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Antioxidant and antimicrobial activities of *Shorea kunstleri*

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PEER REVIEW

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Comments

This is a commendable research work in which author has successfully demonstrated the *in vitro* antioxidant and antimicrobial characteristics of the stem bark of the *S. kunstleri*. The antioxidant activity was assessed based on DPPH and ferric thiocyanate assays and antimicrobial activity was determined by following disc diffusion method and MIC was determined by using 96-well microtiter plates. MIC, MBC and MFC values were successfully determined with respect to know the true antimicrobial nature of this plant.
Details on Page 643

ABSTRACT

Objective: To evaluate antioxidant and antimicrobial activities of stembark of *Shorea kunstleri* (*S. kunstleri*) together with analysis of phytochemical and total phenolic contents.
Methods: Extraction was conducted with different solvent polarity of *n*-hexane, dichloromethane (DCM) and methanol by using Soxhlet extraction. Total phenolic content was determined using Folin-Ciocalteu method. Free radical scavenging activity and inhibition of lipid peroxidation were evaluated with DPPH radical scavenging and ferric thiocyanate assays, respectively. Antimicrobial activities were performed using disc diffusion method, minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration.
Results: *S. kunstleri* stembark extracts revealed presence of steroids, terpenoids, saponins, flavonoids, and phenolic compounds. Methanol extract exhibited the highest total phenolic content and free radical scavenging activity resulting in phenolic content of (8.340±0.003) g GAE/100 g of extract and (95.90±1.07)% DPPH inhibition (IC_{50} value of 18.6 μg/mL), respectively. Ferric thiocyanate assay of *n*-hexane, DCM, and methanol extracts indicated lipid peroxidation inhibitory activity of (74.20±0.35)%, (74.00±0.10)%, and (72.80±0.27)%, respectively. In antimicrobial and antifungal tests, methanol extract showed inhibition against *Staphylococcus aureus* (*S. aureus*), *Candida albicans*, and *Candida tropicalis* with inhibition zones of 10-12, 18-22, and 18-19 mm, respectively. The MIC test of methanol extract showed highest inhibition against *Candida albicans* and *S. aureus* (0.04 and 0.08 mg/mL, respectively) while DCM extract exhibited the highest activity towards *Candida tropicalis* (MIC value of 0.63 mg/mL). Taken together, MBC test of methanol extract strongly demonstrated bactericidal effect against *S. aureus* with MBC value of 0.08 mg/mL.
Conclusions: The study demonstrated that stembark extracts of *S. kunstleri* possessed antioxidant and antimicrobial properties.

KEYWORDS
Shorea kunstleri, Phytochemicals, Antioxidant, Antimicrobial

1. Introduction

It has been widely acclaimed that higher plant species possessed antioxidant capacity. It was reported that 250 000 flowering plant species are present worldwide[1]. Of them, 35 000 can be found in Southeast Asia[2] with over 6 000 species to exhibit medicinal value. Moreover, in the Indo-Malayan region, it was estimated that

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6 000 plant species have been utilized in traditional medical systems[3] attributed to their active components. Currently, there is a rising interest towards emerging oxidant species in biological systems and its functions in development in chronic disorders. Elevated reactive oxygen species such as mitochondrial superoxide in endothelial cell[4] and endoplasmic reticulum stress[5] followed by reduced antioxidant defense mechanism provoked cellular and enzymes damage as well as lipid peroxidation[6]. Accordingly, current emphasis has directed on biochemically protective features of antioxidant in cells and organisms containing them. Antioxidants may provide defense mechanism by suppressing lipid peroxidation and hydroperoxide formation, inhibiting Fenton and Haber-Weiss-type reactions and scavenging free radicals[7]. Polyphenols functions to defend against ultraviolet radiation, pathogens[8] or as antioxidants, via neutralization of reactive oxygen/nitrogen species, which generated from metabolic processes that lead to protection against cancers, cardiovascular diseases, neurodegenerative diseases, osteoporosis, diabetes[9], asthma and hypertension[10]. *Shorea kunstleri* King. (family Dipterocarpaceae) (*S. kunstleri*) known as red balau[11] or *balau laut merah* are widely distributed in Malaysia and Indonesia[12], which inhabited leached sandy clay soils. To date, the information concerning *in vitro* antioxidant potential of the plant seemed limited, thus the present study had focused on the evaluation of antimicrobial and antioxidant properties of various extracts from stembark of *S. kunstleri*.

