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Full Length Article

In Vitro Biological Investigations on *Syzygium polyanthum* Cultivars

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Abstract

Syzygium polyanthum (Wight) popularly known as *Serai Kayu* and *Serai Kayu Hutan* is consumed in Peninsular Malaysia as Ulam and as a remedy to hypertension, diabetes, endometriosis, cancer and postpartum. The medicinal property of *Serai Kayu* and *Serai Kayu Hutan* was highlighted by the local traditional healers but not yet ascertained scientifically. Radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), α-glucosidase inhibition and antimicrobial assay was used to evaluates their biological activity. The ethanolic leaves extract of *Serai Kayu Hutan* showed a high antioxidant activity in terms of radical scavenging activity, reducing power and alpha-glucosidase inhibition at 1.99 µg/mL, 36.94 mmol/g and 1.6 µg/mL, respectively. All the tested parts of *Serai Kayu* and *Serai Kayu Hutan* exhibited activity against all the tested microorganisms with a moderate of zone inhibition of (4–24 mm). Both the *Serai Kayu* and *Serai Kayu Hutan* exhibited the potentials to be utilized in the pharmaceutical and food industry and proved claimed traditional medical practitioners. © 2019 Friends Science Publishers

Keywords: Antioxidant; α-glucosidase; Radical scavenging; Medicinal use

Introduction

Plants produced natural substance or chemical as secondary metabolites which are very important in prevention, pollination and adaptation (Jaberian *et al*., 2013). The secondary metabolites produced by the plant's parts are generally utilized in many aspects of day to day life of a man. These chemical produces are known to have wide biological application either processed or not processed (Abdulrahman *et al*., 2018a; Mostafa *et al*., 2018).

Nature has design systems of human to contain great variety of cells, which involve different molecules within them. The molecules are made up of different atoms linked through a chemical bonding. Under normal circumstances the bonds between the atoms does not split in a situation whereby it leaves the molecules only with an odd unpaired electron. But the free radicals are formed as a result of the splitting of weak bond. These unpaired free radicals electron is quite unstable, there nature makes them to look for any compound within to react in other to gain stability (Floegel *et al*., 2011; Javadi *et al*., 2014). Usually they attack the closest stable compound, which make it to lose it electrons and become free radicals in the body system resulting in the damaging of the cells which has serious negative effect. Mitochondrial oxidative phosphorylation has been considered as the main producer of free radicals electron through oxygen metabolism as a byproduct (Knezevic *et al*., 2016). Pathogenic microorganisms are becoming resistance to present conventional antibiotics which is very alarming (Gowri and Vasantha, 2010; Ali *et al*., 2015).

Today pathogenic microorganisms are constantly developing drug resistance against numerous drugs that are constantly used in order to overcome their challenges in human body (Ramli *et al*., 2017). Utilization of the whole plants and plant parts to treat diverse ailments has long being in practice all over the world both in developing and developed countries through traditional medicinal system (Jaberian *et al*., 2013). Due to many problem associated with the resistance of microbes toward the antibiotics, the present day medicine have led to the much interest on plants with promising traditional history (Abdulrahman *et al*., 2018a). Medicinal plants constituents thousands of valuable compounds with therapeutic potentials. The usefulness of ethnobotany cannot be overemphasized if standardized in vitro methods that can validate traditional herbalist claim. Therefore, standard method must be establish for extraction and exploration of biological activity (Das *et al*., 2010).

In Asia medicinal plants have contributed significantly in the traditional medicinal system. Malaysia is a tropical country with diverse flora and fauna as a result of almost

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rain, high humidity and hot temperature throughout the year. The condition makes it favourable for many medicinal plants to be grown in the country. Myrtaceae is a pan tropical family of trees and shrubs with nearly about 55 thousand species, classified into 2 sub-family, 17 tribes and 142 genera. They are mainly found in large amount of number in Central America, South America, Australia and southern hemisphere. *Syzygium polyanthum* (Wight) Walp is a tree ranging between 22 m tall with glabrous or greenish brown bark. Opposite leaves with eliptic apex angle and cuneate base shape. *S. polyanthum* grows in low land areas, widely distributed in tropical, subtropical region of the world. The plants have several local names it depends on the location of the plant species. In Malaysia the species has two cultivars known as *Serai Kayu* and *Serai Kayu Hutan* (Abdulrahman *et al*., 2018b).

From the literature survey, there is no comprehensive report with respect to the utilization of *S. polyanthum* cultivars (*Serai Kayu* and *Serai Kayu Hutan*) by the Malay ethnic group in Terengganu state Peninsular Malaysia for treating of various ailments and improvements of health status. In this present study we examine the potential of using plants parts of *S. polyanthum* cultivars *Serai Kayu* and *Serai Kayu Hutan* extract of ethanolic and aqueous extract as antioxidant, α-glucosidase inhibition and antimicrobial activity.

Materials and Methods

Taxonomic Identification

Taxonomic identification and herbarium deposition, herbarium specimens of *Serai Kayu* and *Serai Kayu Hutan* were collected from natural habitat and home gardens. The species were identified and fully confirmed by a botanist at the Universiti Sultan Zainal Abidin (UniSZA), after which the identified herbarium specimen was further taken to the Universiti Kebangsaan Malaysia (UKMB) and the University of Malaya (KLU) (herbarium) for further identification, and finally deposited at Unisza, UKMB and KLU, herbaria, respectively. The following vocher number were given Unisza 00395, Unisza 00396, UM-KLU49443, UM-KLU49444, UKMB 40352, UKMB 40353, respectively.

