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Chemical constituents of leaves *Dialium cochinchinense* Pierre

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Abstract

The genus *Dialium* belongs to the Caesalpinioid**eae** family, consisting of approximately 30 species distributed in the tropical regions**.** Secondary metabolites from the Dialium genus have been reported to exhibit various biological activities including antioxidant, cytotoxicity and antimicrobial activities**.** This work describes the isolation and characterization of five compounds from the leaves of *Dialium cochinchinense* Pierre. Their structures were established by spectroscopic analysis, including MS and NMR spectra. Accordingly, the isolated compounds were identified to be lupeone (1), *β*-sitostenone (2), *β*-sitosterol (3), daucosterol (4), and dihydrokaempferide (5). To the best of our knowledge, this is the first report of the isolation of compounds 1 and 5 from the genus *Dialium*.

Keywords

Dialium cochinchinense, Caesalpinioideae, Phytochemistry, Terpenoids, Flavonoids

Introduction

Dialium is a genus of legume in the Caesalpinioideae family. The genus consists of approximately 30 species, which most occur in Africa (Junior *et al.*, 2016) with some species in Asia (Schmidt & Nguyen, 2005). Previous phytochemical studies of the Dialium genus revealed the presence of steroids, terpenoids, polyphenols, and saponin (Awantu *et al.*, 2011; Adeleye *et al.*, 2014; Ayessou *et al.*, 2014; Tuo *et al.*, 2015; Ijoma & Ajiwe, 2017; Moronkola *et al.*, 2017). Some bioactivities studies of this genus have reported cytotoxic (Awantu *et al.* , 2011), antimicrobial (Orji *et al.*, 2012; Ajiboye *et al.*, 2015; Ijoma *et al.*, 2016), antioxidant (Ogu *et al.*, 2013), and anticancer activities (Prakash *et al.*, 2013).

Dialium cochinchinese is a significant and well-recognized tree in Vietnam. It is distributed in many regions, but is especially predominant in the Kon Tum and Gia Lai provinces. In Vietnam, several parts of the plant have been used in folk medicine for the treatment of various diseases. The *D.cochinchinense* fruits are also

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used as a supplemental food to improve appetite and digestion. Traditional practitioners use the leaves and fruits of *D. cochinchinese* to treat waterborne parasitic diseases, fever, and malaria. Our previous screening program on plants belonging to the *Dialium* genus in Vietnam found that the ethyl acetate extract of the leaves of *D. cochinchinese* (at a concentration of 1 μg/mL) showed a potent cytotoxic effect against KB cancer cell line. In this study, the isolation and identification of five compounds from the leaves of *D. cochinchinense* were presented. This study aimed to investigate the chemical composition of the leaves of *D. cochinchinense*. This is the first report on the phytochemical of the leaves of this plant.

Materials and Methods

General procedures

All chromatographic solvents were purchased from Sigma-Aldrich (Merck) or redistilled before use. The silica gel plates (Merck silica gel $60F_{254}$) and RP-18 modified silica gel coated with fluorescent indicator $F_{254}S$ plates were used for thin-layer chromatography (TLC). The spots were visualized first under UV light (254nm and 365nm) and by heating silica gel plates sprayed with cerium (IV) sulfate reagent. Column chromatography (CC) was carried out on silica gel (40-63µm, Kieselgel 60, Merck) and on sephadex LH-20 (Merck). A Model Themo Scientific Mel-Tem 3.0 instrument was used for determining the melting points of isolated compounds. NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer. ESI-MS was performed on a LTQ Orbitrap XL^{TM} instrument. The chemical formulas were drawn using ChemBioOffice Ultra Version 14.0 software.

Plant materials

The leaves of *D. cochinchinense* Pierre were collected in Kon Chu Rang (Gia Lai, Vietnam) in 2005 and were identified by Dr. Nguyen Quoc Binh (Vietnam Academy of Science and Technology). The samples were washed, removed damaged leaves, dried at 40°C, powdered and vacuum sealed in plastic bags, then, stored in the sample store room.