2. Materials and methods

2.1. Plant material

Stembarks of *S. kunstleri* were collected in June 2012 from Bukit Pelindung, Kuantan, Pahang, Malaysia. Taxonomic identification was made by Dr. Norazian Mohd Hassan (Faculty of Pharmacy, IIUM) against voucher specimen number of PIIUM-0201, and deposited in the Herbarium, Kulliyyah of Pharmacy, IIUM, Malaysia.

2.2. Extraction

Stembarks were chopped and into small pieces and dried at 40 °C for 48 h using laboratory drier (Memmert, Germany) and then it was pulverized using a grinder. A total of 1.05 kg of powdered stembark was extracted using Soxhlet method for 8-12 h with *n*-hexane, dichloromethane (DCM) and methanol, successively. The extract was then collected and filtered through Whatman No. 1 filter paper and concentrated to dryness at 45 °C under reduced pressure using rotary evaporator (IKA, Germany).

2.3. Test microorganisms

The microorganisms used in this study were *Staphylococcus aureus* (ATCC 14778) (*S. aureus*), *Bacillus cereus* (ATCC 25923), *Escherichia coli* (ATCC 25922) (*E. coli*), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (IMR C 523/11 A) (*C. albicans*), and *Candida tropicalis* (IMR C 480/08 A) (*C. tropicalis*).

2.4. Phytochemical screening

Phytochemical screening was performed to investigate phytochemical contents of the extracts. Five tests were carried out[13] to investigate the presence of alkaloid test by thin layer chromatography, terpenoid and steroid tests by Liebermann-Burchard test followed by flavonoid, saponin and phenolic detection.

2.5. Determination of total phenolic content (TPC)

Estimation of total phenolic in the extract of *S. kunstleri* stembarks was performed according to Folin-Ciocalteu method. About 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of sodium carbonate (Na_2CO_3) (2% w/v) was added to 0.5 mL extracts (1 mg/mL). The tubes were shaken thoroughly and the mixture was then incubated in the dark for 90 min at room temperature. The resulting dark blue colour indicated presence of phenolic compounds. Methanol was used as blank. The absorbance was read at 725 nm using a UV-vis spectrophotometer (Infinite M200 NanoQuant). The analyses were done in triplicate. Gallic acid was used as standard to obtain a calibration curve (0.020, 0.040, 0.060, 0.080, 0.100 mg/mL) and total phenolic content of fractions were expressed as gallic acid equivalents in milligram per gram (g GAE/100 g) of dried extract[14]. The total phenolic content was calculated using formula as followed:

$C=cv/m$

Where, C=total phenolic content (g GAE/100 g extract), c=the concentration of gallic acid (mg/mL) established from the calibration curve; v=volume of sample (mL); m=mass of sample (g).

2.6. DPPH radical scavenging activity assay

A total of 10 mg of each extract (*n*-hexane, DCM, methanol) were dissolved in 1 mL of methanol to obtain a stock solution of 10.0 mg/mL. Each sample was prepared for several dilutions. The free radical scavenging activity of *S. kunstleri* extracts was determined based on the ability to scavenge DPPH free radical[15]. A test sample solution (200 μL) was added to 3.8 mL of 50 μmol/L DPPH methanolic solution (to give a final concentration of 500, 250, 125, 62.5, 31.3, and 15.7 μg/mL). The mixture was then vortexed, and incubated for 30 min at room temperature. The scavenging activity was evidenced by purple discoloration to yellow which further measured at 517 nm. Vitamin C and E were used as positive controls while methanol served as blank. Analyses were done in triplicate. The difference in absorbance between a test sample and a control (methanol) was expressed as percentage inhibition. The ability of sample to scavenge DPPH radical was calculated as below[15]:

$$\text{Percent inhibition (I \%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Where, Absorbance of control=Absorbance of DPPH + methanol. Antioxidant activity was expressed as IC_{50} (inhibitory concentration in μg/mL of samples, or standard, necessary to reduce the initial DPPH by 50% as compared to the negative control).

