Malaysian Journal of Chemistry, 2025, Vol. 27(1), 236-241
(Special Issue: *International Conference in Organic Synthesis, ICOS 2024*)

Phytochemical Constituents from the Twigs of *Dipterocarpus kerrii* (Dipterocarpaceae) and their Antibacterial Activity

Siti Zakirah Azahar[1,3], Nursyafiqah Syahzanani Mohd Nizam[1], Puteri Nursyamellia Dayana Suhaimi[1], Mohd Helmy Yusof[2], Nik Fatini Nik Azmin[1], Nurunajah Ab. Ghani[1,3], Norizan Ahmat[2] and Aisyah Salihah Kamarozaman[2,3]*

[1]Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]Centre of Foundation Studies, Universiti Teknologi MARA, Cawangan Selangor, Kampus Dengkil, 43800 Dengkil, Selangor, Malaysia
[3]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA, Cawangan Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia
*Corresponding author (email: aisyah2910@uitm.edu.my)

Dipterocarpus is the third most diverse genus within the Dipterocarpaceae family. In addition to its importance in timber production, this genus exhibits a wide spectrum of bioactive properties due to the presence of resveratrol oligomers, terpenoids, flavonoids, and other phenolic compounds. In this study, *Dipterocarpus kerrii* was selected for phytochemical investigation and the examination of the antibacterial properties of its crude extract against *Bacillus cereus* (*B. cereus*) and *Staphylococcus aureus* (*S. aureus*). Acetone maceration of the granules (650 g) from the twigs of *D. kerrii* yielded a crude acetone extract (178 g). Fractionation of the crude extract via VLC resulted in four semi-purified fractions (DK 1-4). Purification of the fractions using several chromatographic methods has yielded two pure compounds. The structural elucidation of the purified compounds was accomplished by spectroscopic analyses and comparison with literature data. The compounds were identified as apigenin (**1**) (5 mg) and scopoletin (**2**) (4 mg). The antibacterial activity of the crude acetone extract was assessed using disk diffusion and minimum inhibitory concentration (MIC) test. The results indicated that the crude acetone extract of *D. kerrii* demonstrated antibacterial properties against both *B. cereus* and *S. aureus*. At 50 mg/disc, the inhibition zones on *B. cereus* and *S. aureus* were 11.0 $\pm$ 1.00 and 13.6 $\pm$ 1.15 mm, respectively. Meanwhile, the MIC values of *B. cereus* and *S. aureus* were 1.56 mg/mL and 0.78 mg/mL, respectively.

Keywords: *Dipterocarpus kerrii*; phytochemical constituents; antibacterial activity; *Bacillus cereus*; *Staphylococcus aureus*

Received: September 2024; Accepted: December 2024

The *Dipterocarpus* is known as "keruing" by the locals and is a member of the Dipterocarpaceae family, which consists of 75 species. *Dipterocarpus* is the third largest genus in the family of 16 genera after *Shorea* (194) and *Hopea* (104) and is widely distributed in tropical regions of Asia, particularly in Southeast Asia, such as Malaysia, Indonesia, Cambodia, Thailand and Philippines [1]. Although the tree of *Dipterocarpus* is a source of "keruing" timber, it was also reported to exhibit a variety of potential pharmacological properties such as antioxidant, antibacterial [2], anti-inflammatory [3], cytotoxic [4], antidiabetic and anti-plasmodial [5]. Methanol extract from the leaves of *D. turbinatus* showed significant antidiabetic activity by inhibiting alpha-amylase activity at 50% concentration with an IC_{50} value of 38.40 µg/mL [6]. A chemical constituent isolated from the heartwood of *D. costatus*, named 20(*S*)-29-hydroxy-17α,20-peroxy-28-norlupan-3-one, exhibited potent anti-plasmodial activity against chloroquine-resistant strain FcB1 of *Plasmodium falciparum* with an IC_{50} value of 3.74 µM compared to chloroquine used as positive control (IC_{50} of 73 nM) [7]. In addition, a resveratrol oligomer isolated from the acetone extract of the bark of *D. hasseltii*, known as hopeaphenol, showed strong inhibition against murine leukemia P-388 cells [8]. The twig extract of *D. alatus* displayed the lowest MIC and MBC against Methicillin-Resistant *Staphylococcus aureus* (MRSA) at 250 and 500 µg/mL, respectively, while a terpene, α,β-gurjunene isolated from this plant, showed good antibacterial activity against the same bacteria strain with MIC and MBC value at 250 µg/mL [9].