Extraction and Isolation

The phytochemical tests were conducted utilizing standard procedures with some modifications as described by Ijoma et *al*. (Ijoma & Ajiwe, 2017). The leaves of *D. cochinchinense* are taken for phytochemical analysis to study the presence of steroids, terpenoids, anthraquinones, phenolic glycosides, saponins and polyphenols. Dried and grounded leaves of *D. cochinchinense* (1.0kg) were successively extracted with *n*-hexane, ethyl acetate, and methanol by maceration (4 times x 4L) for one day at 30°C and then ultrasounded for 30min. The *n*-hexane, ethyl acetate, and methanol solution were concentrated under reduced pressure to dry and to obtain the *n*-hexane (3.7g, 0.37%), ethyl acetate (16.4g, 1.64%) and MeOH (17.5g, 1.75%) crude extracts.

The ethyl acetate crude extract from the leaves (16 g) was chromatographed on a silica gel column (70 x 80cm) by gradient elution with *n*-hexane in the EtOAc system (1:0 to 0:1, each 500 mL) to obtain four fractions (EA1÷EA4). Fraction EA1 (610mg) was subjected to column chromatographic separation over silica gel (2.5 x 60 cm) and eluted with *n*-hexane-EtOAc (1:0 to 0:1, 100mL) as the mobile phase to give ten subfractions (EA1.1÷EA1.10). Fraction EA1.3 (35mg) was further purified on a silica gel column chromatography (1.0 x 40cm) using *n*-hexane-CH_2Cl_2 (100:1, 120mL) to concentrate compound **1** (5.3mg). Fraction EA2 (504mg) was chromatographed on a silica gel column using *n*-hexane-EtOAc (99:1 to 0:1, each 100mL) to provide four subfractions (EA2.1÷EA2.4). Fraction EA2.2 (65mg) was then purified by a silica gel column using *n*-hexane-EtOAc (9/1, 150mL) to yield compounds **2** (3.0mg). Fraction EA4 (1.81g) was chromatographed on a silica gel column using a gradient solvent of *n*-hexane-EtOAc (10:1 to 0:10, each 150mL) as eluent, to yield seven fractions (EA4.1-EA4.7). Fraction EA4.3 (377mg) was further subjected to column chromatography (2.0 x 60cm) with *n*-hexane-EtOAc (9:1, 280mL) to give compound **3** (103mg).

The methanol crude extract (17.0g) was chromatographed on a silica gel column and eluted with a gradient solvent system of *n*-hexane-CH_2Cl_2, and MeOH. A total of 150 fractions (20mL each) were collected and then combined based upon similar TLC profiles to yield 10 main fractions (Me1-Me10). Fraction Me6 (553mg) was further subjected to column chromatography (2.0 x 60cm) and eluted with a CH_2Cl_2-EtOAc-MeOH (85:15:5, 550mL) to give compound **4** (92.2mg). Fraction Me9 (1.3g) was subjected to column chromatographic separation (2.5 x 60cm) over silica gel eluting with a $CHCl_3$-MeOH-formic acid system (5:1:0.1, 750mL) to obtain eight subfractions (MeF9.1-MeF9.8). Fraction MeF9.8 was purified by column chromatographic separation over Sephadex LH-20 with MeOH/CH_2Cl_2 (9/1) as the mobile phase to yield compound **5** (2.0mg).

The structures of compounds **1-5** were identified by comparison of their physical and spectra with the literature values, and also with standards by TLC.