2.7. Ferric thiocyanate (FTC) assay

The antioxidant activity of *S. kunstleri* extract was evaluated according to the FTC method with slight modification. A total of 4 mg of extracts were dissolved in 4 mL ethanol, 4.1 mL of 2.5% linoleic acid, 8 mL of 0.02 mol/L phosphate buffer (pH 7.0), and 3.9 mL distilled water. The mixtures were wrapped with aluminium foil and placed in lab oven at 40 °C. Then, 0.1 mL of the mixture was added to 9.7 mL of 75% ethanol followed by addition of 0.1 mL of 30% ammonium thiocyanate. After 3 min, 0.1 mL of 0.02 mol/L ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. The absorbance was measured at 500 nm at 24-hour interval until the absorbance of the control reached maximum value. The inhibition of lipid peroxidation in percentage was calculated using the following equation[16]:

$$\text{Percent inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

Where, A_0=Absorbance of the control reaction, A_1=Absorbance in the presence of extracts or standard compound.

2.8. Disc diffusion method

S. kunstleri extracts of 0.10 g (*n*-hexane, DCM, methanol) were weighed and dissolved in 1.0 mL DMSO. A volume of 100 μL suspension containing the microbe was spread on agar plates. After that, 10 μL of each sample solution was applied to the blank discs of 6 mm diameter and placed onto agar plates. All tests were made in triplicate. Gentamicin and fluconazole were used as positive control for bacteria and fungi, respectively. DMSO served as negative control. The agar plates were incubated for 18 h (bacteria) and 72 h (fungi) at 37 °C. The antimicrobial activity was evaluated by presence of clear inhibition zones around discs[17]. Significant results were further used for determination of minimum inhibitory concentration (MIC) using microdilution method, minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC).

2.9. MIC

MIC was determined using broth microdilution method. The test was performed in sterile 96-well microplates. A volume of 200 μL inoculate containing 10^8 CFU/mL of bacteria and 10^6 spores/mL of fungi were added to column A followed by 10 μL sample solution. Then, 100 μL of sterile Mueller-Hinton (bacteria) and Sabouraud dextrose broths (fungi) were placed in each well, started from column B to H. An eight times serial dilution was prepared from the initial concentration with continuous mixing. Each mixture was resuspended three times and 100 μL was orderly transferred to the next well. Agar plates were incubated at 37 °C for designated time (18 and 72 h for bacteria and fungi, respectively). The MIC is defined as the lowest concentration of a given sample able to inhibit any microbial growth, which assessed via turbidity and pellet formation at bottom of the well[18].

2.10. MBC and MFC

MBC/MFC are defined as the lowest concentration of a given sample to kill a microorganism. Samples from the MIC test which did not show any bacterial or fungal growth after incubation period were diluted in respective broth at 1:4 ratio, which then subcultured onto Mueller Hinton and Sabouraud dextrose agar plates. All plates were incubated at 37 °C for 18 h (bacteria) and 72 h (fungi). The MBC and MFC were determined as the lowest concentration of extract which retard any visible bacterial or fungal colony growth on agar plate after incubation period[18].

2.11. Statistical analysis

All data are representative of at least three independent experiments, unless otherwise stated in respective tests. Values are expressed as means±SD. Statistical analyses were conducted using Student's *t*-test. A value of $P<0.05$ was considered to indicate statistical significance. The statistical package IBM SPSS statistics version 19 for

Windows was used in the analysis.