Sample Extraction

The collected plants parts, leaves, bark and root from *Serai Kayu* and *Serai Kayu Hutan* were washed using tap water to remove dirty, stains and latex. The washed parts were arranged accordingly and cover with a tissue paper for air drying until it dried completely. Dry samples of leaves, bark and root were grinded to powder using grinding machine. The powder form of the plants samples were weighed (100 g). Ethanolic and aqueous extraction of the plant samples were carried out using Soxhlet extraction. Whatman no 2-filter paper was used to filter the product of the extraction. Evaporation machine was used (E-Z-2-Elite) in other to obtained crude compound from both the ethanolic and aqueous extract of the plant parts. The vacuum was set at 40ºC and pressure of the solvent at 300 and 72 for the ethanolic and aqueous extracts, respectively. The obtained crude extract from the leaves, bark and root was kept in oven for overnight to obtain the extract yield (Wang *et al*., 2010; Ramli *et al*., 2017).

$$\text{Yield of Extract} = \text{Dried extract (g)} / \text{weight of dried material (g)} \times 100$$

6.4.1.2 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity

A 200 μL of DPPH methanolic solution (0.004% w/v) was added to 100 μL of leaves, bark, root (ethanolic and aqueous) and essential oil at 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 μg/mL concentration, respectively. The mixture was kept for 30 min in the dark at room temperature and reduction of DPPH was measured at 517 nm. The percentage of scavenging activity was evaluated by comparing with the control (100 μL methanol + 200 μL DPPH). Quercetin was used as standards. The radical scavenging activity was calculated using the formula:

$$\text{Inhibition (\%)} = [(Ao - A1)/ Ao)] \times 100$$

Where, Ao is the absorbance of the control reaction and A1 is the absorbance of the sample itself.

The inhibitory concentration at 50% (IC_{50}) values (extract concentration that cause 50% scavenging of DPPH radical) were determined from the graph of scavenging percentage against the extract concentrations (1.56, 3.13, 6.25, 12.5, 25, 50 and 100 μg/mL). All analyses were done in three different experiments (Jaberian *et al*., 2013; Poojary *et al*., 2015; Tan and Lim, 2015).

Ferric Reducing Antioxidant Power (FRAP) Assay

A working FRAP solution was prepared by mixing 300 m*M* acetate buffer, 10 m*M* (2,4,6-tri (2-Pyridyl) –S-triazine) TPTZ in 40 m*M* HCl and 20 m*M* $FeCl_3$ in the ratio of 10:1:1 and warmed at 37°C for 10 min in a water bath before use. A 285 μL of the working FRAP solution was added to 15 μL of plant samples (ethanolic and aqueous) and essential oil of 100 μg/mL, respectively and incubated at room temperature in the dark for 30 min. Absorbance was read at 517 nm. $FeSO4.7H_2O$ with concentration between 125 to 1000 μM was used as a standard and the results were expressed as micromoles of Fe^{2+} equivalents per gram of dried extract mmol Fe^{2+}/g (Mayur *et al.*, 2010).

α-glucosidase Inhibition Assay

The alpha glucosidase method was performed. A 10 μL of extracts from leaves, bark and root samples at 0, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 μg/mL were mixed with 50 μL of

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0.1 M phosphate buffer (pH 7.0), 25 μL of alpha-glucosidase in buffer (0.2 U/mL) were added into well plate and incubated for 10 min at 37°C to initiate the reaction. A 25 μL of 0.5 m*M* 4-nitrophenyl alpha-D-glucopyranoside (pNPG) substrate was added to complete the reaction and incubated for another 30 min at 37°C. The reaction was terminated by adding 100 μL of 0.2 M sodium carbonate solution. Quercetin was used as positive control (Mayur *et al*., 2010). The absorbance was measured at 410 nm. The percentage of inhibition was calculated using the following formula: The Percentage of inhibition was calculated using the formula:

Inhibition (%) [Control abs – sample abs)/control abs] x 100

Antimicrobial Activity

Sample preparation: Stock solutions of leaves, bark and root (ethanolic and aqueous) extracts 10,000 μg/mL was prepared in DMSO, the following concentrations were prepared from the stock solutions 1000, 2000 and 4000 μg/mL, respectively.

Media preparation: Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) were prepared according to the manufacturer instruction, it was autoclaved at 121ºC cooled to room temperature and poured in to the sterile disposable Petridish under aseptic conditions. The plates were stored at 4ºC before use.

Test Microorganisms

Both Gram negative bacteria *i.e*., *Escherichia coli* (ATCC 33591), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella typhi* (ATCC 14028), *Enterobacter* (ATCC 13048), *Pseudomonas aeruginosa* (ATCC 27853) and Gram positive bacteria *i.e*., *Staphylococcus aureus* (ATCC 33591), *Enterococcus faecalis* (ATCC 24212), *Listeria monocytogenes* (ATCC 7644), *Bacillus* (ATCC 14579) and *Staphylococcus epidermidis* (ATCC 12228) bacteria were provided by the Microbiology Department of Universiti Sultan Zainal Abidin *(*UniSZA). Microbial stock culture using inoculation loop were streak on Mueller Hinton agar plates and incubated at 37ºC for overnight. The following day, they were subculture until a fresh colony was obtained and inoculated on Mueller Hinton broth and incubate for overnight at 200 rpm (Poojary *et al*., 2015).

Disk Diffusion Method

Solidified mueller hinton plates was seeded with 200 μL of microbial inoculums 1.10^6 (CFU) mL^{-1}. A whatman no 1 filter paper discs (6 mm) were impregnated with 20 μL of different concentrations of 1000, 2000 and 4000 μg/mL of the plant parts (ethanolic and aqueous) extract. The impregnated disc was placed with the aid of sterile forceps on the plates. Plates were incubated at 37ºC for 24 h. Zone of inhibition was recorded in triplicate (Tan and Lim, 2015; Mostafa *et al*., 2018).