Lupeone (1): $C_{30}H_{48}O$, colourless needles from *n*-hexan-CH_2Cl_2, mp 168-170°C. ^{1}H-NMR (500 MHz, $CDCl_3$, δ_H, *J*/Hz): 0.80 (3H, s, H-28), 0.93 (3H, s, H-25), 0.96 (3H, s, H-26), 1.02 (3H, s, H-27), 1.08 (6H, s, H-23 and H-24), 1.68 (3H, s, H-30), 4.57 (1H, d, *J* = 2.5, H-29b), 4.69 (1H, d, *J* = 2.5, H-29a). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 14.5 (C-27), 15.1 (C-26), 15.9 (C-25), 18.0 (C-28), 19.3 (C-30), 19.7 (C-6), 21.0 (C-24), 21.5 (C-11), 25.2 (C-12), 26.8 (C-23), 27.5 (C-15), 29.9 (C-21), 33.6 (C-7), 34.2 (C-2), 35.5 (C-16), 36.9 (C-10), 38.2 (C-13), 39.6 (C-1), 39.9 (C-22), 40.8 (C-8), 42.9 (C-14), 43.0 (C-17), 47.3 (C-4), 47.9 (C-19), 48.3 (C-18), 49.8 (C-9), 54.9 (C-5), 109.4 (C-29), 150.9 (C-20), 218.2 (C-3).

***β*-Sitostenone (2):** $C_{29}H_{48}O$, white solid, mp 93-94°C. ^{1}H-NMR ($CDCl_3$, 500 MHz, δ_H, ppm, *J*/Hz): 0.70 (3H, s, H-18), 0.81 (3H, d, *J* = 6.8, H-27), 0.83 (3H, d, *J* = 6.8, H-26), 0.84 (3H, t, *J*= 7.4, H-29), 0.91 (3H, d, *J* = 6.5, H-21), 1.17 (3H, s, H-19), 5.71 (1H, s, H-4). ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 11.9 (C-29), 12.0 (C-18), 17.4 (C-19), 18.7 (C-21), 19.0 (C-27), 19.8 (C-26), 21.0 (C-11), 23.1 (C-28), 24.2 (C-15), 26.1 (C-23), 28.2 (C-16), 29.2 (C-25), 32.1 (C-7), 32.9 (C-6), 33.9 (C-2), 34.0 (C-22), 35.6 (C-1), 35.7 (C-8), 36.1 (C-20), 38.6 (C-10), 39.6 (C-12), 42.4 (C-13), 45.8 (C-24), 53.8 (C-9), 55.9 (C-14), 56.0 (C-17), 123.7 (C-4), 171.6 (C-5), 199.6 (C-3).

***β*-sitosterol (3)**: $C_{29}H_{50}O$ white powder, mp 140-142°C. ^{1}H-NMR (500 MHz, $CDCl_3$), δ, ppm, *J*/Hz): 0.68 (3H, s, H-18), 0.82 (3H, d, *J* = 7.0, H-26), 0.83 (3H, d, *J* = 7.0, H-27), 0.85 (3H, t, *J* = 7.0, H-29), 0.92 (3H, d, *J* = 7.0, H-21), 1.01 (3H, s, H-19), 3.52 (1H, m, H-3), 5.35 (1H, t, *J* = 3.0, H-6). ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 12.0 (C-29), 12.0 (C-18), 18.8 (C-21), 19.1 (C-26), 19.4 (C-27), 19.8 (C-19), 21.1 (C-11), 23.1 (C-28), 24.3 (C-15), 26.1 (C-23), 28.3 (C-16), 29.2 (C-25), 31.7 (C-2), 31.9 (C-7), 31.9 (C-8), 34.0 (C-22), 36.2 (C-20), 36.5 (C-10), 37.3 (C-1), 39.8 (C-12), 42.3 (C-4), 42.4 (C-13), 45.9 (C-24), 50.2 (C-9), 56.1 (C-17), 56.8 (C-14), 71.8 (C-3), 121.7 (C-6), 140.8 (C5).

