	9±0.000^{b}	14±0.578^{a}
	50	8±0.578^{b}	11±0.578^{a}	-	6±0.000^{c}	12±0.578^{a}
	25	7±0.578^{b}	10±0.578^{a}	-	6±0.000^{b}	9±0.578^{a}
	Control	14±0.000	14±0.000	14±0.000	14±0.000	14±0.000
S. aureus	100	12±0.000^{a}	10±0.578^{b}	12±0.000^{a}	10±0.578^{b}	12±0.000^{a}
	75	10±0.578^{b}	9±0.578^{c}	12±0.578^{a}	9±0.578^{c}	10±0.000^{b}
	50	8±1.000^{a}	9±0.578^{b}	10±0.000^{a}	8±0.578^{b}	10±0.578^{a}
	25	6±0.000^{b}	8±0.578^{a}	9±0.578^{a}	7±0.000^{a}	9±0.578^{a}
	Control	18±0.000	18±0.000	18±0.000	18±0.000	18±0.000
B. subtilis	100	-	10±0.000^{b}	15±1.000^{a}	14±0.578^{a}	14±0.000^{a}
	75	-	8±0.000^{b}	13±0.578^{a}	13±0.578^{a}	13±0.000^{a}
	50	-	8±0.000^{b}	12±0.578^{a}	12±0.578^{a}	12±0.578^{a}
	25	-	8±0.000^{b}	11±0.578^{a}	11±0.578^{a}	11±1.154^{a}
	Control	18±0.000	18±0.000	18±0.000	18±0.000	18±0.000
E. coli	100	-	-	-	-	11±0.000^{a}
	75	-	-	-	-	10±0.578^{a}
	50	-	-	-	-	9±0.000^{b}
	25	-	-	-	-	8±0.578^{b}
	Control	18±0.000	18±0.000	18±0.000	18±0.000	18±0.000
C. albicans	100	-	-	-	-	-
	75	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	-	-
	Control	18±0.000	18±0.000	18±0.000	18±0.000	18±0.000
A. niger	100	-	-	-	-	-
	75	-	-	-	-	-
	50	-	-	-	-	-
	25	-	-	-	-	-
	Control	18±0.000	18±0.000	18±0.000	18±0.000	18±0.000

Values are means of triplicate, different superscript in a same row indicate significant different at $p<0.05$

certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than other gram-positive bacteria. This could be the beginning for further research on the screening approach by taking into consideration the extracts preparation and the mechanism of action. *E. coli* in this study also exhibited the same pattern in which it was resistant to almost all extracts. However, *S. resinosa* methanol extract is capable of moderately inhibiting *E. coli* growth. Although, *S. resinosa* methanol extract could inhibit bacteria, it was still unable to inhibit the two fungi *C. albicans* and *A. niger*.

S. resinosa showed good antimicrobial activity in which its methanol extract inhibited the growth of all Gram-positive and Gram-negative bacteria but not the fungi. It also gave good IC_{50} values 0.125 μg mL^{-1}, that may be regarded as good antioxidant agent. There were differences in cell wall structure between Gram-positive and Gram-negative bacteria, with the gram-negative outer membrane acting as a barrier to many environmental antimicrobial substances, including

antibiotic. The results from the antimicrobial activity of five *Shorea* species may come from resveratrol oligomers which constitute as the major compound.

CONCLUSIONS

As a conclusion, *S. resinosa* methanol extract exhibited good antimicrobial activity against *S. pyogenes*, *S. aureus*, *B. subtilis* and *E. coli* while *S. macroptera* showed the highest inhibition in both FTC and TBA assays. The results of this study have revealed the potential of Malaysian *Shorea* as antioxidant and antimicrobial agents.

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REFERENCES

Achuthan, C.R., B.H. Babu and J. Padikkala, 2003. Antioxidant and hepatoprotective effects of *Rosa damascena*. J. Pharm., 41: 357-361.