The prior study in 2024 on the twigs of *D. kerrii* has reported the isolation of two resveratrol oligomers namely (-)-laevifonol and (+)-α-viniferin as well as the potential of its crude acetone extract against free radicals such as 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and hydroxyl radicals with the IC_{50} values of 11.20 ± 0.54 and 32.37 ± 0.28 µg/mL, respectively [10]. In continuation of the previous study, herein we discuss the addition of two phenolic

†Paper presented at the International Conference in Organic Synthesis 2024 (ICOS 2024)

compounds and the antibacterial activity of the twigs of *D. kerrii*.

EXPERIMENTAL

Sample Collection

The twigs of *D. kerrii* were collected from the Jengka Reserve Forest, Universiti Teknologi MARA, Jengka Campus, Cawangan Pahang. The species was identified by Mr. Nik Hazlan Nik Hashim and the voucher specimen (FSG5) was deposited at the Forest Research Institute Malaysia (FRIM) herbarium.

Extraction

A 650 g sample of the dried twigs was macerated in 5 liters of acetone at room temperature for 24 hours. This process was repeated thrice. The extract was then filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator at 50 °C and 100 rpm to yield the crude acetone extract (178 g).

Fractionation and Isolation

Thin layer chromatography (TLC) was performed on aluminium plates coated with Merck Kiesel gel 60 F254 silica gel. Spots were applied with a fine glass capillary tube, and a gradient solvent system of *n*-hexane and ethyl acetate was used to increase the polarity. The spots were visualized under UV light at 254 nm and 356 nm. For vacuum liquid chromatography (VLC), silica gel 60 (MERCK 1.07747) was used, while silica gel with a 70-230 mesh size was employed for sample preparation. In column chromatography (CC), silica gel 60 (0.2-0.5 mm, MERCK 1.07733) was used to prepare the column, and silica gel 60 (0.040-0.063 mm, MERCK 1.09385) was used for the sample preparation. The crude extract (10.9 g) was subjected to vacuum liquid chromatography (VLC) with the solvent system *n*-hexane: ethyl acetate (hex: EtOAc) in increasing polarity to give four semi-purified fractions, DK1-4. Fraction DK2 (4.5 g) was further purified using column chromatography using eluent hex: chloroform in ascending polarity, which afforded two pure compounds, **1** (5 mg) and **2** (4 mg).

Phytochemical Screening

Phytochemical screening methods were employed to qualitatively confirm the presence of key compound classes, including alkaloids, flavonoids, saponins, tannins, and terpenoids, following the procedures outlined by [11].

Bacterial Strains

Antibacterial properties were tested by using Gram-positive bacteria such as *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778. These bacteria were obtained from the microbe collection of the Faculty of Applied Sciences, UiTM Shah Alam.

