

# Evaluation of Antimicrobial, Antioxidant and Cytotoxic Activities of *Dialium cochinchinensis* Seed Extract

L. T. K. BUI, Q. T. NGUYEN<sup>1</sup>, L. M. DAO<sup>1</sup>, H. L. NGUYEN, M. V. LAM AND C. T. HOANG<sup>2,3\*</sup>

Department of Food Technology, HUTECH Institute of Applied Sciences, Ho Chi Minh City University of Technology (HUTECH), <sup>1</sup>Faculty of Biology & Biotechnology, University of Science, Vietnam National University – Ho Chi Minh City, <sup>2</sup>Department for Management of Science and Technology Development, <sup>3</sup>Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam

**Bui et al.: Antimicrobial, antioxidant and cytotoxic activities of *Dialium cochinchinensis***

This study was undertaken to evaluate the phytoconstituents present in the seed extract of *Dialium cochinchinensis* for antioxidant, antimicrobial and cytotoxic properties. Results showed the presence of polyphenols, flavonoids, alkaloids, saponins, coumarins and a polyuronid compound in the *Dialium cochinchinensis* seed extract. The total polyphenol and flavonoid contents of the dry extract were  $133.5 \pm 2.87$  mg GAE/g and  $227.0 \pm 23.20$  mg rutin equivalent/g, respectively. *Dialium cochinchinensis* seed extract exhibited antioxidant property in 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid and potassium ferricyanide reducing antioxidant power methods. Moreover, *Dialium cochinchinensis* seed extract exerted inhibitory effect on Gram-positive bacteria. Finally, *Dialium cochinchinensis* seed extract was found to be toxic to Vero cell lines and the pathogens with selectivity index ranging from 0.01 to 0.04. In conclusion, *Dialium cochinchinensis* seed extract could be considered as a new source of natural antioxidant and antimicrobial agents for research and application.

**Key words:** *Dialium cochinchinensis*, antioxidant activity, phytochemical screening, antimicrobial, cytotoxicity

*Dialium cochinchinensis* Pierre belongs to the genus *Dialium* and consists of about 30 species, in which 10 species are found in the tropical rain forests of Southeast Asian including Myanmar, Thailand, Indochina, Malaysia, Laos and Vietnam (Nghe An, Ha Tinh, Kon Tum, Dong Nai, Binh Duong, Binh Phuoc, Ho Chi Minh, and Ninh Thuan provinces)<sup>[1]</sup>. In Vietnam, *D. cochinchinensis* is listed in IUCN Red list and Vietnam Red Book<sup>[2]</sup>.

*D. cochinchinensis* can grow up to 35 meters high with the bole is about 16-18 m high, 80-100 cm diameter, and rarely 150 cm. The bark generally is grey or whitish, the inner bark is 6-8 mm thick, hard, brownish, exuding a little transparent resin, soon turning red. This is one

of the valuable woody trees, being used for furniture, railway sleepers, heavy construction (staircases, doors and window frames), bridges, ship- and boat-building, vehicles, chopping board, sports equipment, cart axles, agriculture implements, and oil and sugar mills. Fruit is a pod, ovoid, slightly laterally compressed, 15-20×8-15 mm, finely pubescent to velvety<sup>[1]</sup>. The edible pulp is sweet and sour that has laxative effect<sup>[2]</sup>. Seeds are

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms

\*Address for correspondence

E-mail: hoangthanhchi@tdtu.edu.vn

Accepted 15 July 2019

Revised 23 March 2019

Received 24 January 2019

Indian J Pharm Sci 2019;81(5):975-980

either 1 or 2, elliptic, 9×6×3 mm, longitudinally striate and covered by whitish pulp<sup>[1]</sup>. The seeds have usually been thrown out after using fruits that can become a potential resource for industry. To our knowledge, there is still no publication about the bioactivity of *D. cochinchinensis*. However, there are some studies about the bioactivity of *D. guineense*, one of the other *Dialium* genus species. The bark extract of *D. guineense* exhibited antibacterial activity<sup>[3]</sup>, while the leaf extracts have antioxidant and antimicrobial activities<sup>[4]</sup>. The dichloromethane fraction of *D. guineense* fruit coat exerted wound-healing and antimicrobial properties<sup>[5]</sup>. Moreover, the phenolic extract of *D. guineense* pulp enhanced reactive oxygen species detoxification in aflatoxin B(1) hepatocarcinogenesis<sup>[6]</sup>. In this study, the antioxidant, antimicrobial and cytotoxicity of *D. cochinchinensis* seeds (DCS) were evaluated for the first time.