Agar Well Diffusion Method

Mueller Hinton agar seeded was inoculated with 200 μL microbial loads of 1.10^6 (CFU) mL^{-1}. The plates divide into five equal quadrants and a well of 6 mm were cut using a sterile cork borer. A 100 μL of different concentrations of 1000, 2000 and 4000 μg/mL of the above mentioned plants parts (ethanolic and aqueous) were introduced into the well and incubated for 24 h at 37ºC. All experiment was carried out in triplicate of three different time and measured the zone of inhibition using ruler in millimeter (Tan and Lim, 2015).

Statistical Analysis

The data was analyzed using Statistical Analysis System (SAS) software (University version 9.4). Completed Randomized Design (CRD) was employed as the experimental design with three replication. One way repeated ANOVA procedure was carried out and means were subjected to post hoc Duncan's Multiple Range Test (DMRT) to find out significant differences in the means at $p \leq 0.05$ level.

Results

Yield of Extract

Ethanolic extract was found to give higher yield than the aqueous extract. High yield was found from ethanolic leaves extract of *Serai Kayu Hutan* (13.2%), follwed by *Serai Kayu* (10.4%), respectively (Table 1).

DPPH Free Radical

Radical scavenging activities of DPPH was determined on the leaves, bark, root of ethanolic and aqueous extracts and essential oil of *Serai Kayu* and *Serai Kayu Hutan*. The highest scavenging activities were recorded from the ethanolic leaf extract of *Serai Kayu Hutan* with IC_{50} value of 2.34 μg/mL, followed by essential oil from Serai Kayu leaf 2.7 μg/mL, *Serai Kayu Hutan* essential oil 3.43 μg/mL, ethanolic *Serai Kayu* root 5.02 μg/mL, then aqueous leaves extract of *Serai Kayu Hutan* 10.37 μg/mL, respectively (Table 2). The result obtained showed significant difference among the treatment.

FRAP Assay

Ethanolic extract of *Serai Kayu Hutan* was found to have the highest ability of converting Fe^{3+} to Fe^{2+} with 37.85 and 37.32 mmol/g of the leaf and bark, respectively, then aqueous leaves extract of *Serai Kayu Hutan* 36.94 mmol/g, aqueous *Serai Kayu Hutan* bark 35.46 mmol/g, aqueous *Serai Kayu Hutan* root 28.08 mmol/g and ethanolic leaf extract of *Serai Kayu* 27.76 mmol/g, respectively. The result obtained show significant difference among the treatment.

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Table 1: Percentage of yield and color of different extracts from leaf, bark and root of *Serai Kayu* and *Serai Kayu Hutan*

Plants parts	Extracts	Extraction yields (%)	Colour
SHL	Ethanol	13.2	Greenish gum
SKL	Ethanol	10.4	Greenish solid
SHL	Aqueous	9.4	Brownish solid
SKL	Aqueous	7.6	Brownish semi solid
SHB	Ethanol	6.2	Greenish solid
SKB	Ethanol	5.6	Dark brownish solid
SHB	Aqueous	5.4	Brownish solid
SKB	Aqueous	3.6	Brownish solid
SHR	Ethanol	5.6	Reddish solid
SKR	Ethanol	5.8	Reddish solid
SHR	Aqueous	3.6	Reddish solid
SKR	Aqueous	4.2	Reddish solid

Note: SKL: *Serai Kayu* Leaves, SHB: *Serai Kayu Hutan* Bark, SKB: *Serai Kayu* Bark, SHB: *Serai Kayu Hutan* Bark , SKR: *Serai Kayu* Root, SHR: *Serai Kayu Hutan* Root. Extraction was carried out in 3 triplicate

Table 2: Percentage of inhibition and IC_{50} value of ethanolic, aqueous extracts and essential oil from the leaves, bark and root of *Serai Kayu* and *Serai Kayu Hutan* inhibition of radical scavenging activity (DPPH) at concentration 100 μg/mL

Sample	Percentage of inhibition	IC_{50} Value (μg/mL)
Serai Kayu Leaves E	85.16 ± 1.16b, c, d, e	10.89h
Serai Kayu H. Leaves E	89.65 ± 0.36a	2.34l
Serai Kayu Bark E	56.00 ± 0.55h	63.49d
Serai Kayu H. Bark E	86.10 ± 0.18b, c, d, e	64.90c
Serai Kayu Root E	85.88 ± 0.24b, c, d, e	5.02i
Serai Kayu H. Root E	88.18 ± 0.90a,b	14.36g
Serai Kayu Leaves A	83.19 ± 0.14e, f	50.62e
Serai Kayu H. Leaves A	86.23 ± 0.04a, b, c, d, e	10.37h
Serai Kayu Bark A	74.92 ± 1.86 g	73.52a
Serai Kayu Hutan Bark A	83.59 ± 6.28d, e	67.17b
Serai Kayu Root A	84.38 ± 1.95c, d, e	23.56f
Serai Kayu Hutan Root A	88.32 ± 0.58a, b	2.96k
Essential oil K	80.28 ± 0.81f	2.71k
Essential oil H	86.93 ± 0.10a, b, c, d	3.43j
Quercetin	87.34 ± 0.97a, b, c	5.24i

Note: Values are the Means ± Standard deviation of three replicates of three different experiment. Values with same alphabet have no significant difference at $p \leq 0.05$. E=Ethanolic, A=Aqueous, H=Hutan, K=Kayu

α-glucosidase Inhibition

The α-glucoside inhibitory activity of the essential oil, ethanolic and aqueous extracts of the above plant parts of *Serai Kayu* and *Serai Kayu Hutan* was investigated using 100 μg/mL sample. The percentage inhibition and the inhibition concentration at 50 (IC_{50}) were calculated (Table 4). The IC_{50} values were not detected in ethanolic bark extract, aqueous bark extract of *Serai Kayu*, ethanolic root extract of *Serai Kayu Hutan* and essential oil of *Serai Kayu* and *Serai Kayu Hutan*, respectively (Table 4). Table 4 revealed the ethanolic leaves extracts of *Serai Kayu Hutan* with the highest IC_{50} Value 19.66 μg/mL exhibited the best α-glucoside inhibition as compared to all other part and control (quercetin).