Aniya, Y., C. Miyagi, A. Nakandakari, S. Kamiya, N. Imaizumi and T. Ichiba, 2002. Free radical scavenging action of the medicinal herb *Limonium wrightii* from the Okinawa islands. Phytomedicine, 9: 239-244.

Chiang, L.C., W. Chiang, M.Y. Chang, L.T. Ng and C.C. Lin, 2003. Anti-leukemic activity of selected natural products in Taiwan. Am. J. Chinese Med., 31: 37-46.

Chu, Y., J. Sun, X. Wu and R.H. Liu, 2002. Antioxidant and antiproliferative activities of common vegetables. J. Agric. Food Chem., 50: 6910-6916.

Dai, J.R., Y.F. Hallock, J.H. Cardellina and M.R. Boyd, 1998. HIV-inhibitory and cytotoxicity oligostilbenes from the leaves of *Hopea malibato*. J. Nat. Prod., 61: 351-353.

Diplock, A.T., 1997. Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease? Free Radic. Res., 27: 511-532.

Geran, R.I., N.H. Greenberg, M.M. Macdonald, A.M. Schumache and B.J. Abbot, 1972. Protocols for screening chemical against animal tumor and other biological systems. Cancer Chemo. Reprod., 3: 1-103.

Gill, N.S., J. Bajwa, P. Sharma, K. Dhiman and S. Sood *et al*., 2011. Evaluation of antioxidant and antiulcer activity of traditionally consumed *Cucumis melo* seeds. J. Pharmacol. Toxicol., 6: 82-89.

Hirano, Y., R. Kondo and K. Sakai, 2001. Compounds inhibitory to rat liver 5α-reductase from tropical commercial wood species: Resveratrol trimers from Melapi (*Shorea* sp.) heartwood. J. Wood Sci., 47: 308-312.

Huang, D., B. Ou and R.L. Prior, 2005. The Chemistry behind antioxidant capacity assays. J. Agric. Food Chem., 53: 1841-1856.

Ito, T., Y. Akao, H. Yi, K. Ohguchi and K. Matsumoto *et al*., 2003. Antitumor effect of resveratrol oligomers against human cancer cell lines and the molecular mechanism of apoptosis induced by vaticanol C. Carcinogenesis, 24: 1489-1497.

Jose, J.K. and R. Kuttan, 1995. Antioxidant activity of *Emblica officinalis*. J. Clin. Biochem. Nut., 19: 63-70.

Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen, 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962.

Karaman, I., F. Sahin, M. Gulluce, H. Oigutcu, M. Sengul and A. Adyguzel, 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol., 85: 231-235.

Kelmanson, J.E., A.K. Jager and J. van Staden, 2000. Zulu medicinal plants with antibacterial activity. J. Ethnopharmacol., 69: 241-246.

Khanahmadi, M., S.H. Rezazadeh and M. Taran, 2010. *In vitro* antimicrobial and antioxidant properties of *Smyrnium cordifolium* Boiss. (Umbelliferae) extract. Asian J. Plant Sci., 9: 99-103.

Kitanaka, S., T. Ikezawa, K. Yasukawa, S. Yamanouchi, M. Takido, H.K. Sung and I.H. Kim, 1990. (+)-α-viniferin, an anti-inflammatory compound from *Caragana chamlagu* root. Chem. Pharm. Bull., 38: 432-435.

Lin, J.M., C.C. Lin, M.F. Chen, T. Ujiie and A. Takata, 1995. Scavenging effects of *Mallotus repandus* on active oxygen species. J. Ethnopharmacol., 46: 175-181.

Masika, P.J. and A.J. Afolayane, 2002. Antimicrobial activity of some plants used for the treatment of livestock diseases in Eastern Cape, South Africa. J. Ethnopharmacol., 83: 129-134.

Masoodi, M.H., B. Ahmed, I.M. Zargar, S.A. Khan, S. Khan and P. Singh, 2008. Antibacterial activity of whole plant extract of *Marrubium vulgare*. Afr. J. Biotechnol., 7: 86-87.

