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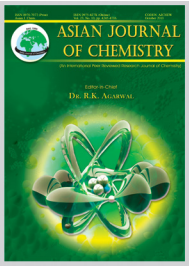

Evaluation of Antioxidant and Antimicrobial Activities of *Drypetes perreticulata* Gagnep. Extracts

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The aim of this paper was to screen the petroleum ether, dichloromethane, ethyl acetate and methanol extracts of *D. perreticulata* leaves and stem. In this study, antioxidant, antimicrobial effects of *D. perreticulata* leaves and *D. perreticulata* stem were evaluated using DPPH and FRAP, disc diffusion method, MIC and MTT cytotoxicity assays, respectively. Results showed that petroleum ether, dichloromethane, ethyl acetate, methanol extracts of leaves and stem of *D. perreticulata* exhibited moderate antioxidant and antibacterial activity. EC_{50}, FRAP value of DPSEA were 325.7 µg/mL and 3503.3 ± 63.2 µ mol Fe^{2+}/g in DPPH, FRAP method, respectively. All the extracts showed no inhibitory effect on tested fungi strain, *Candida albicans*. The petroleum ether extracts exhibited a broad spectrum cytotoxicity activity, in which IC_{50} were from 76.79 to 682.5 µg/mL. Results demonstrated that the extracts prepared from *D. perreticulata* possess antioxidant activity *in vitro* and antibacterial properties. It is concluded that the *D. perreticulata* extracts can be useful as a natural antioxidant and a probable antibacterial agent.

Key Words: Antioxidant, Antimicrobial, *Drypetes perreticulata*, Extracts.

INTRODUCTION

Drypetes perreticulata Gagnep., a big tree, is one of the many species of the genus *Drypetes* (Euphorbiaceae) encountered in China. In Hainan rain forests more than 10 species of *Drypetes* have been identified[1]. These plants are well known in folk medicine of Africa and Hainan. Many of them are used to treat various diseases including tumors, gonorrhea, toothache, dysentery, coryza, sinusitis, boils and swellings in West and Central Africa[2-5]. This background prompted us to explore the bioactivities of the species, *D. perreticulata* (DP). Hence, for selecting crude plant extracts with potential useful properties, *in vitro* screening methods have been used for further in-depth chemical elucidation and pharmacological investigations.

The activities have been selected because of their great medicinal relevance. Within the recent years, a greater interest in the antioxidant activity of plant extracts exists because of free radicals *e.g.* reactive oxygen species (ROS) that can be responsible for several diseases, for example, heart disease, stroke, arteriosclerosis and cancer, as well as the aging process[6,7]. In addition infection rates have increased and antibiotic resistance has become an increasing therapeutic problem[8,9].

However, to date, there are few reports on the antioxidant activities and antimicrobial effects of *D. perreticulata*. Therefore, this study was conducted to evaluate the antioxidant, antimicrobial activities of the petroleum ether (MSO), dichloromethane (DCM), ethyl acetate (EA) and methanol (MeTH) extracts of *Drypetes perreticulata*. using ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), disc diffusion, minimal inhibitory concentration (MIC) determination and methyl thiazolyl tetrazolium (MTT) cytotoxicity assay methods of analysis.

EXPERIMENTAL

Plant materials: The stems and leaves of *D. perreticulata* (Euphorbiaceae) were harvested from Bawangling rain forest (Southwest Hainan) in December 2010. The botanical identification of the plant was done at the College of Materials and Chemical Engineering, Hainan University, where the voucher specimens were conserved under the reference number 1112-1115/*D. perreticulata.*

Ascorbic acid (Vc), ceftazidime, butylated hydroxytoluene (BHT), 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma Chemical Co. DPPH was procured from A Johnson Matthey Company. Other chemical reagents and all solvents used were of analytical grade which were procured from Guangzhou Chemical Reagent factory.

Test organisms and culture medium: *Staphylococcus aureus* ATCC 1339, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 14886, *Escherichia coli* ATCC 8739 and *Candida albicans* (Clinical Isolate, Hainan provincial people's

hospital, Hainan, China). Nutrient agar (NA, Difco, USA) was used for the activation of microorganisms. Mueller Hinton agar (MHA, Difco) was used for the agar diffusion method. Mueller Hinton Broth (MHB, Difco) was used for the determination of the MIC and MTT cytotoxicity assay methods on these species.