Disc Diffusion Assay

The antibacterial activity of *D. kerrii* extract was tested by using the Kirby-Bauer disc diffusion method [12]. The crude extract was dissolved in dimethyl sulfoxide (DMSO). Blank discs (6 mm diameter) were loaded with 10 µL of crude extract with a final concentration of 50 mg/disc. Chloramphenicol served as a positive control and 1% DMSO was used as the negative control. A microbial suspension was prepared according to the 0.5 McFarland standard, resulting in a concentration of 5 x 10^6 CFU/mL. This suspension was streaked on the entire surface of Mueller Hinton (MH) agar plates with a sterile swab. The impregnated discs with extracts, along with positive and negative controls, were placed on the agar. The plates were incubated at 37 °C for 18-24 hours. The experiment was performed in triplicate and the antibacterial activity was evaluated by measuring the diameter of the inhibition zones around the discs.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) was determined using the microdilution method as per the Clinical and Laboratory Standards Institute protocol [12]. Hundred µL of Mueller Hinton broth was added to the wells in columns A2 to A10, B2 to B10, and C2 to C10 of a sterile 96-well microtiter plate. Next, 200 µL of extract, initially at a concentration of 100 mg/mL, was added to the first wells (A1, B1, C1). Two-fold serial dilutions were carried out by transferring 100 µL from one well to the next, resulting in extract concentrations ranging from 0.195 to 100 mg/mL. A suspension of *S. aureus*, adjusted to 5×10^6 CFU/mL, was prepared, and 10 µL of this suspension was added to each well. Growth control wells contained only Mueller Hinton broth and bacterial suspension (without extracts), while sterility control wells contained Mueller Hinton broth and extracts (without bacteria) to check for contamination or inherent antibacterial activity. A blank control with serial dilutions of extracts in uninoculated. Mueller Hinton broth was also prepared to detect any potential colour interference from the extracts. The plates were incubated at 37 °C for 24 hours. The MIC was recorded as the lowest concentration where no visible growth or turbidity was observed. The procedure was repeated for *B. cereus*. After the determination of MIC, MBC was assessed. Ten µL samples from the MIC clear wells were transferred onto Mueller Hinton agar. The inoculum was streaked evenly on the agar plates with a sterile loop. The plates were then incubated at 37 °C for 24 hours. The MBC was the lowest concentration at which no visible bacterial growth was observed on the agar plate.

RESULTS AND DISCUSSION

Isolated Compounds

Compound **1** was obtained as a yellowish crystal. The UV absorption in MeOH at λ_{max} 267 and 336 nm showed the presence of phenolic compound, and the IR spectrum demonstrated absorption at ν_{max} 3335 (OH), 1710 (C=O) and 1619 (aromatic C=C) cm^{-1}. Two *ortho*-coupled doublet signals at δ_H 7.93 (d, *J* = 9.0 Hz, H-2',6') and 7.01 (d, *J* = 9.0 Hz, H-3',5') indicated the presence of a 1,4-disubstituted aromatic ring (B). The two *meta*-coupled doublet signals appeared at δ_H 6.53 (d, H-6) and 6.25 (d, H-8) with coupling constant *J* = 2.0 Hz, representing a 1,2,4,6-tetrasubstituted aromatic ring (A). A singlet signal at δ_H 6.64 (H-3) represents an olefinic proton at ring C which indicates the characteristic of a flavone skeleton.

The ^{13}C-APT NMR spectrum showed 15 signals which correspond to the skeleton of a flavonoid. The presence of four oxyaryl carbons at δ_c 161.1 (C-5), 162.4 (C-7), 157.9 (C-9) and 160.5 (C-4') and two quaternary aromatic carbons at δ_c 105.2 (C-10) and 122.2 (C-1') supported the presence of rings A and B. Meanwhile, the signal at δ_c 164.2 belongs to C-2 of ring C. Methines aromatic signals were observed at δ_c 128.3 (C-2'/6'), 115.9 (C-3'/5'), 98.8 (C-6) and 93.8 (C-8). The signal at δ_c 182.2 (C-4) indicated the presence of a carbonyl group, which is attached to ring C. A signal of C-3 was observed at δc 103.1, supporting that this compound is a flavone-type structure (**Table 1**). The comparison of the ^{1}H and ^{13}C-APT NMR spectra of compound **1** with literature data [13] led to the identification of apigenin (**1**) (**Figure 1**).

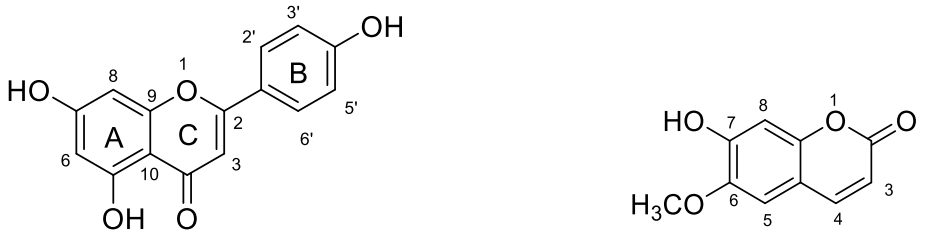
Compound **2** was obtained as a pale yellowish crystal. The UV absorption in MeOH at λ_{max} 228 and 345 nm showed the characteristic of a coumarin class of compound. It is supported by the IR absorptions at ν_{max} 3425 (OH), 2944, 2832 (C_{sp}^3- H stretch), 1703 (C=O) and 1455 (C=C Ar) cm^{-1}. The ^{1}H NMR spectrum displayed two singlet signals at δ_H 7.19 (H-5) and 6.78 (H-8), which indicated the presence of a 1,2,4,5-tetrasubstituted aromatic ring. A pair of doublet signals at δ_H 6.17 (*J* = 9.5 Hz, H-3) and 7.86 (*J* = 9.5 Hz, H-4) suggested the occurrence of olefinic protons in a *trans* configuration. A singlet signal at δ_H 3.87 with the integration of three protons corresponds to the methoxy group (OCH_3-6a).