3. Results

3.1. Phytochemical screening

The phytochemical analysis of *S. kunstleri* extracts revealed presence of terpenoids, steroids, flavonoids, saponin and phenols (Table 1). These phytochemicals are known to exert biological activities in medicinal plants and may be responsible for the antioxidant activities of the extract used in the study. There was no alkaloid in these extracts.

Table 1

Phytochemicals in the extracts of *S. kunstleri* stembark.

Extract	Test/s				
	Alkaloid	Terpenoid and steroid	Flavonoid	Saponin	Phenols
n–Hexane	–	+	–	–	+
DCM	–	+	–	–	+
Methanol	–	+	+	+	+

+: presence; –: absence.

3.2. TPC

Total phenolic was estimated from the calibration curve of gallic acid equivalent, Y=6.3441X, R^2=0.998 0. From the result, phenolic content was detected in all extracts, but at specified amount. Methanol extract demonstrated highest level of phenolic compounds, 8.34 g GAE/100 g extract, followed by DCM, 1.71 g GAE/100 g extract and *n*–hexane, 1.25 g GAE/100 g extract. Polyphenolics constituent in *S. kunstleri* served as antioxidants as well as free radical scavenger.

3.3. DPPH radical scavenging activity

DPPH assay was performed to detect antioxidant activity of the extract. Most antioxidant may possess proton–radical scavenging action and the mechanism could be identified via discoloration of purple DPPH radicals into yellow DPPH in a dose–dependent pattern. It was observed that methanolic extract exhibited the highest scavenging activity (IC_{50} value of 18.6 μg/mL) compared to vitamin C and E (IC_{50} values of 21.8 and 48.2 μg/mL, respectively) (Table 2). Extracts of *n*–hexane and DCM showed high inhibition percentage at lowest initial concentration, 15.7 μg/mL. Thus, highest scavenging activity was observed in methanolic extract followed by *n*–hexane and DCM.

3.4. FTC method

At Day 6, it was found that the absorbance of the control solution reached maximum level, indicated formation of lipid peroxides. Low absorbance indicated high inhibition level of lipid peroxidation, attributed to high antioxidant activity. Figure 1 showed reducing capability of the extracts via elevated conversion of Fe^{3+} to Fe^{2+} in concentration–dependent manner. Mean absorbance at Day 6 was used to calculate percent inhibition of lipid peroxidation as tabulated in Table 3. The percentages inhibitions of linoleic acid peroxidation of all samples ranged from 72.79% to 74.21%. It was found that *n*–hexane, DCM, and methanol extracts of *S. kunstleri* showed almost identical potential against lipid peroxide inhibition activity [(74.21±0.35)%, (74.02±0.10)%, and (72.79±0.27)%, respectively)]. Thus, there were no statistically significant difference in lipid peroxide inhibition values of the three sample of *S. kunstleri* extract (P>0.05).

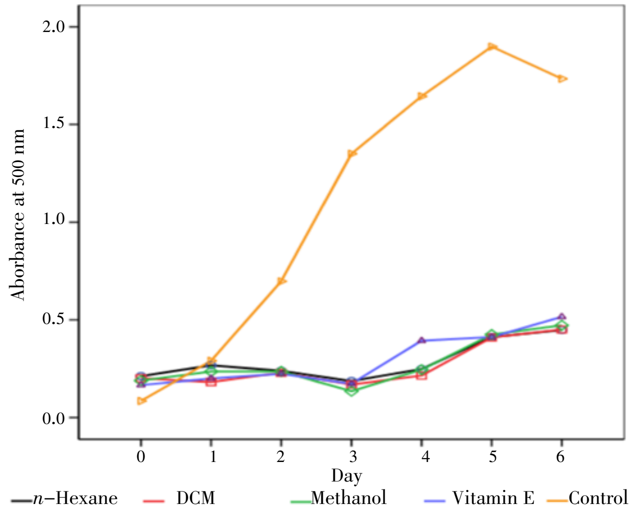

Figure 1. Antioxidant activity of *S. kunstleri* extracts by FTC method.

Table 2

Percentage inhibition values of *S. kunstleri*, vitamin C and E by DPPH radical scavenging activity assay.