Daucosterol (4): $C_{35}H_{60}O_6$, white solid, mp 272-273°C. ^{1}H-NMR (500 MHz, DMSO-*d*$_6$, δ_H, *J*/Hz): 0.65 (3H, s, H-18), 0.79 (3H, t, *J* = 7.0, H-29), 0.82 (3H, s, H-26), 0.83 (3H, s, H-27), 0.90 (3H, d, *J* = 6.5, H-21), 0.95 (3H, s, H-19), 3.50 (1H, m, H-3), 5.35 (1H, brs H-6). ^{13}C-NMR (125 MHz, DMSO-*d*$_6$): δ_C 11.7 (C-29), 11.8 (C-18), 18.6 (C-21), 19.1 (C-27), 19.7 (C-19), 19.8 (C-26), 20.6 (C-11), 22.6 (C-28), 23.9 (C-15), 25.5 (C-23), 27.8 (C-16), 28.7 (C-25), 29.3 (C-2), 31.4 (C-7), 31.4 (C-8), 33.4 (C-22), 35.5 (C-20), 36.2 (C-10), 36.8 (C-1), 38.2 (C-12), 41.9 (C-4), 39.9 (C-12), 41.9 (C-13), 45.1 (C-24), 49.6 (C-9), 55.4 (C-17), 56.2 (C-14), 61.1 (C-6′) 70.1–77.0 (C-2′-C-5′), 73.4 (C-3), 100.8 (C-1′), 121.2 (C-6), 140.5 (C-5).

Dihydrokaempferide (5): $C_{16}H_{14}O_6$, yellow amorphous powder. ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 3.82 (3H, s, OCH_3-4′), 4.56 (1H, d, *J* = 11.5, H-3), 5.02 (1H, d, *J* = 12.0, H-2), 6.06 (1H, d, *J* = 2.0, H-6), 6.12 (1H, d, *J* = 2.0, H-8), 6.92 (2H, d, *J* = 9.0, H-3′ and H-5′), 7.42 (2H, d, *J* = 8.5, H-2′ and H-6′), 11.18 (1H, s, OH-5). This compound was compared to the standard by TLC.

Results and Discussion

From the leaves of *D. cochinchinense*, five compounds (**1-5**) (**Figure 1)** were isolated and their structures were elucidated.

Compound **1** appeared as colorless needles from *n*-hexan-CH_2Cl_2. Its ESI-MS showed an ion peak at *m/z* 425 $[M+H]^+$ corresponding to the molecular formula of $C_{30}H_{48}O$. The ^{1}H-NMR spectrum of **1** exhibited two doublet protons for two olefinic methylene protons at δ_H 4.69 (H-29a) and 4.57 (H-29b), representing the exocyclic double bond protons, and seven methyl groups at δ_H 1.68 (H-29), 1.08 (H-23 and H-24), 1.02 (H-27), 0.96 (H-26), 0.93 (H-25), and 0.80 (H-28). The ^{13}C- and DEPT-NMR spectrum of **1** showed thirty carbon signals including seven methyl groups (δ_C 14.5, 15.1, 15.9, 18.0, 19.3, 21.0), five methine groups (δ_C 38.2, 47.9, 48.3, 49.8, 54.9), eleven methylene groups (δ_C 19.7, 21.5, 25.2, 27.5, 29.9, 33.6, 34.2, 35.5, 39.6, 39.9, 109,4), five high-field quaternary carbons (δ_C 36.9, 40.8, 42.9, 43.0, 47.3), one olefinic quaternary carbon (δ_C 150.9), and one carbonyl carbon (δ_C 218.2). The ^{13}C-NMR spectrum of compound **1** showed a saturated carbonyl group at δ_C 218.2 and the alkene carbons at δ_C 150.4 and 108.8; suggesting the presence of a lupane triterpene having a carbonyl group in its structure. Our spectroscopic data was consistent with published ones (Wang *et al.*, 2011). Therefore, compound **1** was determined to be lupeone.

Compound **2** appeared as a white solid. The ^{1}H-NMR spectrum of **2** showed a singlet olefinic proton at δ_H 5.74 (H-4). The ^{1}H-NMR spectrum also revealed singlet protons at δ_H 1.18 and 0.70 (each 3H, s) corresponding to two methyl groups at C-10 (H_3-19) and C-13 (H_3-18), respectively. Nine doublet protons at δ_H 0.91 (J = 6.5), 0.83 (J = 6.8) and 0.81 (J = 6.8) were demonstrated for methyl groups at C-20 (H_3-21), and C-25 (H_3-26 and H_3-27). Three triplet protons (J = 7.4) at δ_H 0.84 were observed for another methyl group at C-28 (H_3-29). The ^{13}C-NMR spectrum of **2** showed the resonances of 29 carbons, including one carbonyl group at δ_C 199.6 (C-3) and one olefinic methine carbon at δ_C 123.7 (C-4). In addition, its DEPT-NMR showed signals of six methyl carbons (δ_C 11.9, 12.0, 17.4, 18.7, 19.0, 19.8), eleven methylene groups (δ_C 21.0, 23.1, 24.2, 26.1, 28.2, 32.1, 32.9, 33.9, 34.0, 35.6, 39.6), five methine groups (δ_C 29.2, 35.7, 36.1, 45.8, 53.8, 55.9, 56.0), and three quaternary carbons (δ_C 38.6, 42.4, 171.6). On this basis, the structure of compound **2** was determined to be β-sitostenone.