Antimicrobial Screening

Five strains each of Gram positive and Gram negative bacteria was tested using disk diffusion method from the ethanolic and aqueous extract of leaves, bark and root of *Serai Kayu* and *Serai Kayu Hutan* of three different concentrations of 1000, 2000 and 4000 μg/mL, respectively. All the tested part has shown a significant zone of inhibition except for the strain of Listeria monocytogenes where no zone of inhibition was observed at both concentration of the ethanolic and aqueos extract of *Serai Kayu* and *Serai Kayu Hutan*, respectively. (Table 5 and 6).

Similarly agar well method was used against the five strains of gram positive and gram negative bacteria using the same concentrations of 1000, 2000 and 4000 μg/mL, respectively to further evaluate the antimicrobial activity of ethanolic and aqueous extracts of body parts of *Serai Kay*u and *Serai Kayu Hutan* to further confirmed the results of disk diffusion method.

Table 3: Fe^{2+} formed from the ethanolic, aqueous extract and essential oil of the leaves, bark and root of *Serai Kayu* and *Serai Kayu Hutan*

Sample	Fe^{2+}/(mmol/g)
Serai Kayu Leaves E	27.76 ± 0.1b
Serai Hutan Leaves E	37.32 ± 0.2a
Serai Kayu Bark E	3.64 ± 0.2f
Serai Hutan Bark E	37.85 ± 0.9a
Serai Kayu Root E	26.11 ± 0.8b
Serai Hutan Root E	35.58 ± 0.6a
Serai Kayu Leaves A	11.43 ± 9.9de
Serai Hutan Leaves A	36.94 ± 2.3a
Serai Kayu Bark A	4.48 ± 0.2f
Serai Hutan Bark A	35.46 ± 0.6a
Serai Kayu Root A	18.38 ± 0.3c
Serai Hutan Root A	28.08 ± 1.5b
Essential oil K	11.99 ± 0.4d
Essential oil H	7.19 ± 0.9ef
Quercetin	27.03 ± 0.1b

Note: Values are the Means ± Standard deviation of three replicate of different three experiment.Values with same alphabet have no significant difference at $p \leq 0.05$. E=Ethanolic, A=Aqueous, H=Hutan, K=Kayu

Table 4: Percentage of inhibition and IC_{50} value of ethanolic and aqueous extracts from the leaves, bark and root of *Sera Kayu* and *Serai Kayu Hutan* against α-glucosidase at concentration 100 μg/mL

Sample	Percentage of inhibition	IC_{50} Value (μg/mL)
Serai Kayu leaves E	75.87 ± 0.5c	41.32f
Serai Kayu Hutan leaves E	79.85 ± 1.8a	19.66i
Serai Kayu bark E	27.35 ± 0.1j	ND
Serai Kayu Hutan bark E	75.46 ± 0.6c	49.14e
Serai Kayu root E	51.37 ± 0.5g	94.80b
Serai Kayu Hutan root E	43.60 ± 0.3h	ND
Serai Kayu leaves A	73.18 ± 0.5d	74.24b
Serai Kayu Hutan leaves A	77.88 ± 2.0b	39.86g
Serai Kayu bark A	38.55 ± 1.6i	ND
Serai Kayu Hutan bark A	51.50 ± 0.7g	98.08a
Serai Kayu Root A	51.75 ± 0.1g	97.95a
Serai Kayu Hutan root A	62.44 ± 2.0e	57.40d
Essential oil K	27.89 ± 0.1j	ND
Essential oil H	26.34 ±0.1j	ND
Quercetin	58.99 ± 0.4f	28.12 h

Note: Values are the Means ± Standard deviation of three replicate of three different experiment
Values with same alphabet have no significant difference at $p \leq 0.05$. E=Ethanolic, A=Aqueous, H=Hutan, K=Kayu and ND= Not Detected

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Table 5: Antimicrobial evaluation of ethanolic and aqueous extracts of leaves, bark and root of *Serai Kayu* and *Serai Kayu Hutan* using disk diffusion against Gram positive bacteria