The ^{13}C-APT NMR spectrum revealed the presence of three oxyaryl carbons at δ_c 151.9 (C-7), 151.7 (C-8a) and 146.0 (C-6), as well as a quaternary aromatic carbon at δ_c 112.0 which belongs to C-4a. There were two methine olefinic carbons observed at δ_c 113.3 (C-3) and 144.7 (C-4) as well as two methine aromatic signals at δ_c 109.9 (C-5) and 103.7 (C-8). A signal demonstrated at the most downfield region, δ_c 161.3 (C-2) indicates the presence of a carbonyl group in the structure. The signal at δ_c 56.7 (C-6a) exhibited the presence of one methyl carbon which belongs to the methoxy group that is attached to an aromatic ring. Comparison of the ^{1}H and ^{13}C-APT NMR spectra with literature data [14] led to the identification of compound **2** as scopoletin (**Figure 1**).

Table 1. NMR spectroscopic data of compounds **1** and **2**.

Position	**1**		Position	**2**	
	δ_H (mult, *J* in Hz)	δ_C		δ_H (mult, *J* in Hz)	δ_C
1	-	-	1	-	-
2	-	164.2	2	-	161.3
3	6.64 (s)	103.1	3	6.17 (d, 9.5)	113.3
4	-	182.2	4	7.86 (d, 9.5)	144.7
5	-	161.1	4a	-	112.0
6	6.53 (d, 2.0)	98.8	5	7.19 (s)	109.9
7	-	162.4	6	-	146.0
8	6.25 (d, 2.0)	93.8	6a-OCH_3	3.87 (s)	56.7
9	-	157.9	7	-	151.9
10	-	105.2	8	6.78 (s)	103.7
1'	-	122.2	8a	-	151.7
2'/6'	7.93 (d, 9.0)	128.3	-	-	-
3'/5'	7.01 (d, 9.0)	115.9	-	-	-
4'	-	160.5	-	-	-

NMR spectra recorded at 600 MHz (^{1}H) and 150 MHz (^{13}C-APT) in acetone- d_6



1 **2**

Figure 1. Structure of compounds **1** and **2**.

Table 2. Phytochemical screening of *D. kerrii* crude extract.

Crude	Screening Test				
	Shinoda Test (Flavonoids)	Mayer's Test (Alkaloids)	Foam Test (Saponins)	Ferric Chloride Test (Tannins)	Salkowski Test (Terpenoids)
D. kerrii	+	+	+	+++	++

(+): present
(++): moderately present
(+++): significantly present

Phytochemical Screening

The crude acetone extract of *D. kerrii* was found to contain all the major classes of compounds. Specifically, tannins were present in a significant amount (+++), indicating a high concentration in the crude extract. In contrast, flavonoids, alkaloids, and saponins were found in relatively low amounts (+). Additionally, the Salkowski test revealed a moderate presence (++) of terpenoids in the crude extract (**Table 2**).