DCS were collected from trees at Kon Chur Rãng conservation garden, Son Lang, Kbang District, Gia Lai-Kon Tum Province, Vietnam. DCS were washed and oven-dried until fully dry after a week at 40°. The dry DCS were ground to a fine powder in a blender. The powder was macerated with 80 % ethanol in the ratio of 1:10 (w/v). The mixture of powder and ethanol was continuously swirled for 4 d at room temperature and filtered through a Whatman filter paper. The filtrate concentrated in a rotary evaporator at 40° to obtain the crude extract. The crude extract was kept at -20° till further analysis. The phytoconstituents were evaluated according to the method described by Ciulei<sup>[7]</sup>.

The total polyphenol content (TPC) of the DCS extract was determined using Folin-Ciocalteu reagent<sup>[8]</sup> following a slightly modified method of Nunzia *et al.*<sup>[9]</sup>. A volume of 200 µl extract in concentration of 1000 µg/ml was mixed with 200 µl Folin-Ciocalteu reagent 100 %. The mixture was incubated at room temperature for 5 min and 1600 µl of sodium carbonate solution (5 %, w/v) was added. The reaction mixture was incubated at 40° for 20 min and the absorbance was measured at 765 nm. Gallic acid 0 to 500 µg/ml was used to develop a standard curve. The content of TPC was expressed as mg/g gallic acid equivalent (GAE) of dry extract.

The total flavonoid content (TFC) of the DCS extract was determined using aluminium chloride colorimetric method<sup>[10]</sup>. Rutin 0 to 500 µg/ml was used to develop a standard curve. Rutin solution (500 µl) was added

to 2000 µl distilled water and 150 µl of 5 % NaNO<sub>2</sub>. After incubated at room temperature for 6 min, 150 µl of 10 % AlCl<sub>3</sub> solution was added and then incubated for 6 min at room temperature. A volume of 2000 µl of 4 % NaOH and 200 µl distilled water was added to the mixture and incubated for 15 min at room temperature before distributed into 96-well plates and absorbance was measured at 510 nm. The extract with concentration of 1000 µg/ml was determined to determine TFC as mg/g rutin equivalent (RE) of dry extract.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was carried out according to the method of Ghatak *et al.* with slightly modification<sup>[11]</sup>. DPPH solution (0.3 mM) was prepared and reacted with the DCS extract at ratio of 1:1. The absorbance of the mixture was measured at 517 nm after incubation at 37° for 30 min. Vitamin C was used as a positive control for this assay. The ability to scavenge DPPH radical was calculated using following formula, % DPPH radical scavenging = (sample-negative control)/negative control)×100.

2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS, 2.6 mM) was allowed to react with K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (7.4 mM) at ratio of 3:1 in a dark room for 16 h at room temperature to become the solution of ABTS\*(+). ABTS\*(+) solution was diluted with distilled water to obtain an absorbance of 1.00±0.02 at 734 nm. After mixing 750 µl of diluted ABTS\*(+) solution with 150 µl of extract, the absorbance was measured at 734 nm. Vitamin C was used as positive control for this assay. The ability to scavenge ABTS\*(+) radical was calculated using following formula, % ABTS\*(+) radical scavenging = ((sample-negative control)/negative control)×100.

Reducing power of DCS extract was detected based on potassium ferricyanide reducing antioxidant power (PFRAP) assay<sup>[12]</sup>. A volume 1 ml of extract was added 2.5 ml of phosphate-buffered saline (PBS, pH 6.6) and 2.5 ml potassium ferricyanide 1 %, mixed well and incubated for 20 min at 50°. The mixture was added to 2.5 ml of 10 % trichloroacetic acid and incubated for 10 min at room temperature. The upper layer (2.5 ml) was taken, 2.5 ml distilled water was added followed by 0.5 ml of 0.1 % FeCl<sub>3</sub>. The absorbance of the resulting blue-green colour was measured at 700 nm. Vitamin C was used for positive control.