SPP	P	E	Conc µg/mL	ZI(mm)				
				S.A	E.F	L.M	B.C	S.E
S. H	L	E	1000	10.3 ± 0.5h	10.2 ± 0.1i	-	9.8 ± 0.2i	11.6 ± 0.4e
	L	E	2000	15.4 ± 0.4d	13.4 ± 0.2g	-	11.5 ± 0.6h	14.1 ± 0.8c
	L	E	4000	17.8 ± 0.2b	17.1 ± 0.5c	-	14.4 ± 1.0f	21.9 ± 0.3a
	L	A	1000	-	-	-	-	-
	L	A	2000	-	-	-	-	-
	L	A	4000	4.0 ± 1.0m	-	-	-	-
	B	E	1000	7.5± 0.3k	13.5 ± 0.5g	-	13.0 ± 0.0g	-
	B	E	2000	8.7 ± 0.6j	16.7 ±0.3d	-	17.7 ± 0.2d	-
	B	E	4000	12.5 ± 0.5f	18.3 ± 0.3b	-	22.6 ± 0.5b	-
	B	A	1000	-	-	-	-	-
	B	A	2000	-	-	-	-	-
	B	A	4000	-	-	-	-	-
	R	E	1000	9.8 ± 0.3h, i	10.6 ± 0.5i	-	12.6 ± 0.5g	10.2 ± 0.4f
	R	E	2000	10.2 ± 0.4h	15.1 ± 0.1f	-	15.3 ± 0.3e	13.5 ± 0.4d
	R	E	400	14.6 ± 0.5e	17.6 ± 0.1c	-	21.3 ± 1.1c	17.7 ± 0.5b
	R	A	1000	-	-	-	-	
	R	A	2000	-	-	-	-	
	R	A	4000	-	-	-	-	4.7 ± 0.1i
S. K	L	E	1000	12.7 ± 0.3f	15.8 ± 0.8e	-	-	-
	L	E	2000	16.6 ± 0.5c	17.0 ± 1.0d	-	-	3.9 ± 0.3j
	L	E	4000	19.0 ± 1.0a	21.7 ± 0.5a	-	-	7.8 ± 0.3g
	L	A	1000	-	-	-	-	-
	L	A	2000	-	-	-	-	-
	L	A	4000	-	-	-	-	-
	B	E	1000	7.6 ± 0.5k	13.2 ± 0.4g	-	-	7.9 ± 0.1g
	B	E	2000	9.2 ± 0.4j	13.6 ± 0.5g	-	-	10.7 ± 0.5f
	B	E	4000	11.4 ± 0.4g	18.3 ± 0.5b	-	-	13.3 ± 0.5d
	B	A	1000	-	-	-	-	-
	B	A	2000	-	-	-	-	-
	B	A	4000		-	-	-	-
	R	E	1000	6.2 ± 0.5l	12.6 ± 0.2h	-	11.2 ± 0.4h	6.0 ± 1.0h
	R	E	2000	8.8 ± 0.5j	15.5 ±0.3e,f	-	12.8 ± 5.6g	14.2 ± 0.4c
	R	E	4000	11.4 ± 0.4g	16.8 ± 0.1d	-	23.3 ± 0.5a	22.3 ± 0.5a
	R	A	1000	-	-	-	-	-
	R	A	2000	-	-	-	-	-
	R	A	4000	-	-	-	-	-
Std			10	18.0 ± 0.1b	15.3±0.5e,f	-	18.2 ± 0.2d	-

Note: S/N= Serial Number, Data are means of three replicates (n = 3) ± standard deviation of three different experiments. Means in the vertclal column with the same alphabet are no significantly different at $p \leq 0.05$.S.H: Serai Kayu Hutan, S.K: Serai Kayu, L: Leaves, B: Bark, R: Root, E: Ethanol, A: Aqueous, *S.A: Staphylococcus aureus, E.F: Enterococcus faecalis, L.M: Listeria monocytogenes, B.C: Bacillus, S.E: Staphylococcus epidermidis*, SPP: Species, P: Parts, E: Extracts and STD:Amphicilin

Table 6: Antimicrobial evaluation of ethanolic and aqueous extracts of leaves, bark and root of *Serai Kayu* and *Serai Kayu Hutan* using disk diffusion against Gram negative bacteria

SPP	P	E	Conc µg/mL	ZI(mm)				
				E.C	E.B	P.A	K.P	S.T
S.H	L	E	1000	11.2±0.2h,i	12.6 ±0.2h	16 ± 0.8e	9.1 ± 0.1h	9.3.8 ±0.1n
	L	E	2000	11.9 ± 0.6h	15.9±0.3c,d	22.3 ± 0.3b	12.1 ± 0.7f	11.7± 0.4m
	L	E	4000	15.6±0.2e,f	19.5 ± 0.3a	24.3 ± 0.3a	14.0 ± 0.3e	14.8±0.1g,h
	L	A	1000	-	-	-	-	-
	L	A	2000	-	-	-	-	-
	L	A	4000	10.2± 02k,l	5.7 ± 0.2k	10.5 ± 0.3i	-	-
	B	E	1000	12.8 ±0.1g	14.1 ±0.3f,g	12.1 ± 0.5h	12.1 ± 0.1f	13.6±0.2j,k
	B	E	2000	15.5±0.4e,f	16.3 ±0.5c,d	16.1 ± 0.1e	14.8±0.1c,d	15.5±0.2e,f
	B	E	4000	20.9 ± 0.3b	17.7 ± 0.4b	21.7 ± 0.6c	16.8 ± 0.1a	18.1 ± 0.1b
	B	A	1000	-	-	-	-	-
	B	A	2000	7.2 ± 01o	-	-	-	-
	B	A	4000	11.3±0.4i,j	-	-	4.8 ± 0.3i,j	-
	R	E	1000	9.6±0.2m,l	12.3 ± 0.5h	10.8±0.5i	12.5 ± 0.2f	12.1±2.0m
	R	E	2000	12 ± 0.8h	14.8 ± 0.1e	14.5±0.7f	13.7 ± 0.3e	14.3±0.0h,i
	R	E	4000	16 ± 0.3e	18.2 ± 0.1b	17.7±0.3d	15.0±0.1c,d	15.7±0.3e,f
	R	A	1000	-	-	-	-	-
	R	A	2000	-	-	-	-	-
	R	A	4000	-	-	9.2±0.3j	4.3 ± 0.1j	-
S.K	L	E	1000	9.3 ± 0.4m	12.0 ±0.1h,i	12.8±0.5g	10.2 ± 0.3g	12.8±02l
	L	E	2000	10.8±0.4k,j	14.5 ±0.1e,f	14.7±0.3f	12.7 ± 0.3f	14.0±0.1i,j
	L	E	4000	13.1 ± 0.3g	16.1 ±0.1c,d	22.3±0.3b,c	16.5 ± 0.4a	15.9±0.5d,e
	L	A	1000	-	-	-	-	-
	L	A	2000	-	-	-	-	-
	L	A	4000	-	-	-	-	8.2 ± 0.1o
	B	E	1000	8.5 ± 0.4n	11.0±0.0j	17.2 ± 0.4d	12.3 ± 0.3f	14.2 ± 0.2i
	B	E	2000	18.0 ± 0.3d	14.2±0.1e,f,g	22.6 ± 0.5b	15.4±1.2b,c	17.7±0.2b,c
	B	E	4000	19.1 ± 0.5c	16.5±0.4c	24.2 ± 0.6a	15.8±0.1b	21.6 ± 0.5a
	B	A	1000	-	-	-	-	-
	B	A	2000	-	-	-	-	-
	B	A	4000	7.2 ± 0.1o	-	-	5.1 ± 0.2i	6.9 ± 0.2p
	R	E	1000	14.9 ± 0.7f	11.5 ± 0.2i,j	9.3 ± 0.3j	13.5 ± 0.2e	13.4 ± 0.4k
	R	E	2000	15.4 ±02e,f	13.8 ± 0.1g	12.4 ± 0.2g	14.7 ± 0.5d	15.3±0.4f,d
	R	E	4000	20.9 ± 0.7b	15.8 ± 0.1d	16.3 ± 0.3e	16.7 ± 0.3a	17.4 ± 04c
	R	A	1000	-	-	-	-	-
	R	A	2000	-	-	-	-	-
	R	A	4000	8.5 ± 0.2n	-	-	-	-
Std			10	25.8 ± 0.8a	-	16.0 ± 0.1e	-	16.3 ± 0.3d