Concentration (μg/mL)	Percentage inhibition (%)						IC_{50} (μg/mL)
	500	250	125	62.5	31.3	15.7	
n–Hexane	45.05±2.35	42.04±5.03	46.01±5.34	45.30±2.26	42.12±0.35	39.55±2.62	NA
DCM	24.85±0.71	27.75±0.38	31.91±2.02	37.13±5.67	53.09±5.66	58.72±5.66	36.25±4.60
Methanol	95.25±0.95	95.93±1.07	93.73±1.49	71.40±0.45	90.91±4.71	71.29±4.43	18.60±0.00
Vitamin C	93.76±2.81	94.00±3.90	86.89±3.90	78.92±2.03	62.02±5.60	48.89±0.62	21.75±2.47
Vitamin E	98.20±1.40	97.88±1.27	82.27±1.27	56.97±0.25	39.41±0.93	31.75±0.86	48.17±1.04

Data are presented as mean±SD of three independent experiments, performed in triplicate. Control (methanol)=0.5735±0.0100. NA: not applicable.

Table 3

Percentage inhibition of extracts of *S. kunstleri* on lipid peroxidation.

Sample	Absorbance at 500 nm (Day 6)	Percentage inhibition (%)
n–Hexane extract	0.4479±0.0060	74.21±0.35
DCM extract	0.4506±0.0018	74.02±0.10
Methanol extract	0.4720±0.0047	72.79±0.27
Vitamin E	0.5158±0.0092	69.93±0.99
Control (ethanol)	1.7346±0.0411	–

3.5. Disc diffusion method

Among all strains tested, *S. aureus*, *C. albicans* and *C. tropicalis* were inhibited by methanol extract of *S. kunstleri*. It was shown that methanol extract of *S. kunstleri* possessed antibacterial and antifungal properties, evidenced by inhibition zones of 10–12 and 18–22 mm, respectively (Table 4). However, no activity was observed against Gram–negative bacteria of all extracts.

Table 4

Diameter of inhibition zone via disc diffusion test in mm for *S. kunstleri* extracts.

Microorganisms	Sample			Drugs	
	n–Hexane	DCM	Methanol	Gentamicin	Fluconazole
S. aureus	7.0±0.0	7.0±0.0	10.7±1.2	22.0±0.0	NA
Bacillus cereus	–	–	–	22.0±0.0	NA
Pseudomonas aeruginosa	–	–	–	21.0±0.0	NA
E. coli	–	–	–	25.0±0.0	NA
C. albicans	–	–	20.0±2.0	NA	35.0±0.0
C. tropicalis	7.7±0.6	7.0±0.0	18.7±0.6	NA	25.0±0.0

Mean diameter of inhibition zone (mm) includes disc diameter of 6 mm and calculated from triplicate tests for each microorganism used. Values for zone of inhibition are presented as mean±SD. NA: not applicable.

3.6. MIC

The results showed different MIC values of each extract which were determined by bacterial and fungal growth at designated concentrations (Table 5).

Table 5

MIC values of *n*–hexane, DCM, and methanol extract of *S. kunstleri* stembark.

Extract	Microorganism	Concentration of serial dilution (mg/mL)							
		5.00	2.50	1.25	0.63	0.32	0.16	0.08	0.04
n–Hexane	*S. aureus*	+	–	–	–	–	+	+	+
	C. tropicalis	–	–	–	–	+	+	+	+
DCM	*S. aureus*	+	–	–	–	–	+	+	+
	C. tropicalis	–	–	–	–	+	+	+	+
Methanol	*S. aureus*	+	+	+	–	–	–	–	+
	C. tropicalis	–	–	–	+	+	+	+	+
	C. albicans	+	+	+	+	–	–	–	–

+ indicated growth observed; – indicated no growth.