Nazri, N.A.A.M., N. Ahmat, A. Adnan, S.A.S. Mohamad and S.A.S. Ruzaina, 2011. *In vitro* antibacterial and radical scavenging activities of Malaysian table salad. Afr. J. Biotechnol., 10: 5728-5735.

Norizan, N., N. Ahmat and Z. Shameeri, 2011. Isolation of three oligostilbenes from the bark of *Shorea bracteolate*. Planta Medica, 77: 1334-1334.

Oboh, G. and A.A. Akindahunsi, 2004. Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. Nutr. Health, 18: 29-36.

Oboh, G., 2005. Effect of blanching on the antioxidant on the antioxidant property of some tropical green leafy vegetables. Food Sci. Technol., 38: 513-517.

Oseni, O.A. and A.A. Akindahunsi, 2011. Some phytochemical properties and effect of fermentation on the seed of *Jatropha curcas* L. Am. J. Food Technol., 6: 158-165.

Ottolenghi, A., 1959. Interaction of ascorbic acid and mitochondrial lipid. Arch. Biochem. Biophys., 79: 355-363.

Rice-Evans, C.A., N. Miller and G. Paganga, 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci., 2: 152-159.

Sarker, S.D., P. Whiting, L. Dinan, V. Sik and H.H. Rees, 1999. Identification and ecdysteroid antagonist activity of three resveratrol trimers (Suffruticasols A, B and C) from *Paeonia suffruticosa*. Tetrahedron, 55: 513-524.

Shylesh, B.S. and J. Padikkala, 1999. Antioxidant and anti-inflammatory activity of *Emilia sonchifolia*. Fitoterapia, 70: 275-278.

Sotheeswaran, S., M.U.S. Sultanbawa, S. Surendrakumar and P. Bladon, 1983. Polyphenols from dipterocarp species. Copalliferol A and stemonoporol. J. Chem. Soc., 1: 159-162.

Sung, S.H., S.Y. Kang, K.Y. Lee, M.J. Park and J.H. Kim *et al*., 2002. (+)-α-Viniferin, a stilbenetrimer from *Caragana chamlague*, inhibit acetylchlorinesterase. Biol. Pharm. Bull., 25: 125-127.

Tanaka, T., T. Ito, K. Nakaya, M. Iinuma and Y. Takahashi *et al*., 2000. Vaticanol D, a novel resveratrol hexamer isolated from *Vatica rassak*. Tetrahedron Lett., 4: 7929-7933.

Tripathi, Y.B., S. Chaurasia, E. Tripathi, A. Upadhyay and G.P. Dubey, 1996. *Bacopa monniera* Linn. as an antioxidant: Mechanism of action. Indian J. Exp. Biol., 34: 523-526.

Vani, T., M. Rajani, S. Sarkar and C.J. Shishoo, 1997. Antioxidant properties of the ayurvedic formulation Triphala and its constituents. Int. J. Pharm., 35: 313-317.

Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem., 46: 4113-4117.

Yang, J.H., H.C. Lin and J.L. Mau, 2002. Antioxidant properties of several commercial mushrooms. Food Chem., 77: 229-235.

Zakaria, M., 1991. Isolation and characterization of active compounds from medicinal plants. Asia Pac. J. Pharmacol., 6: 158-220.

Zawawi, N.K.N. and N.A. Ahmat, 2011. Isolation of trimer stilbenoids from the bark of *Shorea maxwelliana*. Planta Medica, 77: 1333-1333.

Zin, Z.M., A. Abdul-Hamid and A. Osman, 2002. Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. Food Chem., 78: 227-231.

Zongo, C., A. Savadogo, L. Ouattara, I.H.N. Bassole and C.A.T. Ouattara *et al*., 2010. Polyphenols content, antioxidant and antimicrobial activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae): A medicinal plant from Burkina Faso. Int. J. Pharmacol., 6: 880-887.