Four Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538 and ATCC 25923, *Rhodococcus equi* ATCC 6939 and *Listeria monocytogenes* ATCC 13932) and

5 Gram-negative bacteria (*Escherichia coli* ATCC 25922 and ATCC 8739, *Listeria monocytogenes* ATCC 13932, *Proteus mirabilis* ATCC 25933, *Citrobacter freundii* ATCC 8090 and *Salmonella enterica* ATCC 14028) were used. Agar well diffusion method was adopted for antibacterial activity assessment<sup>[13]</sup>. The bacteria were reactivated in tryptic soy broth (Acumedia, Neogen, US) at 37° for 20 h before adjusted turbidity to 0.5 McFarland equivalent to  $(1-2) \times 10^8$  CFU/ml<sup>[14]</sup>. The adjusted bacterial biomass was spread out as a microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm is punched aseptically with a sterile cork borer and a volume of 50 µl of the extract at different concentration is introduced into the well. The agar plates were incubated at 37° overnight. The appearance of the inhibition zones indicated antibacterial property of extracts. Ampicillin was used as positive control and ethanol was used as a solvent control.

The microbial biomass prepared in Mueller-Hinton broth (MHB; HiMedia, India) after dilution of standardized microbial suspension adjusted to 0.5 McFarland scale<sup>[14]</sup>. Then, the adjusted biomass was diluted with MHB to obtain a concentration of  $(1-2) \times 10^5$  CFU/ml. A volume of 50 µl diluted bacterial biomass was transferred into each well in 96 plates. Fifty microlitres of extracts in different concentration was added into each well. The plate was incubated at 37° for 20 h. The MIC was read as the least concentration that inhibited the growth of the test organisms. In this study a volume of 30 µl resazurin 0.02 % (Sigma-Aldrich, Merck, Germany) was added to each well to identify the MIC which was the least concentration that still in the blue colour<sup>[15]</sup>. The MBC was determined by sub-culturing the test dilutions onto TSA dish and incubated further for 18-24 h. The lowest dilution that yielded no single bacterial colony on the TSA dish was taken as MBC<sup>[16]</sup>.

The toxic effects of the DCS extract on African green monkey kidney (Vero) cells Vero cell lineage (ATCC CCL-81<sup>TM</sup>) was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Vero cells were grown in Dulbecco's modified Eagle's medium (Sigma-Aldrich, Ho Chi Minh City, Vietnam) supplemented with 10 % foetal bovine serum, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, 1 % sodium pyruvate and 1 % L-glutamine (Biobasic, TBR Technology Corporation, Ho Chi Minh City, Vietnam) in a humidified incubator of 5 % CO<sub>2</sub> at 37°. The Vero cells were distributed

into 96-wells microplates ( $3 \times 10^4$  cells per 100 µl per well) and incubated at 37° for 24 h. Then, 100 µl of DCS extract at different concentrations was added starting with 400 µg/ml in successive serial dilutions to 25 µg/ml. Dimethyl sulfoxide (DMSO) 0.1 % was used as a control. After 48 h incubation, 20 µl of MTT solution (5 mg/ml in PBS) was added and the plates were incubated at 37° for 2 h. Then, 100 µl DMSO was added to each well to dissolve the MTT-formazan crystals. Cytotoxicity was expressed as the concentration of test sample resulting in a 50 % reduction of absorbance compared to untreated cells (LC<sub>50</sub> values). The selectivity index (SI) was expressed as LC<sub>50</sub>/MIC value. The SI values indicate the plant extract's relative safety. The index value is a measure of the extract's beneficial affects at a low dose versus its harmful effects a high dose. A high SI is an indication of a large safety margin between beneficial and toxic dose.

All experiments were conducted in triplicate. The data were analysed using GraphPad Prism version 7.04. Significant differences were determined by p-value with p-value was less than 0.001. Data were presented as mean±standard error of mean (SEM).

The traditional medicinal herbs are used as cures because of pharmacological actions, which could be attributed to phytoconstituents such as alkaloids, flavonoids and tannins. Some of them have potential to reduce the risk and progression of acute and chronic diseases such as cancer, cardiac and stroke mostly due to antioxidant effect<sup>[17]</sup>.

All results of phytochemical analysis are showed in the Table 1. The results showed the presentation of polyphenols, flavonoids, alkaloids, saponins, coumarins and a polyuronid compound in the DCS extract. Plant

**TABLE 1: SECONDARY METABOLITES GROUPS EXISTING IN DCS**

Natural products	Fractions		
	Diethyl ether	Ethanol	Water
Alkaloid	+	+	+
Coumarin	+		
Flavonoid	+	+	+
Cardiac glycoside		+	-
Saponin		+	+
Polyphenol		+	+
Anthocyanin		±	-
Polyuronid			+
Reducing sugar		+	-
Organic acid		-	-

'+' Present, '-' absent '||' no need to detect

polyphenols are well known antioxidants due to suitable structure for scavenging free radicals<sup>[18]</sup>. The tests also indicated that cardiac glycoside, anthocyanin and reducing sugar compound have a high probability of appearing in the sample.