Note: Data are means of three replicates (n = 3) ± standard deviation of three different experiments. Means in the vertical column with the same alphabet are no significantly different at $p \leq 0.05$.S.H: Serai Hutan, S.K: Serai Kayu L: Leaves, B: Bark, R: Root, E: Ethanol, A: Aqueous, ZI: Zone of inhibition, mm: millimeter, *E.C: Escherichia coli, E.C: Escherichia coli, K.P: Klebsiella pneumoniae, S.T: Salmonella typhi*, SPP: Species, P: Parts, E: Extracts and STD:Amphicilin

All the tested part showed a significant zone of inhibition except for strain of *Listeria monocytogenes* (Table 7 and 8).

Discussion

High yield recorded from the ethanolic extracts might be as a results of the ability of the solvent to extracts more compounds from the samples (Table 1). In this context Ramli *et al*. (2017) reported high yield from the ethanolic leaves extract of Indonesian *S. polyanthum* (8.21%). The findings from the study disagrees with Poojary *et al*. (2015) where they report high yield of extract from the root and bark of aqueous extract 10.43% from the studies of extractions, phytochemical, characterization and biological studies of *Mammea suriga*. The study also disagrees with Javadi *et al*. (2014) where they report a high yield percentage from the aqueous extract as against the ethanolic extracts. The disparity in the yield extract of different medicinal plant parts is strongly dependent on the type of extraction, nature of extraction solvents, nature of compounds presents and the polarity of the metabolites (Sultana *et al*., 2009).

DPPH is one of the widely used methods adopted previously by many researchers in other to establish the antioxidant potential of a particular plant parts extract. The method is a stable with a potential of the DPPH free radical accept an electron from the tested antioxidant plant extract to become non radical. The method has the ability to decolorize to yellow from purple as a result of the electron acceptance from the antioxidant sample (Sultana *et al*., 2009; Tan and Lim, 2015). In the present study the ethanolic and aqueous extract of leaves, bark, root and essential oil of *Serai Kayu* and *Serai Kayu Hutan* were measured (Table 2). The activity of the extract established in the different parts of the plant was due to the present of some compound in

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Table 7: Antimicrobial evaluation of ethanolic and aqueous extracts of leaves, bark and root of *Serai Kayu* and *Serai Kayu Hutan* using agar well against Gram positive bacteria

Spp	P	E	Conc μg/mL	ZI(mm)				
				Microorganisms				
				S.A	E.F	L.M	B.C	S.E
S.H	L	E	1000	9.0 ± 1i	14.5 ± 0.2k	-	7.8±0.1h	10.4±0.6e
	L	E	2000	10.9±0.1f,g	16.5 ± 0.2h	-	10.7±0.6g	14.6±1.1b
	L	E	4000	15.6 ± 0.5d	18.1 ± 0.7d	-	13.1±0.1f	16.9±0.3a
	L	A	1000	-	-	-	-	-
	L	A	2000	-	-	-	-	-
	L	A	4000	4.2 ± 0.1l	-	-	-	-
	B	E	1000	7.0 ± 0.1j	13.8 ± 0.1l	-	17.2±0.5d	-
	B	E	2000	9.3 ± 0.5h,i	17.1±0.1f,g	-	23.3±1.1b	-
	B	E	4000	14.9 ± 0.1d	19.3±0.5c	-	28.3±0.5a	-
	B	A	1000	-	-	-	-	-
	B	A	2000	-	-	-	-	-
	B	A	4000	-	-	-	-	-
	R	E	1000	9.2 ± 0.4h,i	12.1±0.2n	-	15.4±0.8e	9.0 ± 0.5f
	R	E	2000	10.1±0.1g,h	16.2±0.1i	-	18.6±0.5c	12.1±0.2d
	R	E	4000	16.6 ± 0.4c	18.1±0.1d	-	23.6±1.1b	15.9±0.1a
	R	A	1000	-	-	-	-	
	R	A	2000	-	-	-	-	
	R	A	4000	-	-	-	-	4.0 ± 0.2h
S.K	L	E	1000	12.3 ± 0.5e	16.8±0.2g,h	-	-	-
	L	E	2000	17.6±0.5b	17.6±0.5e	-	-	4.1 ± 0.1h
	L	E	4000	22.9±0.1a	20.3±0.3a	-	-	8.2 ± 0.4f
	L	A	1000	-	-	-	-	-
	L	A	2000	-	-	-	-	-
	L	A	4000	-	-	-	-	-
	B	E	1000	7.3 ± 0.5j	13.6 ± 0.5l	-	-	7.0 ± 0.0g
	B	E	2000	10.1±0.2g,h	17.3 ± 0.5f	-	-	9.0 ± 0.1f
	B	E	4000	11.3 ± 0.4f	19.6 ± 0.5b	-	-	13.3±1.1c
	B	A	1000	-	-	-	-	-
	B	A	2000	-	-	-	-	-
	B	A	4000	-	-	-	-	-
	R	E	1000	6.0 ± 0.1k	12.6± 0.2m	-	15.5±0.5e	-
	R	E	2000	8.5 ± 0.4i	15.5 ± 0.3j	-	18.5±0.1c	-
	R	E	4000	10. ± 1.0g,h	16.8±0.1g,h	-	28.3±0.3a	-
	R	A	1000	-	-	-	-	-
	R	A	2000	-	-	-	-	-
	R	A	4000	-	-	-	-	-
Std			10	18.0 ± 0.1b	15.3 ± 0.5j	-	18.2±0.2c	-