The air-dried plants were separated into leaves and stems. To perform the extractions with the four different solvents (*i.e.* petroleum ether, dichloromethane, ethyl acetate, methanol), extracts of *D. perreticulata*. leaves (PDL) and stems (PDS) were prepared with a system ASE 150 from Dionex Corporation (Sunnyvale, CA, USA) according to Miguel *et al.*[10] and Andrew *et al.*[11] with modifications. The extraction cell (100 mL), which contained a stainless steel frit and a cellulose filter at the outlet, was loaded with *D. perreticulata*. (50 g) and sand (2 g). The parameters of ASE method, which were set and maintained electronically, were: 120 ºC, 10 MPa, two 5-min static cycles with 60 % flush volume and 120 s purge time. After the extraction, the solvents were removed from the extracts using a rotary evaporator at 45 ºC, respectively to give the crude dried extract. The different dried extracts were stored at 4 ºC until tested.

Determination of antioxidant activity

DPPH radical scavenging assay: In order to measure antioxidant activity, DPPH free radical scavenging assay was used. The radical scavenging activity of extracts was determined following the methods of Changwei *et al.*[12] and AS Al-Zubairi *et al.*[13] with slight modification.

Briefly, *D. perreticulata*. extracts stock solutions were prepared at 25.6 mg/mL in dimethyl sulfoxide (DMSO) and different concentrations (0.2-25.6 mg/mL) of each extract were prepared. The sample solution (100 µL) with various concentrations was added to 100 µL of 0.1 mM DPPH in 95 % ethanol in microtiter 96-well plates. Control contained DPPH solution and solvent without test sample. Blank contained test sample, 95 % ethanol solution without DPPH solution. The mixture was shaken vigorously and allowed to stand at room temperature, the absorbance was measured at 517 nm using xMarK (BIO-RAD, USA). Ascorbic acid and BHT were used as positive control. The measurement was performed in triplicate. Activity of scavenging (%) was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = [A_0\text{-}(A\text{-}A_b)/A_0]\times 100$$

where, A_0 was the absorbance of the control, A was the absorbance of test sample and A_b was the absorbance of blank. The correlation between each concentration and its percentage of scavenging was plotted and the EC_{50} was calculated by interpolation. The activity was expressed as EC_{50} (the effective concentration of each extract that scavenges 50 % of DPPH radicals).

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous-blue-coloured form in the presence of antioxidants. It is a relatively simple method frequently used in the assessment of antioxidant activity of various fruits, vegetables and some biological samples[14]. Briefly, the FRAP assay was done according to Iris and Strain[15] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2{\cdot}3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine solution in 40 mM HCl and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL 2,4,6-tripyridyl-s-triazine solution and 2.5 mL $FeCl_3{\cdot}6H_2O$ solution and then warmed at 37 ºC before using. The methanolic extract was not fully soluble in ethanol (even after sonication for 5 min), the extract was dissolved in DMSO with less than 1 % (v/v), while other extracts were appropriately diluted with ethanol and immediately used in the antioxidant assay. A 20 µL sample which was prepared at 0.2 mg/mL, was mixed with 150 µL of FRAP reagent and the absorbance of the reaction mixture was measured at 593 nm using xMarK (BIO-RAD) after incubation at 37 ºC for 0.5 h. The results could be expressed in micromole of Fe^{2+} equivalent. The standard curve was linear between 25 and 1500 µM Fe^{2+}. Results are expressed in µM Fe^{2+}/g fresh mass. All the measurements were taken in triplicate and the mean value ± standard error (SE) are reported.