It was shown that methanol extract of *S. kunstleri* exhibited highest activity against *C. albicans* and *S. aureus*, with MIC value of 0.04 and 0.08 mg/mL, respectively. DCM and *n*–hexane extracts of *S. kunstleri* stembark demonstrated moderate activity, with MIC value of 0.32 mg/mL. However, among these extracts, DCM displayed highest activity against *C. tropicalis* (0.63 mg/mL) whereas *n*–hexane and methanol extracts moderately inhibited *C. tropicalis* growth, with MIC values of 0.63 and 1.25 mg/mL, respectively.

3.7. MBC and MFC

MBC values signified bactericidal or bacteriostatic ability of the *S. kunstleri* extracts. The methanol extract demonstrated bactericidal effect against *S. aureus*, with MBC value of 0.08 mg/mL. Taken together, both *n*–hexane and methanol extracts revealed fungistatic activity against *C. tropicalis* at MFC value of 1.25 mg/mL. However, DCM extract marked the lowest fungal inhibition activity against *C. tropicalis* with MFC value of 5.00 mg/mL (Table 6).

Table 6

MBC and MFC of *n*–hexane, DCM, and methanol extract of *S. kunstleri* stembark.

Sample	Microorganisms	Well	Concentration of serial dilution (mg/mL)	Observation (colony count)
n–Hexane extract	*C. tropicalis*	A	5.00	33.00±39.25
		B	2.50	5.00±2.83
		C	1.25	>3
DCM extract	*C. tropicalis*	A	5.00	>37
Methanol extract	*S. aureus*	D, E, G	0.63, 0.32, 0.08	Clear
		F	0.16	55.00±67.17
	C. tropicalis	A	5.00	5.00±2.52
		B	2.50	39.00± 2.33
		C	1.25	6.00±0.00
Gentamicin (positive control)	*S. aureus*	A, B, C	5.00, 2.50, 1.25	Clear
Fluconazole (positive control)	*C. tropicalis*	H	0.04	>64
	C. albicans	–	–	–
DMSO (control)	*S. aureus*	B, D, F	2.50, 0.63, 0.16	–

4. Discussion

It was found that *n*–hexane and DCM extracts contained terpenoids, steroids and phenols. While, flavonoids were detected in methanol extract. Polyphenols are well known as major compounds with antioxidant activity. High phenolic content in plant may exert strong scavenging ability of free radical and other reactive oxygen species due to hydroxyl groups (aromatic ring), singlet and triplet oxygen quenchers and inhibition of peroxidation[19]. Thus, potential health benefits of phenolic substances attributed to the compounds action as reducing agent or antioxidants prior to their capability to donate single electron or hydrogen atom for reduction. Phenolic compounds are a large and diverse group of molecules that include many different families of aromatic secondary metabolites in plants. These compounds are the most abundant secondary metabolites in plants and can be classified into non–soluble compounds such as condensed tannins, lignins, and cell wall–bound hydroxycinammic acids, and soluble compounds such as phenolic acids, phenylpropanoids, flavonoids, and quinines[20].

DPPH radical scavenging assay was performed to identify antioxidant properties of the extracts. It was shown that *S. kunstleri* exhibited a potent proton–donating ability to DPPH. Phenolic compounds in methanol extract were able to transfer labile hydrogen atoms to DPPH. Bleaching of purple–coloured DPPH was observed with increasing sample concentration. Formation of dark purple colour slowly bleached into stable compound (yellow) by reacting with an antioxidant agent. Capability of extract to scavenge DPPH was measured in percentage inhibition (%). Higher percentage inhibition of DPPH absorbance shows higher antioxidant effect compared to the lower IC_{50}[21].

FTC assay measures the amount of lipid peroxide formed in 24–hour interval for six days via assessment. High inhibition of lipid peroxidation resulted in increased antioxidant activity. Radical chain reactions could be terminated when substances exhibiting high reducing tendencies donated electrons that may react with free radicals, thus converted them to a more stable products in the process. Since most of secondary metabolites in plants and fruits contained phenolic compounds which may potentiate radical scavenging and inhibit lipid hydroperoxide, phenolic antioxidants, via release of lipid alkoxyl radical may initiate free radical chain oxidation, as a result of O–O bond cleavage[22]. This activity however depends on the molecular structure, number and position of the hydroxyl group. Antioxidants are able to alter peroxidation kinetics by modifying the lipid packing order. They stabilize membranes by decreasing membrane fluidity (in a concentration–dependent manner) and hinder the diffusion of free radicals and restrict peroxidative reaction[23].