Note: Data are means of three replicates (n = 3) ± standard deviation of three different experiments. Means in the vertical column with the same alphabet are no significantly different at $p \leq 0.05$. S.H: Serai Kayu Hutan, S.K: Serai Kayu, L: Leaves, B: Bark, R: Root, E: Ethanol, A: Aqueous, *S.A: Staphylococcus aureus, E.F: Enterococcus faecalis, L.M: Listeria monocytogenes, B.C: Bacillus, S.E: Staphylococcus epidermidis*, SPP: Species, P: Parts, E: Extracts and STD:Amphicilin

each of the extract such as the hydroxyl group, methoxyl group, phenolics compounds, flavonoids compound and many other structure that might be present (Dudonne *et al*., 2009; Sultana *et al*., 2009; Tan and Lim, 2015).

The assay of ferric reducing power antioxidant was carried out in order to determine the reducing power of antioxidant. The ethanolic and aqueous extracts determination of antioxidant based on their reducing ability did not vary greatly when compared with the scavenging activity of the tested plant parts (Table 2 and 3). The variation documented from DPPH and FRAP assay might be as a result of the manner the phenolics compounds act or respond toward the type of the assay. Dudonne *et al*. (2009) reported similar results in comparative study of antioxidant based on different *in vitro* methods.

Table 8: Antimicrobial evaluation of ethanolic and aqueous extracts of leaves, bark and root of *Serai Kayu* and *Serai Kayu Hutan* using agar well against Gram negative bacteria

Spp	P	E	Conc μg/mL	ZI(mm)				
				Microorganisms				
				E.C	E.B	P.A	K.L	S.T
S.H	L	E	1000	18.1±0.1h	13.2 ± 0.4i	12.4 ± 0.2j	8.7 ± 0.2k	8.4 ± 0.1l
	L	E	2000	20.1± 0.1f	15.4 ± 0.3f	15.8 ± 0.1g	9.4 ± 0.2j	11.3±0.3j
	L	E	4000	22.1± 1.3e	17.5 ± 0.4d	22.1 ± 0.1c	9.4 ± 0.8j	15.3±0.6e,f
	L	A	1000	-	-	-	-	-
	L	A	2000	-	-	-	-	-
	L	A	4000	14.2± 0.1j	5.1 ± 0.1m	9.1 ± 0.1m	-	-
	B	E	1000	9.8 ± 0.1n	10.6 ±0.5j,k	9.9 ± 0.1l	10.4±0.4h	12.2 ± 0.2i
	B	E	2000	21.1±0.3e	13.8±0.2ghi	12.6 ± 0.3j	14.5± 0.3e	14.6 ± 0.2g
	B	E	4000	27.9±0.3a	17.3 ± 0.5d	16.3 ± 0.3f	16.7± 0.1b	19.5 ± 0.4b
	B	A	1000	-	-	-	-	-
	B	A	2000	8.3 ± 0.1p	-	-	-	-
	B	A	4000	13.1±0.1k	-	-	4.1 ± 0.1m	-
	R	E	1000	7.8 ± 0.2q	14.0 ±0.2gh	11.1 ± 0.1k	14.8±0.1d,e	10.7 ± 0.5k
	R	E	2000	12.6± 0.6l	16.3 ± 0.4e	13.1 ± 0.2i	16.2 ± 0.1c	13.3 ± 0.0h
	R	E	4000	16.2± 0.1i	18.3 ± 0.5c	18.6 ± 0.3d	17.8 ± 0.1a	15.9±0.3d,e
	R	A	1000	-	-	-	-	-
	R	A	2000	-	-	-	-	-
	R	A	4000	-	-	9.2 ± 0.3m	4.3 ± 0.1m	-
S.K	L	E	1000	7.8 ± 0.1q	8.1 ± 0.1l	12.5 ± 0.4j	9.9 ± 0.1i	8.7 ± 02l
	L	E	2000	10.7±0.4m	13.5 ±0.2h,i	13.7 ± 0.6h	13.4 ± 0.3f	11.4 ± 0.3j
	L	E	4000	12.3 ± 0.2l	19.4 ± 0.7b	22.3±0.3b,c	16.5±0.4b,c	15.3±0.6e,f
	L	A	1000	-	-	-	-	-
	L	A	2000	-	-	-	-	-
	L	A	4000	-	-	-	-	7.1 ± 0.1m
	B	E	1000	10.0±0.3n	8 ± 0.3l	17.2 ± 0.4e	11.2±0.1g	14.4 ± 0.4g
	B	E	2000	18.8±0.1g	10.2± 0.1k	22.6 ± 0.5b	13.3 ± 0.2f	15.8±0.1d,e
	B	E	4000	22.7± 0.6c	20.7 ± 0.4a	24.2 ± 0.6a	15.0 ± 0.0d	19.5 ± 0.5b
	B	A	1000	-	-	-	-	-
	B	A	2000	-	-	-	-	-
	B	A	4000	9.2 ± 0.2o	-	-	5.1 ± 0.2l	6.0 ± 0.1n
	R	E	1000	8.6 ± 0.3p	11.1 ± 0.1j	7.5 ± 0.2n	13.7 ± 0.4f	15.0±0.0f,g
	R	E	2000	13.1± 0.5k	14.2 ± 0.0g	12.3 ± 1.1j	14.9±0.3d,e	17.1 ± 0.1c
	R	E	4000	19.9± 0.5f	15.7 ±0.6e,f	16.1±0.7f,g	16.6±0.3b,c	20.5 ± 0.2a
	R	A	1000	-	-	-	-	-
	R	A	2000	-	-	-	-	-
	R	A	4000	10.5±0.1m	-	-	-	-
Std			10	25.8± 0.8b	-	16.0±0f,g	-	16.3 ± 0.3d