Determination of antimicrobial activity

Disc diffusion method: Both bacteria and yeast strains were tested following the National Committee for Clinical Laboratory Standards (NCCLS)[16,17]. Briefly, sterile paper discs (6 mm in diameter, Whatman No.1) were immersed in 10 µL of crude extract at 20 mg mL^{-1} (200 µg/disc), ceftazidime at 3 mg/mL (30 µg/disc) prepared using water. The discs were evaporated at 37 ºC in a sterile room for 18 h. The microbial suspensions were then diluted to a turbidity of approximately 0.5 McFarland (-1.5 × 108 CFU/mL). Next, the MHA was poured into Petri dishes and inoculated with 100 µL of the suspension. Ceftazidime was used as the positive control and discs treated with DMSO were used as the negative control. The plates were then placed in an incubator (Sanyo, Japan) at 37 ºC for 24 h, after which the diameter of the zone of inhibition around each of the discs was measured and recorded. Each experiment was performed in triplicate.

Determination of MICs: The MIC values were determined for microorganisms following the NCCLS[16,17]. Microbial strains were inoculated on MHA plates and were incubated at 37 ºC for 24 h. Four-five colonies were transferred from each solid culture to saline solution (3 mL) and then the solution was adjusted to 0.5 McFarland standard turbidity. The appropriate working suspension for each microorganism was prepared containing 5×10^5 CFU/mL of microbial. The extracts were prepared at 25.6 mg/mL in DMSO. Concentrations for each extract ranged from 2.56 to 0.01 mg/mL. A 0.4 mL volume of the extract sample was transferred to the first cuvette containing 1.6 mL MHB, other cuvette added 1 mL MHB prior and serial 2-fold dilutions were performed; the remaining 1 mL was discarded. A 1 mL volume of working suspension was added to each cuvette. Nine samples and one antimicrobial drug control, ceftazidime (2.56-0.01 mg/mL, or 20-0.078125 µg/mL, respectively), were included in each cuvette. One additional cuvette was used as growth controls where no drug was added. Cuvettes were incubated at 37 ºC for 24 h and the growth was visually examined. Each experiment was performed in triplicate.

MTT assay: Microorganism suspension (5×10^5 CFU/mL) was plated out into 96-well microtiter plate. Plant

extracts were initially dissolved in DMSO as mentioned earlier. The cytoxicity profiles of the extracts were assessed using methyl thiazolyl tetrazolium microculture tetrazolium viability assay as described by Mosmann[18] and Abate *et al*.[19] with some modifications. Thereafter, various concentrations of the plant extract samples were plated out in triplicates. Each plate included untreated microorganism controls and a blank microorganism-free control. After 24 h of incubation at 37 ºC in an ambient atmosphere, 10 µL methyl thiazolyl tetrazolium (MTT) (5 mg/mL) was added to each well and re-incubated for further 4 h. Then, the media was removed and DMSO was added into each well to solubilize the formazan crystals. Finally, the absorbance was read at wavelength of 570 nm using xMark (BIO-RAD) and the percentage cell viability was calculated with the appropriate controls taken into account. The concentration which inhibited 50 % of cellular growth (IC_{50} value) was determined and the inhibitory rate of cell proliferation was calculated by the following formula:

$$\text{Growth inhibition (100 \%)} = \frac{OD_{control} - OD_{treated}}{OD_{control}} \times 100$$

where, OD is the optical density. Cytotoxicity of the sample towards the microorganism cells was expressed as IC_{50} values (*i.e.* the extract concentration reducing the absorbance of treated cells by 50 % in respect to untreated microorganism).

Statistical analysis: All the results were expressed as mean ± standard error of means. The data were statistically analyzed by one-way analysis of variance followed by Duncan's multiple range tests using SAS 8.0 Software. The $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Radical scavenging activities: DPPH is a stable free radical that shows maximum absorbance at 517 nm in ethanol. Owing to rapid hydrogen acceptable ability of DPPH, it reacts with antioxidants and gets converted into 1,1-diphenyl-2-picrylhydrazine and hence shows decrease in absorbance. Fig. 1 shows the results of DPPH radical scavenging activity of all extracts at various concentrations. With increasing extracts concentrations, the scavenging effect on DPPH radical increased. At a concentration of 1.6 mg/mL, the scavenging effects of DPSMSO, DPSDCM and DPSEA (59.9, 57.3 and 75.7 %, respectively) are similar and their effects were much higher than that of DPLMSO, DPLDCM, DPLEA, DPLMeTH and DPSMeTH (28.7, 30.7, 40.6, 12.8 and 17.5 %, respectively). As is shown in Fig. 1, ascorbic acid and BHT exhibited higher activity than all extracts at low concentration. The effective concentration (EC_{50}, meaning the concentration that scavenges 50 % of the initial DPPH radical) values for DPLMSO, DPLDCM, DPLEA, DPLMeTH, DPSMSO, DPSDCM, DPSEA, DPSMeTH, ascorbic acid and BHT were 6087.8, 5195.0, 3679.6, 11050.7, 757.1, 464.9, 325.7, 7169.9, 10.73 and 47.13 µg/mL, respectively. *D. perreticulata* stem (DPS) extracts showed significant antioxidant activity which was comparable to that of *D. perreticulata* leaves (DPL) extracts ($P < 0.05$). These results revealed that *D. perreticulata* extracts probably contained substances that were proton donors and could react with free radicals to convert them to stable diamagnetic molecules.