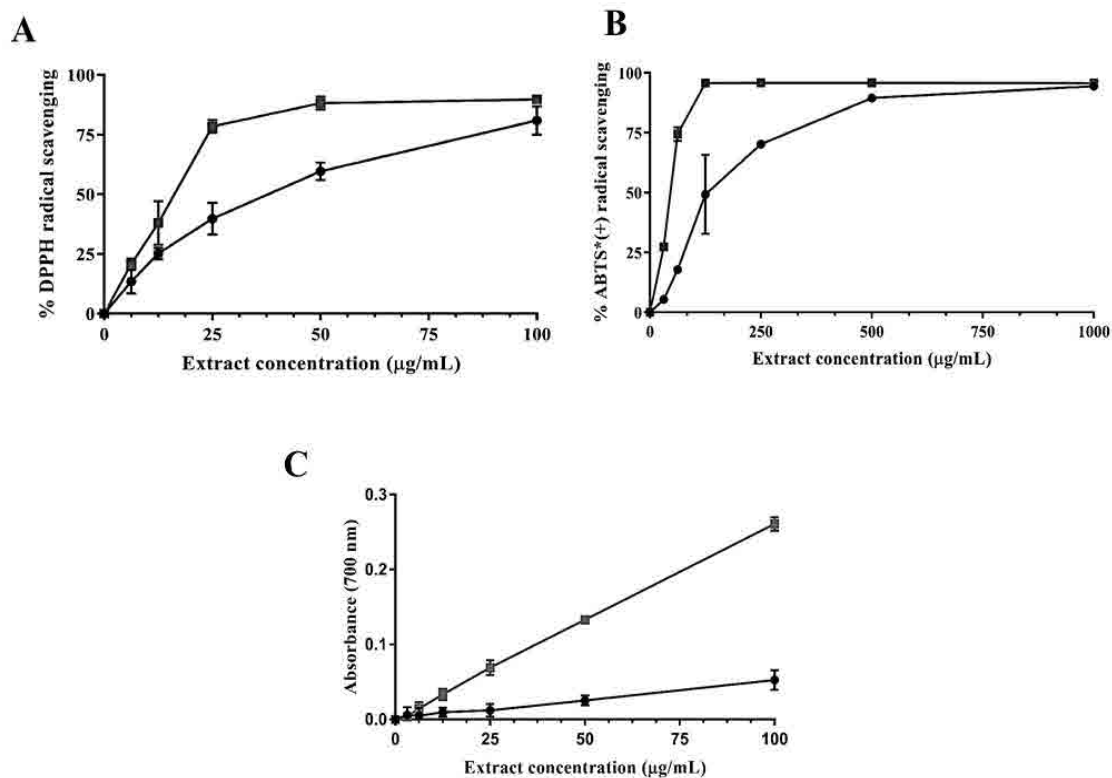
Moreover, the total TPC and TFC of dry extract were found to be  $133.5 \pm 2.87$  mg GAE/g and  $227.0 \pm 23.20$  mg RE/g dry, respectively. These results indicated that DCS has a high level of total polyphenol and flavonoid. The antioxidant property of DCS was determined using DPPH and ABTS radical scavenging methods. DPPH free radical scavenging activity is one of the most common methods based on the change of colour from purple into yellow when the radicals contact the antioxidants<sup>[18]</sup>. DCS extract potential to scavenge DPPH free radical was evaluated and compared with vitamin C. The results showed in fig. 1A and B indicated that DCS extract exhibited antioxidant effect as compared to vitamin C. The half maximal inhibitory concentration ( $IC_{50}$ ) of DPPH radical scavenging assays were inferred by nonlinear regression analysis. Although vitamin C exhibited higher scavenging activity, the higher the DCS extract concentration the stronger effect was expressed and

approached asymptote vitamin C curve with quite low  $IC_{50}$  ( $33.6 \pm 1.66$   $\mu\text{g/ml}$ , fig. 1).