The identification of compound **2** was confirmed by the comparison of its ^{1}H- and ^{13}C-NMR spectral data with the published values for β-sitostenone (Prachayasittikul *et al.*, 2009).

Compound **3** was isolated as a white powder. The ^{1}H-NMR spectrum of **3** revealed one multiplet proton at δ_H 3.52 (H-3). The typical H-6 of the steroidal skeleton appeared as a multiplet at δ_H 5.35 for one proton. The ^{1}H-NMR spectrum also showed six methyl groups which were similar in the ^{1}H-NMR spectrum of compound **2**. Moreover, the ^{13}C- and DEPT-NMR spectra displayed twenty-nine carbon signals including: 6 sp^3 methyls at δ_C 12.0, 12.0, 17.4, 18.7, 19.0, 19.8), 11 sp^2 methylens at δ_C 21.1, 23.1, 24.3, 26.1, 28.3, 31.7, 31.9, 34.0, 37.3, 39.8, 42.4), 9 sp^2 methines at δ_C 29.2, 31.9, 36.2, 45.9, 50.9, 56.1, 56.8, 71.3, 121.7), and 3 quaternary carbons (δ_C 36.5, 42.2, 140.8). All these properties confirmed that the structure of **3** was β-sitosterol (Mahato & Kundu, 1994).

Compound **4** was determined by comparing its spectral data with the spectral data of compound **3,** and with previously reported values. The ^{1}H-NMR spectrum of compound **4** showed six signals at δ 0.65 (H-18), 0.79 (H-29), 0.82 (H-26), 0.83 (H-27), 0.90 (H-21), and 0.95 (H-19) for methyl groups (-CH_3). One proton at C-3 appeared as a multiplet at δ_H 3.50 ppm. A doublet proton at δ_H 5.35 ppm was the characteristics of a trisubstituted double bond between quaternary carbon (C-5) and methine carbon (C-6). The proton signals at δ_H 3.0-4.0 ppm indicated the presence of protons for the sugar moiety. ^{13}C-NMR indicated that compound **4** consisted of 35 carbons. From DEPT spectra, the carbon signals of **4** additional six carbons in comparison to that of β-sitosterol (**3**) including

five oxygenated methine carbons and one oxygenated methylene carbon. These carbinol signals were assigned for the carbons of glucopyranosyl group. These data indicate the structure of *β*-sitosterol glucoside, in which a O-glucosyl group binding to C-3 of *β*-sitosterol. Above ^{1}H-NMR and ^{13}C-NMR data of **4** revealed it to be *β*-sitosterol-D-glucoside (daucosterol) which was confirmed by Rahmana *et al.* (2009).

Compound **5** was isolated as a yellow amorphous powder. Its ^{1}H-NMR spectrum presented signals of six aromatic protons, including an A_2B_2 system [δ_H 6.92 (2H, d, *J* = 9.0, H-3′ and 5′), 7.42 (2H, d, *J* = 8.5, H-2′ and 6′)] and two doublets with meta-coupling at δ_H 6.06 (*J* = 2.0, H-6) and 6.12 (*J* = 2.0, H-8). The ^{1}H-NMR spectrum also exhibited three protons for two doublet signals at δ_H 4.56 (*J* = 11.5, H-3), 5.02 (*J* = 12.0, H-2), one methoxy group at δ_H 3.82 (OCH_3-4′) and one intramolecular chelated hydroxyl group at δ_H 11.84 (OH-5). The NMR data of **5** was characteristic of a flavonoid which is similar to dihydrokaempferide (Hattori *et al.*, 2011).