The MIC and MBC values revealed almost identical bactericidal action were against selected microbes[24]. Bactericidal agents may trigger cellular pathways leading to cell death apart from growth inhibition. The actions of bactericidal drugs are irreversible and susceptible microorganisms will die upon exposure[25]. On the contrary, bacteriostatic agents functions to retard bacterial growth and multiplication. The result confirmed that among the three microbial strains tested, *S. aureus* was the most sensitive to methanol extracts comparable to others. This action most probably laid in the fact that absence of outer membrane and periplasmic space in *S. aureus* as seen in Gram–negative bacteria like *E. coli*[26].

In conclusion, methanol extract of *S. kunstleri* stembarks demonstrated highest total phenolic content and scavenging activity as compared to other extracts and vitamin C. In FTC assay, *n*–hexane, DCM and methanol extracts of *S. kunstleri* stembarks indicated no comparable values of lipid peroxide inhibition activity. However, methanol extracts were capable to potentiate growth inhibition against *S. aureus*, *C. albicans* and *C. tropicalis*, attributed to its antimicrobial properties. Hence, extensive evaluation of potential antioxidant and antimicrobial properties of Malaysian *Shorea* species specifically *S. kunstleri* should be embarked using several other models to ascertain effectiveness of antioxidant species. Comparatively, it was shown that methanol extract possessed highest antioxidant capability *in vitro* as compared to others, which may serve as natural antioxidant or food supplements, apart from beneficial stabilizers against oxidative damage in pharmaceutical and medical applications. Since mechanism of action of antioxidants against bacteria seemed complicated, further research should be directed on the relationship between antimicrobial potential and chemical structure of each compound in the extract, together with cytotoxicity assessment.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The antioxidant characteristic of natural products for health benefits and coronary protection as well as cancer has become a major research interest among scientists associated with pharmaceutical industries in order to find more effective and safe drugs. Moreover, plants have proven to be the most promising source of potential antibiotics showing less resistance towards microbes which might be safe alternative of synthetic drugs.

Research frontiers

The present research work illustrates antioxidant and antimicrobial potential of the crude extracts of the stem bark of *S. kunstleri* and was done by estimating different biochemical paradigms and *in–vitro* antioxidant and antimicrobial parameters.

Related reports

Ferric thiocyanate assay and DPPH radical scavenging activity assay are well established procedures to evaluate antioxidant potential of the plant extracts. It is well known fact that the antioxidant characteristic of extracts on DPPH is due to their ability to donate hydrogen and the ferric thiocyanate assay measures the amount of lipid peroxide formed.

Innovations and breakthroughs

In the present work, author has explored the *in vitro* antioxidant and antimicrobial potential of the stem bark of *S. kunstleri*.

Applications

In future studies, antioxidant and antimicrobial potential of this plant could further be evaluated in order to isolate compounds responsible for antioxidant and antimicrobial activities of crude extracts of the stem bark of *S. kunstleri*.

Peer review

This is a commendable research work in which author has successfully demonstrated the *in vitro* antioxidant and antimicrobial characteristics of the stem bark of the *S. kunstleri*. The antioxidant activity was assessed based on DPPH and ferric thiocyanate assays and antimicrobial activity was determined by following disc diffusion method and MIC was determined by using 96-well microtiter plates. MIC, MBC and MFC values were successfully determined with respect to know the true antimicrobial nature of this plant.

References

[1] Synge H, Heywood V. Information systems and databases for the conservation of medicinal plants. In: Akerele O, Heywood VH, Synge H, editors. *Conservation of medicinal plants*. Cambridge: Cambridge University Press; 1991.

[2] Lewington A. *Medicinal plant and plant extracts: a review of their importation into Europe*. Cambridge, UK: Traffic International; 1993.