Antibacterial Activity

The antibacterial activity results indicated that the crude acetone extract of *D. kerrii* inhibited the growth of *S. aureus* and *B. cereus*. At a concentration of 50 mg/disc, the inhibition zones measured were 13.6 $\pm$ 1.15 and 11.0 $\pm$ 1.00 mm for *S. aureus* and *B. cereus*, respectively. **Table 3** shows the zone of inhibition for *D. kerrii* extract against *S. aureus* and *B. cereus*. As stated by [15], the activity was classified as inactive if the inhibition zone was less than 7 mm, weakly active for inhibition between 7 and 10 mm, moderately active for inhibition between 10 and 15 mm, and significantly active if the inhibition is greater than 16 mm. At the inhibition zones of 13.6 mm and 11.0 mm, the crude acetone extract was classified as moderately active against both bacterial strains, as these values fall within the 10 to 15 mm range. The MIC values indicated the lowest concentration of the crude extract required to inhibit the visible bacterial growth. For *S. aureus*, the MIC was 0.78 mg/mL, and for *B. cereus*, it was 1.56 mg/mL. Lower MIC values suggest higher potency of the extract against these bacteria. MIC tests on *S. aureus* and *B. cereus* are shown in **Table 4.**

The MBC values represent the lowest concentration of the extract needed to kill 99.9% of the bacteria. The MBC of the crude acetone extract was found to be 50 mg/mL for *S. aureus* and 25 mg/mL for *B. cereus*. **Table 4** showed MBC test on *S. aureus* and *B. cereus*. These values indicate the extract's effectiveness in reducing bacterial populations to undetectable levels. Hence, *D. kerrii* demonstrated antibacterial properties against both *S. aureus* and *B. cereus.* The observed inhibition zones suggest that the crude extract has moderate anti-bacterial activity. Additionally, the MIC values confirm that relatively low concentrations of the extract are effective in inhibiting bacterial growth, while the MBC values show their capacity to kill the bacteria at higher concentrations. Overall, these results high-light the potential of *D. kerrii* as a source of antibacterial agents.

Table 3. Zone of inhibition for *D. kerrii* extract against *Staphylococcus aureus* and *Bacillus cereus.*

Bacterial strains	Inhibition zone (mm ± SD)
S. aureus	13.6 ± 1.15
B. cereus	11.0 ± 1.00
Chloramphenicol (+ve control)	24.6 ± 0.82

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests on *Staphylococcus aureus* and *Bacillus cereus.*

Bacterial Species	Minimum Inhibitory Concentration (mg/mL)			Minimum Bactericidal Concentration (mg/mL)		
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
S. aureus	0.78	0.78	0.78	50	50	100
B. cereus	1.56	1.56	0.78	25	25	25

*+ve control; chloramphenicol (concentration 1 mg/mL - 0.00195 mg/mL), MIC (7.81 µg/mL)

CONCLUSION

The phytochemical analysis of the twigs of *D. kerrii* successfully isolated two phenolic compounds, which are a flavonoid, namely apigenin (**1**) and a coumarin, scopoletin (**2**). To the best of our knowledge, this is the first report of the presence of apigenin (**1**) and scopoletin (**2**) in this species. In addition, the crude acetone extract of *D. kerrii* demonstrated antibacterial activity against *B. cereus* and *S. aureus* with inhibition zones of 11.0 ± 1.00 and 13.6 ± 1.15 mm, respectively. Meanwhile, the MIC values of *B. cereus* and *S. aureus* were 1.56 and 0.78 mg/mL, respectively. These results suggest that *D. kerrii* may possess antibacterial properties that are effective against these bacterial strains.

ACKNOWLEDGEMENTS

We would like to express our greatest appreciation to the Ministry of Higher Education, Malaysia for the financial support through the Fundamental Research Grant Scheme (FRGS/1/2022/STG04/UITM/02/24), Faculty of Applied Sciences and Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA for providing the research facilities. We are deeply grateful to Mr. Khairilanuar Zainil and Mr. Aksso Rosli for the plant collection.

REFERENCES

1. Ashton, P. S. (1982) Dipterocarpaceae. Flora Malesiana, series 1. *Netherlands: Martinus Nijhoff Publishers*.

2. Zain, W. Z. W. M., Ahmat, N., Norizan, N. H. and Nazri, N. A. A. M. (2011) The evaluation of antioxidant, antibacterial and structural identification activity of trimer resveratrol from Malaysia's Dipterocarpaceae. *Aust. J. Basic Appl. Sci.*, **5(5)**, 926–929.