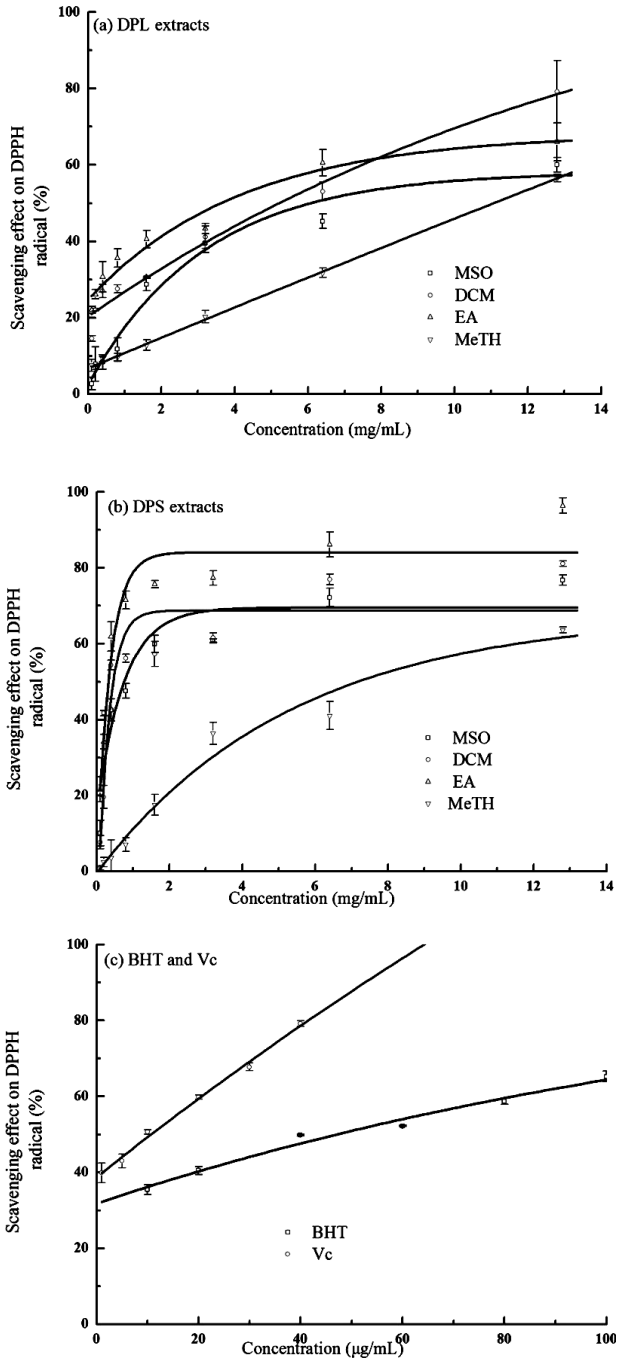

Fig. 1. Scavenging effects on DPPH radical: (a) DPL extracts; (b) DPS extracts; (c) Vc and BHT. Data were expressed as means with standard errors (n = 3)

Reducing power: The FRAP assay results are presented in Table-1 as µMFe^{2+}/g dry weight of extract. We discovered that the FRAP values ranged from 436.7 ± 9.5 to 3503.3 ± 63.2 µMFe^{2+}/g for DPLMeTH and DPSEA, respectively. *D. perreticulata* stem extracts possessed approximately 1.7-fold greater antioxidant capacity than *D. perreticulata* leaves extracts. The reducing powers of ascorbic acid (18030.0 ± 66.1 µMFe^{2+}/g) and BHT (10196.7 ± 118.1 µMFe^{2+}/g) were significantly ($P < 0.05$) higher than that of all extracts. The reducing powers of ascorbic acid and BHT were just 2.7,1.5-fold than *D. perreticulata* stem extracts, respectively. These results indicate that all extracts have potential antioxidant activities.