[3] Perry LM, Metzger J. *Medicinal plants of East and Southeast Asia: attributed properties and uses*. Cambridge: MIT Press; 1980.

[4] Folli F, Corradi D, Fanti P, Davalli A, Paez A, Giaccari A, et al. The role of oxidative stress in the pathogenesis of type 2 diabetes mellitus micro-and macrovascular complications: avenues for a mechanistic-based therapeutic approach. *Curr Diabetes Rev* 2011; **7**: 313-324.

[5] Fiorentino TV, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. *Curr Pharm Des* 2013; **19**: 5695-5703.

[6] Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 1990; **186**: 1-85.

[7] Masoko P, Picard J, Eloff JN. Antifungal activities of six South African *Terminalia* species (Combretaceae). *J Ethnopharmacol* 2005; **99**: 301-308.

[8] Beckman CH. Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiol Mol Plant Pathol* 2000; **57**: 101-110.

[9] Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. *Am J Clin Nutr* 2005; **81**: 317S-325S.

[10] Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev* 2009; **2**(5): 270-278.

[11] Ashton PS. *Annotations to: conservation status listings for Dipterocarpaceae*. 1990.

[12] Forest Research Institute Malaysia. Wood Identification-Red Balau. Selangor Darul Ehsan, Malaysia: Forest Research Institute Malaysia; 2011. [Online] Available from: http://info.frim.gov.my/woodid/DispTimber2.cfm?Name=Red%20Balau [Accessed on 20th June, 2012]

[13] Raaman N. *Phytochemical techniques*. New Delhi: New India Publishing Agency; 2006.

[14] Ismail A, Marjan ZM, Foong CW. Total antioxidant activity and phenolic content in selected vegetables. *Food Chem* 2004; **87**: 581-586.

[15] Ahmad R, Hashi HM, Noor ZM, Ismail NH, Salim F, Lajis NH, et al. Antioxidant and antidiabetic potential of Malaysian *Uncaria*. *Res J Med Plant* 2011; **5**: 587-595.

[16] Gowri SS, Vasantha K. Antioxidant activity of *Sesbania grandiflora* (pink variety) L. Pers. *Int J Eng Sci Technol* 2010; **2**: 4350-4356.

[17] Alim A, Goze I, Cetin A, Atas AD, Vural N, Donmez E. Antimicrobial activity of the essential oil of *Cyclotrichium niveum* (Boiss.) Manden. Et Scheng. *Afr J Microbiol Res* 2009; **3**: 422-425.

[18] Masoko P, Picard J, Eloff J. The antifungal activity of twenty-four southern African *Combretum* species (Combretaceae). *S Afr J Bot* 2007; **73**: 173-183.

[19] Wu LC, Hsu HW, Chen YC, Chiu CC, Lin YI, Ho JA. Antioxidant and antiproliferative activities of red pitaya. *Food Chem* 2006; **95**: 319-327.

[20] Marquez AJ, Stougaard J. *Lotus japonicus handbook*. Germany: Springer; 2005.

[21] Andrade TD, Araújo BQ, Citó AM, Da Silva J, Saffi J, Richter MF, et al. Antioxidant properties and chemical composition of technical Cashew Nut Shell Liquid (tCNSL). *Food Chem* 2011; **126**: 1044-1048.

[22] Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol J Environ Stud* 2006; **15**: 523-530.

[23] Obame LC, Edou P, Bassole IH, Koudou J, Agnaniet H, Eba F, et al. Chemical composition, antioxidant and antimicrobial properties of the essential oil of *Dacryodes edulis* (G. Don) H.J. Lam from Gabon. *Afr J Microbiol Res* 2008; **2**: 148-152.

[24] Duffy CF, Power RF. Antioxidant and antimicrobial properties of some Chinese plant extracts. *Int J Antimicrob Agents* 2001; **17**: 527-529.

[25] Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. *Curr Med Chem* 2003; **10**: 813-829.

[26] Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol* 2004; **94**: 223-253.