3. Chen, Y. S., Chen, C. J., Yan, W., Ge, H. M. and Kong, L. D. (2017) Anti-hyperuricemic and anti-inflammatory actions of vaticaffinol isolated from *Dipterocarpus alatus* in hyperuricemic mice. *Chinese Journal of Natural Medicines*, **15(5)**, 330–340.

4. Le, H. T., Luu, T. N., Nguyen, H. M. T., Nguyen, D. H. T., Le, P. T. Q., Trinh, N. N., Le, V. S., Nguyen, H. D. and Van, H. T. (2021) Anti-bacterial, antioxidant and cytotoxic activities of different fractions of acetone extract from flowers of *Dipterocarpus intricatus* Dyer (Dipterocarpaceae). *Plant Science Today*, **8(2)**, 273–277.

5. Lulan, T. Y., Fatmawati, S., Santoso, M. and Ersam, T. (2020) α-viniferin as a potential antidiabetic and antiplasmodial extracted from *Dipterocarpus littoralis*. *Heliyon*, **6(5)**.

6. Nur, S., Hossain, M. M., Hoque, M., Islam, N., Islam, M. N., Khatun, M. R., Lyzu, C., Seide, V., Attia, S. M., Ahmad, S. F., Al-Mazroua, H. A., Barua, R. and Emran, T. (2024) Antidiabetic, Anti-Inflammatory, Anthelminthic, Cytotoxic, Thrombolytic, Antidiarrheal, and Antipyretic Activity of *Dipterocarpus turbinatus* Leaves:

In Vitro, In Vivo and Computational Insights. *J. Biol. Regul. Homeost. Agents*, **38(7)**, 5437–5453.

7. Satiraphan, M., Thai, Q. D., Pamonsinlapatham, P., Cuyamendous, C., Prost, E., Sittisombut, C., Grellier, P., Garbay, C., Michel, S. and Cachet, X. (2024) 28-Norlupanes and dammaranes from the heartwood of *Dipterocarpus costatus* Gaertn. f. (Dipterocarpaceae) and their cytotoxic and anti-plasmodial activities. *Phytochemistry Letters*, **63**, 31–36.

8. Muhtadi, Hakim, E. H., Juliawaty, L. D., Syah, Y. M., Achmad, S. A., Latip, J. and Ghisalberti, E. L. (2006) Cytotoxic resveratrol oligomers from the tree bark of *Dipterocarpus hasseltii*. *Fitoterapia*, **77**, 550–555.

9. Chatuphonprasert, W., Tatiya-aphiradee, N., Thammawat, S., Yongram, C., Puthongking, P. and Jarukamjorn, K. (2019) Antibacterial and wound healing activity of *Dipterocarpus alatus* Crude extract against methicillin-resistant *Staphylococcus aureus*-induced superficial skin infection in mice. *Journal of Skin and Stem Cell*, **6(1)**.

10. Azahar, S. Z., Adnan, N., Ghani, N. A., Azmin, N. F. N. and Kamarozaman, A. S. (2024) Chemical Constituents and Radical Scavenging Activities from the Twigs of *Dipterocarpus kerrii* King. *Malaysian Journal of Chemistry*, **26(1)**, 8–15.

11. Kamarozaman, A. S., Adnan, N., Saidon, N. A., Rahman, N. F. A., Johari, M. S. M. and Ahmat, N. (2022) Phytochemical screening of Malaysian Dipterocarpaceae species from Kuala Keniam, Taman Negara Pahang. In *IOP Conference Series: Earth and Environmental Science*, **1019**, 1–9.

12. Clinical and Laboratory Standards Institute (2015) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (11th ed.). *CLSI*.

13. Owen, R. W., Haubner, R., Mier, W., Giacosa, A., Hull, W. E., Spiegelhalder, B. and Bartsch, H. (2003) Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food and Chemical Toxicology*, **41(5)**, 703–717.

14. Lin, L. -C., Yang, L. L. and Chou, C. -J. (2002) Constituents from the Stems of *Ecdysanthera rosea*. *J. Chin. Med.*, **13(4)**, 191–195.

15. Doughari, J. H., Pukuma, S. S. and De, D. (2007) Antibacterial activity of some medicinal plants. *African Journal of Biotechnology*, **6(11)**, 1277–1283.