TABLE-1
ANTIOXIDANT CAPACITIES (μMFe^{2+}/g) OF DIFFERENT *D. perreticulata* EXTRACTS ACCORDING TO FRAP ASSAY

μMFe^{2+}/g	MSO	DCM	EA	MeTH	Total
DPL	609.6 ± 41.6a	1592.9 ± 28.2a	1303.3 ± 21.9b	436.7 ± 9.5b	3942.5 ± 101.2b
DPS	438.8 ± 33.1b	1270.0 ± 81.9b	3503.3 ± 63.2a	1405.4 ± 42.5a	6617.5 ± 220.7a

Each value in the table is represented as mean ± SE (n = 3). Different letters in the same column indicate significant difference ($P < 0.05$)

TABLE-2
ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *D. perreticulata* Gagnep. AND CEFTAZIDIME USING DISC DIFFUSION METHOD (INHIBITION ZONES, mm)

Diameter of inhibition (mm)	Microbial strains tested				
	Gram-positive bacteria		Gram-negative bacteria		Fungi
	Sa	Bs	Ec	Pa	Ca
DPLMSO	9.7 ± 0.6bc	8.0 ± 0.0cde	-e	8.0 ± 0.0c	-b
DPLDCM	9.7 ± 0.6bc	8.3 ± 0.6bcd	9.7 ± 0.6bc	8.0 ± 0.0c	-b
DPLEA	-f	8.0 ± 0.0cde	8.0 ± 0.0cd	8.7 ± 0.6bc	-b
DPLMeTH	7.0 ± 0.0e	8.7 ± 1.2bc	7.7 ± 0.6d	-d	-b
DPSMSO	7.3 ± 0.6e	7.0 ± 0.0e	8.7 ± 1.2bcd	9.0 ± 0.0bc	-b
DPSDCM	11.0 ± 1.7b	9.3 ± 1.2b	8.3 ± 0.6bcd	10.0 ± 0.0b	-b
DPSEA	9.0 ± 1.0cd	-f	8.7 ± 1.2bcd	10.3 ± 2.1b	-b
DPSMeTH	7.7 ± 0.6de	7.3 ± 0.6de	10.0 ± 1.7b	9.3 ± 1.5bc	-b
Ceftazidime	18.3 ± 1.5a	22.0 ± 0.0a	21.0 ± 1.0a	22.0 ± 0.0a	20.0 ± 0.0a

The screening of the extracts antibacterial effect was carried out by determining the zone of inhibition using paper disc. Each value in the table is represented as mean ± SE (n = 3), different letters in the same column indicate significant difference ($P < 0.05$). "-" indicated no inhibition zone. Sa, *Staphylococcus aureus*; Bs, *Bacillus subtilis*; Ec, *Escherichia coli*; Pa, *Pseudomonas aeruginosa*; Ca, *Candida albicans*.

Antimicrobial activity

Inhibition zone diameters: The antimicrobial effects of *D. perreticulata* extracts studied are summarized in Table-2. The antimicrobial activities of the different extracts of *D. perreticulata* were evaluated using both Gram-positive (*Staphylococcus aureus, Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and Fungi (*Candida albicans*). The highest antibacterial activity observed was obtained by the DPSDCM extract on *S. aureus*. However, no antimicrobial activity was observed from all extracts against *C. albicans*. The positive control, ceftazidime, had shown zones of inhibition of 18.3 ± 1.5, 22.0 ± 0.0, 21.0 ± 1.0, 22.0 ± 0.0 and 20.0 ± 0.0 mm in *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*, respectively.