ABTS radical scavenging assay was based on the change from dark green into colourless was used. At a concentration of 1000  $\mu\text{g/ml}$  the ABTS scavenging activity of vitamin C were equal to the DCS extract. The  $IC_{50}$  for ABTS scavenging curve was determined as  $140.0 \pm 7.47$   $\mu\text{g/ml}$  (fig. 1).

The reducing power of DCS extract was further investigated by performing FRAP assay. The result (fig. 1C) showed that the reducing power increased with increasing concentration. However, the reducing power of DCS was much lower than that of vitamin C at the same concentration. The results of this study suggested that DCS contained polyphenols and flavonoids that cause the strong antioxidant property. It can be used as a natural source of antioxidants to prevent the progression of diseases.

Many seeds from the plants have proven to be an extremely good source of natural antioxidants, such as grape seeds (*Vitis vinifera*), soybean (*Glycin max*), tamarind (*Tamarindus indica*) seeds<sup>[19]</sup>, *Emblca officinalis* (amla) fruit pulp<sup>[20]</sup> and crab eye seeds



**Fig. 1: Antioxidant activity of the DCS ethanol extracts**

Determination of antioxidant activity of the DCS ethanol extracts by the (A) DPPH, (B) ABTS and (C) FRAP method, using different concentrations, expressed as percent antioxidant activity. Average of analysis obtained in triplicate ( $n=3$ ). (—■—) Vitamin C, (—●—) DCS ethanol extracts



(*Abrus precatorius*)<sup>[21]</sup>. Compared to these seeds (via vitamin C), DCS has a stronger antioxidant activity.

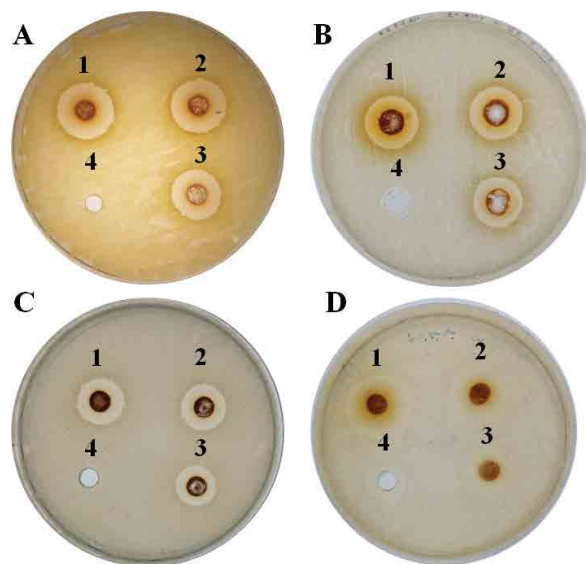
The free radical scavenging activity, reducing power of extract appeared to depend on the present of phenolics, flavonoids or tannin and their concentration<sup>[22]</sup>. In this

investigation, it was found that DCS is rich of TPC and TFC (133.5±2.87 mg GAE/g and 227.0±23.20 mg RE/g, respectively)

The antibacterial activity of DCS ethanol extract is demonstrated by the inhibition zones (Table 2). DCS ethanol extract was more effective on Gram-positive bacteria than the Gram-negative ones (fig. 2). The Gram-positive bacteria such as *S. aureus*, *L. monocytogenes* and *R. equi* showed greater sensitivity to the extracts than the Gram-negative bacteria, *E. coli*, *P. mirabilis*, *S. enterica* and *C. freundii*, which could be due to the presence of outer membrane<sup>[23]</sup>. Outer membrane of Gram-negative bacteria provides a formidable barrier that blocks the penetration of extracts<sup>[23]</sup>.

The effectiveness of an antimicrobial agent is determined by its ability to inhibit or kill bacteria<sup>[3]</sup>. DCS extract showed strong inhibition effect on some bacteria tested. The MICs of DCS extract ranged from 1.56 to 6.25 mg/ml (Table 3). MBCs ranged from 25 to upper 50 mg/ml (Table 3). The observed antibacterial ability of DCS seed extract on the selected bacterial pathogen *in vitro* could imply that the extract may indeed be effective *in vivo* as claimed by traditional healers.