Note: Data are means of three replicates (n = 3) ± standard deviation of three different experiments. Means in the vertical column with the same alphabet are no significantly different. S.H: Serai Hutan, S.K: Serai Kayu, L: Leaves, B: Bark, R: Root, E: Ethanol, A: Aqueous, ZI: Zone of inhibition, mm: millimeter, *E.C: Escherichia coli, E.C: Escherichia coli, K.P: Klebsiella pneumoniae, S.T: Salmonella typhi*, SPP: Species, P: Parts, E: Extracts and STD:Amphicilin

The high activity of the *Serai Kayu Hutan* ethanolic leaf extract might be as results of the presence of high secondary metabolites, that extraction with 95% solvent gives a high antioxidant activity as a result of the compounds that were able to extracts (Ali *et al*., 2015; Poojary *et al*., 2015).

Alpha glucosidase is the main enzyme responsible for catalyzing the digestive end process of carbohydrates. Highest activity of α-glucosidase Inhibition was reported from the leaf of *Serai Kayu Hutan* with the IC_{50} value 19.66 μg/mL (Table 4). The results are in agreement with Javadi *et al*. (2014) where they report a high activity on α-glucosidase of cosmus caudatus of ethanolic extracts. The studies have further confirmed the claimed of the traditional herbalist that both *Serai Kayu* and *Serai Kayu Hutan* are good source of anti diabetes. The high activity of the ethanolic leaves extract might be due the fact

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leaves are primary producers of secondary metabolites before they are transported to the other parts of the plants and the ability of the solvent to extract more polar compound as against the aqueous extract (Ali *et al*., 2015; Poojary *et al*., 2015).

Quite a large number of studies have reported the used of plants extract as antimicrobial agent (Jaberian *et al*., 2013; Ramli *et al*., 2017). The most widely practiced method to test the activity of the extracts against microbial organisms are disk and agar well diffusion methods (Ramli *et al*., 2017). National committee for clinical laboratory system (NCCLS) has accepted the above mentioned methods in foods and drugs test (Ramli *et al*., 2017). In some situation the extract might not show inhibition in one assay but exhibit in another assay as a result of the affinity of the extract toward the assay. Selection of ethanolic and aqueous extract in the following study was based on the reasons that ethanol was one of the permissible solvents in preparation of food for consumption (Ramli *et al*., 2017).

The present study explored two methods to validate and further confirm the activity of the plants parts of *Serai Kayu* and *Serai Kayu Hutan*, respectively. This is due to the fact that sometimes disk diffusion is inaccurate as a results of the inability of the extract to penetrate the disk (Ramli *et al*., 2017). All the extract exhibited a certain degree of activities against the tested bacterial strains. Although increased in the activity was seen with increased in the concentrations of both the ethanolic and aqueous extract, ethanolic extracts exhibit highest activity as compared to the aqueous extracts (Table 5, 6 7 and 8). In this regard Jaberian *et al*. (2013) reported a low activity from aqueous extracts on the tested microorganisms. In this present study all the tested parts manifested activity against the tested microorganisms except on the *Listeria monocytogenes* (Table 5, 6 7 and 8). Ramli *et al*. (2017) reported maximum activity of ethanolic extract of *S. polyanthum* (*Serai Kayu*) leaves collected from Indonesia at 10,000 µg/mL against *L. monocytogenes*. In the present study a higher activity was reported against Gram negative bacterial strains (Table 7 and 8). Gowri and Vasantha (2010) reported greater activity of methanol and aqueous extract of *S. cumin* against seven tested pathogenic microorganisms where the reported increased activity against Gram negative bacteria. The study also concurred with findings of Massaro *et al*. (2014) from the antimicrobial studies against some selected strain of bacteria from *Corymbia torelliana*. The action of the antimicrobial agent might be as a result of the phytochemical compounds that were be extracted from the plant parts with a greater ability to damage or disrupt the cell membrane permeability (Tan and Lim, 2015; Poojary *et al*., 2015; Mostafa *et al*., 2018). The results further confirmed that *S. polyanthum* cultivars can be further enhanced and used as herbal supplements in order to meet the demand of populace and to avoid over sampling of the above said plants species.

Conclusion

The results revealed that *Serai Kayu* and *Serai Kayu Hutan* are good potential of antioxidants caused by free radicals, reducing power antimicrobial against the tested bacterial strain excepts for the *L. monocytogen*. Moreover, *S. polyanthum* cultivars *Serai Kayu* and *Serai Kayu Hutan* are traditional healers and anti α-glucosidase inhibition. The study suggest the possible exploration of the *Serai Kayu Hutan* leaves for developing herbal products and antibiotics against microorganisms. Further studies on in vivo should be carried out on the above plant parts.

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