MICs: The results of the extracts displaying antimicrobial activity are shown in Table-3. No activity against Gram-negative bacteria (*E. coli*) was observed. The DPSEA displayed the strongest activity against *P. aeruginosa*, the MIC value was 320 μg/mL. Most of the extracts tested exhibited no activity against bacteria (MIC > 2560 μg/mL). The antibacterial activity of ceftazidime was significantly ($P < 0.05$) higher than that of all extracts.

Cytotoxic activity: The inhibition concentration (IC_{50}, meaning the concentration that inhibited 50 % of the microbial stains) values for DPLMSO, DPLDCM, DPLEA, DPLMeTH, DPSMSO, DPSDCM, DPSEA, DPSMeTH and ceftazidime were displayed in Table-4. Among the 8 extracts tested for cytotoxicity against 4 strains, only the petroleum ether extract exhibited noticeable activities with IC_{50} values below 1000 μg/mL. The DPLMSO displayed the strongest activity against *Staphylococcus aureus* and *Bacillus subtilis*, the IC_{80} value were 639.1, 650.6 μg/mL, respectively. Also,

TABLE-3
MIC OF THE CRUDE EXTRACT FROM *D. perreticulata* Gagnep. AND CEFTAZIDIME

MIC (μg/mL)	Microbial strains tested			
	Gram-positive bacteria		Gram-negative bacteria	
	Sa	Bs	Ec	Pa
DPLMSO	640b	640c	> 2560a	> 2560a
DPLDCM	> 2560a	> 2560a	> 2560a	> 2560a
DPLEA	> 2560a	> 2560a	> 2560a	> 2560a
DPLMeTH	> 2560a	2560b	> 2560a	> 2560a
DPSMSO	> 2560a	> 2560a	> 2560a	> 2560a
DPSDCM	> 2560a	> 2 560a	> 2560a	> 2560a
DPSEA	> 2560a	> 2560a	> 2560a	320c
DPSMeTH	> 2560a	2560b	> 2560a	640b
Ceftazidime	10c	0.15625d	0.3125b	2.5d

Concentration of samples in assays was expressed as final concentration. Different letters in the same column indicate significant difference ($P < 0.05$). Sa, *Staphylococcus aureus*; Bs, *Bacillus subtilis*; Ec, *Escherichia coli*; Pa, *Pseudomonas aeruginosa*; Ca, *Candida albicans*

the DPSEA and DPSMeTH displayed the strongest activity against *Pseudomonas aeruginosa*, the IC_{80} value were 351.3, 545.6 μg/mL, respectively. The DPLMeTH displayed stronger activity against *Pseudomonas aeruginosa*, the IC_{80} was 2453.2 μg/mL. Most of the extracts tested exhibited no activity against strains (IC_{80} > 2560 μg/mL). The antibacterial activity of ceftazidime was significantly ($P < 0.05$) higher than that of all extracts, the IC_{80} for *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were 5.615, 0.226, 0.28 and 1.952 μg/mL, respectively.

Interest in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants has been increased considerably. Restrictions on the use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and BHT are being imposed due to their carcinogenicity[20];

TABLE-4
IC_{50} OF THE CRUDE EXTRACT FROM *D. perreticulata* Gagnep. AND CEFTAZIDIME

IC_{50} (μg mL)	Microbial strains tested			
	Gram-positive bacteria		Gram-negative bacteria	
	Sa	Bs	Ec	Pa
DPLMSO	180.5e	76.79g	466.6b	682.5b
DPLDCM	308.3e	189.4e	> 2560a	688.3b
DPLEA	> 2560a	332.8d	> 2560a	> 2560a
DPLMeTH	1337.5c	381.4c	> 2560a	> 2560a
DPSMSO	661.1d	142.5f	348c	529.3c
DPSDCM	> 2560a	> 2560a	> 2560a	> 2560a
DPSEA	2018b	> 2560a	> 2560a	72.7e
DPSMeTH	> 2560a	2308b	> 2560a	101.6d
Ceftazidime	1.775f	0.0837h	0.12d	0.447f

Concentration of samples in assays was expressed as final concentration. Different letters in the same column indicate significant difference ($P < 0.05$). Sa, *Staphylococcus aureus*; Bs, *Bacillus subtilis*; Ec, *Escherichia coli*; Pa, *Pseudomonas aeruginosa*; Ca, *Candida albicans*

therefore, a need for identifying alternative natural and safe sources of antioxidants, especially of plant origin, has increased in recent years[21]. In this study, the MSO, DCM, EA and MeTH extracts of *D. perreticulata* leaves and *D. perreticulata* stem were assayed for their antioxidant and antimicrobial properties using DPPH and FRAP, disc diffusion method, MIC and MTT cytotoxicity assay tests.