Fig. 3 shows the cytotoxic effects of DCS extract on Vero cells. The LC<sub>50</sub> value was 63.06 µg/ml. The extract was toxic to the Vero cell lines and the pathogens with SI values ranging from 0.01 to 0.04 (Table 3). With SI



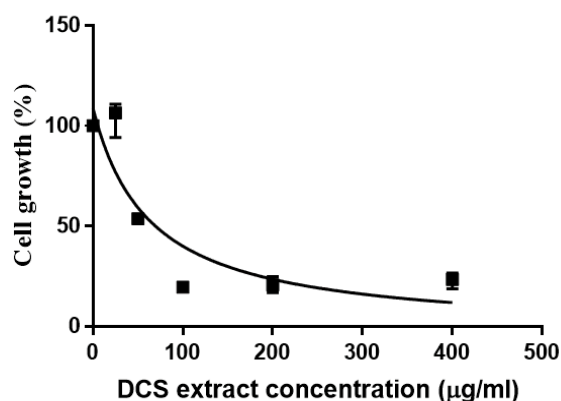
**Fig. 2: Antibacterial activity of DCS ethanol extracts**  
Antibacterial activity of DCS ethanol extract against the bacterial pathogens in a concentration-dependent manner following agar-well diffusion method. The bacteria were treated with 150 (1), 100 (2), 50 mg/ml (3) or solvent ethanol for control (4). (A) *S. aureus* 6538, (B) *S. aureus* 25923, (C) *L. monocytogenes* 13932 and (D) *R. equi* 6939. Ethanol solvent was used as negative control. Data presented are the means of three replicates

**TABLE 2: ANTIMICROBIAL ABILITY OF DCS EXPRESSED AS INHIBITION ZONES (mm)**

Organism	Extract concentration (mg/ml)			
	0	50	100	150
<i>S. aureus</i> ATCC 6538	-	9.00±0.58	10.60±0.58	12.18±0.43
<i>S. aureus</i> ATCC 25923	-	7.13±0.42	8.46±0.49	10.47±0.44
<i>R. equi</i> ATCC 6939	-	6.02±0.19	8.34±0.23	10.50±0.32
<i>L. monocytogenes</i> ATCC 13932	-	6.83±0.42	8.14±0.19	9.03±0.34
<i>P. mirabilis</i> ATCC 25933	-	-	3.49±1.29	5.17±1.00
<i>C. freundii</i> ATCC 8090	-	-	-	-
<i>S. enterica</i> ATCC 14028	-	-	-	-
<i>Escherichia coli</i> ATCC 8739	-	-	-	-
<i>Escherichia coli</i> ATCC 25922	-	-	-	-

**TABLE 3: MICs, MBCs AND SI OF DCS EXTRACT**

Organism	DCS extract			Ampicillin	
	MIC (mg/ml)	MBC (mg/ml)	Selectivity index (SI)	MIC (µg/ml)	MBC (µg/ml)
<i>S. aureus</i> ATCC 6538	3.13	25	0.02	1.56	12.5
<i>S. aureus</i> ATCC 25923	6.25	25	0.01	1.56	3.13
<i>R. equi</i> ATCC 6939	1.56	50	0.04	25	>25
<i>L. monocytogenes</i> ATCC 13932	3.13	>50	0.02	0.78	6.25



**Fig. 3: Effect of DCS extract on cell proliferation of Vero cells**  
Vero cells at a density of  $1 \times 10^4$  cells/ml were treated with 25, 50, 100, 200 or 400 µg/ml DCS extract or ethanol alone (0 µg/ml DCS) as control for 48 h. The cells growth inhibition was evaluated by MTT assay. Results were calculated as the percentage of the control values. Data presented are the means of three replicates (n=3)

values of less than 10 against all the bacteria, it appears that the observed antibacterial activity may be a result of general metabolic toxicity.

### Acknowledgements:

We would like to thank Duong Thi Phung Cac and Ngo Thi Phuong Dung (Ho Chi Minh City, Vietnam) for supporting materials using in this study.