Conclusions

The results demonstrated that the five compounds isolated belonged to three structure classifications, including one terpenoid, three steroids and one flavonoid. They were determined to be lupeone (**1**), *β*-sitostenone (**2**), *β*-sitosterol (**3**), daucosterol (**4**), and dihydrokaempferide (**5**). Other compounds are currently under identification. It is important to continue chemical research on these isolated compounds to evaluate their cytotoxic effects in an attempt to discover a new source of bioactive compounds from the flora in Vietnam.

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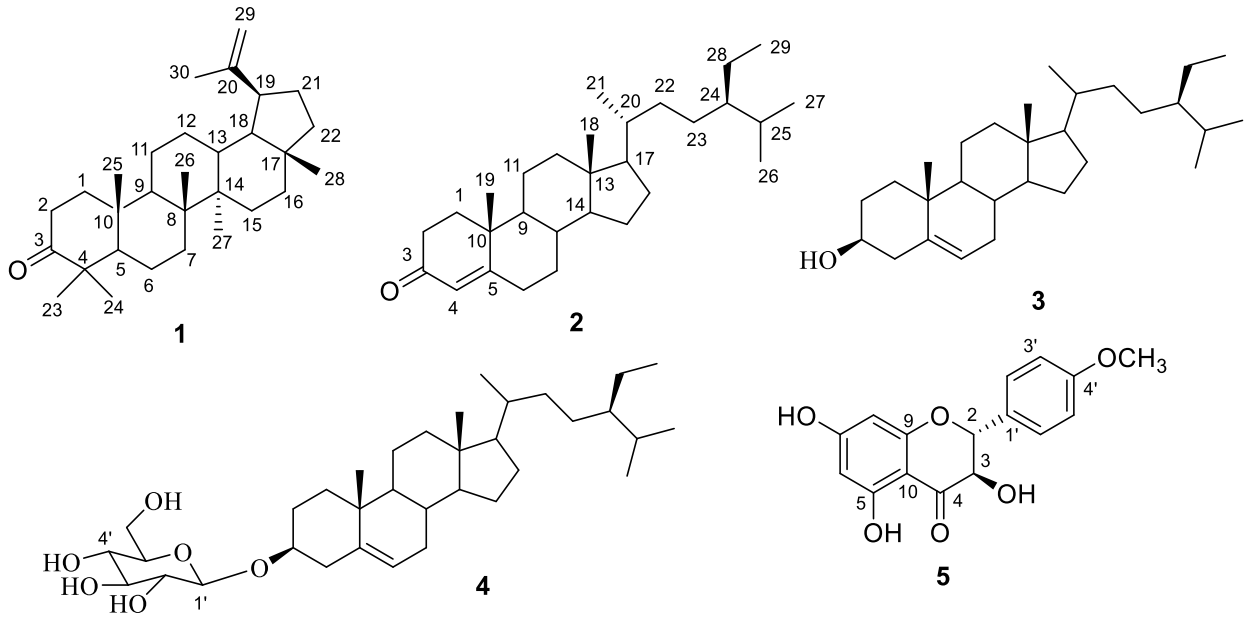

Figure 1. Structures of the compounds isolated from the leaves of *D. cochinchinense*

References

Adeleye A. O., Ajiboye T. O., Iliasu G. A., Abdussalam F. A., Balogun A., Ojewuyi O. B. & Yakubu M. T. (2014). Phenolic extract of *Dialium guineense* pulp enhances reactive oxygen species detoxification in aflatoxin B1 hepatocarcinogenesis. Journal of medicinal food. 17(8): 875-885.

Ajiboye A. E., Ameen M. T. & Adedayo M. R. (2015). Antimicrobial activity and phytochemical screening of the fruit pulp of *Dialium guineense* (Velvet Tamarind) on some microbial isolates. Journal of Microbiology and Antimicrobials. 7(4): 33-41.