The therapeutic benefit of medicinal plants is usually contributed to their antioxidant properties[22-24]. The FRAP assay can react with Fe^{2+} and SH-group containing antioxidants and DPPH method use organic radicals, so it is expected that using these two methods accurately reflects all of the antioxidants in a sample[15,25]. These extracts had shown modest antioxidant power with FRAP assay and those results were also supported with DPPH assay. As a result, the ethyl acetate of DPS showed the highest FRAP value compared with DCM, MSO and MeTH extracts. Therefore, the DPSEA expressed the highest free radical scavenging activity, while the DPLMSO and DPLMeTH extracts were found to have the lowest free radical scavenging activity. However, it must be noted that there was some diversity between the FRAP and DPPH results, the lower FRAP of DPSMSO with higher antioxidant activity compared with DPSMeTH, DPLEA and DPLDCM may be explained by the more polar components of this fraction than the DPSMeTH, DPLEA and DPLDCM. These findings are in agreement with previous investigation of medicinal plants where they showed that the DPPH scavenging activity of the *D. perreticulata* stem was found to be higher than *D. perreticulata* leaves extracts, suggesting that the hydrogen-donating compounds are more likely to be present in stem part of plant. The antioxidant activities of extracts may be due to its proton donating capability as shown in DPPH radical scavenging results. Acting as an electron donor that can react with free radicals, it converts them to more stable products and terminates radical chain reactions. This mechanism may explain its application for the treatment of tumors and other diseases.

In addition to screening the antioxidant properties, we have also investigated the antimicrobial activities of different extracts from *D. perreticulata*. Studies on antimicrobial activities of medicinal plants are currently undergoing rapid progress using different screening methods. In this respect, disc diffusion method and MIC are the first method of choice due to its simplicity and capability of analyzing large number of samples. In addition, several previous publications had exploited these methods to determine antimicrobial activity[16,17]. Results of this study indicated that *D. perreticulata* extracts had modest antibacterial towards the tested bacterial strains. However, the results of antimicrobial activities of extracts indicated that they did not exhibit any activity for fungi (*Candida albicans*). MTT cytotoxicity assay also was used as a method to determine the antibacterial activity of extracts. Results showed that petroleum ether extracts have a high cytotoxicity towards the tested bacterial strains, indicating that petroleum ether extracts will be the best target for further research for the development of antibacterial agents. The high antibacterial effect of the investigated petroleum ether extracts against both Gram-positive and Gram- negative microorganisms may be due to the high content of volatile oil.

The concentration that inhibited 80 % of the microbial stains was the MIC value[17]. We discovered that correlation between IC_{80} obtained from MTT assay and MIC value was positively high ($R^2 = 0.9983$, $P < 0.01$). The results suggested that the cytotoxicity contributed significantly to the antibacterial capacity of the extracts. The results of MTT assay and the constant dilution method were consistent (slope = 0.9568). MTT method is simple, sensitive, specific and reproducible, the results are more objective, more precise than routine direct observing. Hence, MTT method can be used as an application of the antimicrobial susceptibility test methods.

Conclusion

As far as we know, this is the first study concerning the antioxidant capacity and antimicrobial activity of *D. perreticulata.* As this plant is distributed in a large quantity in many tropical regions of the world and its stem extract demonstrated moderate antioxidant activity, reducing power and petroleum ether extracts showed moderate antibacterial activities, our work suggested that the *D. perreticulata* could be utilized as an effective and safe antioxidant and antibacterial source. And the results obtained in the present study are in agreement to a certain degree with the traditional uses of the plants estimated. The obtained results could form a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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