### REFERENCES

- Hoang VS, KhamSeng N, Kessler PJA. Trees of Laos and Vietnam: a field guide to 100 economically or ecologically important species. *Blumea* 2004;49:201-349.
- Do HB, Dang QC, Bui XC, Nguyen TD, Do TD, Pham VH, *et al.* Medicine plant and animal in Viet Nam (Cây thuốc và động vật làm thuốc ở Việt Nam) Tập 1. Hanoi, Vietnam: Nhà xuất bản Khoa học và Kỹ thuật; 2006.
- Olajubu F, Akpan I, Ojo D, Oluwalana S. Antimicrobial potential of *Dialium guineense* (Wild.) stem bark on some clinical isolates in Nigeria. *Int J Appl Basic Med Res* 2012;2:58-62.
- Gideon IO, Joachim E, Ehinobu JM. Antioxidant and antimicrobial activities of *Dialium guineense* (Willd) leaf extract. *Pharm Pharmacol Res* 2013;1:1-7.
- Okeke NC, Udeani TK, Onyebuchi UL. Onyebuchi. Wound-healing and antimicrobial properties of dichloromethane fraction of *Dialium guineense* (Wild) fruit coat. *Res Pharm Sci* 2016;11(3):219-26.
- Adeleye AO, Ajiboye TO, Iliasu GA, Abdussalam FA, Balogun A, Ojewuyi OB, *et al.* Phenolic extract of *Dialium guineense* pulp enhances reactive oxygen species detoxification in aflatoxin B(1) hepatocarcinogenesis. *J Med Food* 2014;17(8):875-85.
- Ciulei I, Grigorescu E, S. U. Medicinal plants, phytochemistry and phytotherapy [Plante medicinale, fitochimie si fitoterapie,

Vol. 1]. Bucharest, Romania: Medical ed.; 1993.

- Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat Protoc* 2007;2(4):875-7.
- Nunzia C, Vincenzo L. The Influence of Initial Carbonate Concentration on the Folin-Ciocalteu Micro-Method for the Determination of Phenolics with Low Concentration in the Presence of Methanol: A Comparative Study of Real-Time Monitored Reactions. *Am J Anal Chem* 2011;2:840-8.
- Samatha T, Shyamsundarachary R, Srinivas P, Nanna RS. Quantification of total phenolic and total flavonoid contents in extracts of *Oroxylum indicum* L.Kurz. *Asian J Pharm Clin Res* 2012;5:177-9.
- Ghatak A, Nair S, Vajpayee A, Chaturvedi P, Samant S, Soley K, *et al.* Evaluation of antioxidant activity, total phenolic content, total flavonoids, and LC-MS characterization of *Saraca asoca* (Roxb.) De.Wilde. *Int J Adv Res* 2015;3(5):318-27.
- Jayanthi P, Lalitha P. Reducing power of the solvent extracts of *Eichhornia crassipes* (mart.) solms. *Int J Pharm Pharm Sci* 2011;3:126-8.
- Finn RK. Theory of Agar Diffusion Methods for Bioassay. *Anal Chem* 1959;31(6):975.
- Poonacha N, Nair S, Desai S, Tuppad D, Hiremath D, Mohan T, *et al.* Efficient Killing of Planktonic and Biofilm-Embedded Coagulase-Negative Staphylococci by Bactericidal Protein P128. *Antimicrob Agents Chemother* 2017;61(8):e00457-17.
- Rampersad SN. Multiple Applications of Alamar Blue as an Indicator of Metabolic Function and Cellular Health in Cell Viability Bioassays. *Sensors* 2012;12:12347-60.
- Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc* 2008;3:163-75.
- Thanh DM, Frentzel H, Fetsch A, Krause G, Appel B, Mader A. Tenacity of *Bacillus cereus* and *Staphylococcus aureus* in dried spices and herbs. *Food Control* 2018;83:75-84.
- Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, *in vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pac J Trop Biomed* 2013;3(8):623-7.
- Waqas MK, Saqib NU, Rashid SU, Shah PA, Akhtar N, Murtaza G. Screening of various botanical extracts for antioxidant activity using DPPH free radical method. *Afr J Tradit Complement Altern Med* 2013;10:452-55.
- Mehrotra S, Jamwal R, Shyam R, Meena DK, Mishra K, Patra R, *et al.* Antihelicobacter pylori and antioxidant properties of *Emblica officinalis* pulp extract: a potential source for therapeutic use against gastric ulcer. *J Med Plants Res* 2011;5:2577-583.
- Okoh SO, Asekun OT, Familoni OB, Afolayan AJ. Antioxidant and Free Radical Scavenging Capacity of Seed and Shell Essential Oils Extracted from *Abrus precatorius* (L). *Antioxidants* 2014;3:278-87.
- Loganayaki N, Siddhuraju P, Manian S. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. *J Food Sci Technol* 2013;50(4):687-95.
- Delcour AH. Outer membrane permeability and antibiotic resistance. *Biochim Biophys Acta* 2009;1794:808-16.