Awantu A. F., Lenta B. N., Bogner T., Fongang Y. F., Ngouela S., Wansi J. D., Tsamo E. & Sewald N. (2011). Dialiumoside, an Olean-18-ene Triterpenoid from *Dialium excelsum*. Zeitschrift für Naturforschung B. 66(6): 624-628.

Ayessou N. C., Ndiaye C., Cisse M., Gueye M., Sakho M. & Dornier M. (2014). Nutrient composition and nutritional potential of wild fruit *Dialium guineense*. Journal of Food Composition and Analysis. 34(2): 186-191.

Hattori H., Okuda K., Murase T., Shigetsura Y., Narise K., Semenza G. L. & Nagasawa H. (2011). Isolation, identification, and biological evaluation of HIF-1-modulating compounds from Brazilian green propolis. Bioorganic and Medicinal Chemistry. 19(18): 5392-5401.

Ijoma K. & Ajiwe V. (2017). Phytochemical Screening of *Dialium Indum* Leaf extract (Velvet Tarmarind). International Journal of Phytopharmacy. 7(1): 06-13.

Ijoma K., Ajiwe V. & Awuzie C. (2016). Antimicrobial Analysis and Structural Elucidation of Active Compounds of *Dialium Indum* Leaves Extract (Valvet Tarmarind). International Journal of Pharmaceutical Chemistry. 6(12): 237-241.

Junior M. J. D. A. F., Pinto R. B. & Mansano V. D. F. (2016). A Taxonomic Revision of the genus Dialium (Leguminosae: Dialiinae) in the Netotropics. Phytotaxa. 283(2): 123-142.

Mahato S. B. & Kundu A. P. (1994). ^{13}C-NMR spectra of pentacyclic triterpenoids-a compilation and some salient features. Phytochemistry. 37(6): 1517-1575.

Moronkola D., Kunle O., Olaoluwa O. & Ogukwe C. (2017). Chemical compositions of *Dialium guineense* Willd. leaf, stem-bark and fruit essential oils. Journal of Complementary and Alternative Medical Research. 1-8.

Ogu G. I., Ezeadila J. & Ehiobu J. M. (2013). Antioxidant and antimicrobial activities of *Dialium guineense* (Willd) leaf extract. Pharmacy and Pharmacology Research. 1(1): 1-7.

Orji J., Alo M., Anyim C. & Okonkwo E. (2012). Antibacterial activities of crude leaf and bark extracts of "icheku" *Dialium guineense* on bacterial isolates from bronchitis patients. IOSR Journal of Pharmacy and Biological Sciences. 1: 21-25.

Prachayasittikul S., Suphapong S., Worachartcheewan A., Lawung R., Ruchirawat S. & Prachayasittikul V. (2009). Bioactive metabolites from *Spilanthes acmella* Murr. Molecules. 14(2): 850-867.

Prakash O., Kumar A. & Kumar P. (2013). Anticancer potential of plants and natural products. American Journal of Pharmacological Sciences. 1: 104-115.

Rahmana S., Muktaa Z. A. & Hossainb M. (2009). Isolation and characterization of β-sitosterol-D-glycoside from petroleum extract of the leaves of *Ocimum sanctum* L. Asian Journal of Food and Agro-Industry. 2(1): 39-43.

Schmidt L. H. & Nguyen V. A. (2005). *Dialium cochichinense* Pierre. Seed Leaflet. 91: 1-2.

Tuo K., Béourou S., Touré A. O., Ouattara K., Silué D., Konan D. T., Adagba M., Koffi D., Yao S. & Djaman J. (2015). Phytochemical Screening and Polyphenolic Contents of *Dialium dinklagei* and *Diospyros monbuttensis*, Two Ivorian Medicinal Plants Used to Treat Malaria. Journal of Advances in Medical and Pharmaceutical Sciences. 2(4): 144-153.

Wang C., Li J., Zhang Y. & Zhang Z. (2011). Chemical constituents from the roots of *Senecio scandens*. Chemistry of Natural Compounds. 47(2): 243-245.