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CHEMICAL CONSTITUENTS OF THE DICHLOROMETHANE FRACTION OF *Pterospermum truncatolobatum* COLLECTED IN LANG SON PROVINCE

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Abstract. Phytochemical studies on the dichloromethane fraction of *Pterospermum truncatolobatum* collected in Lang Son province has led to the isolation and structural elucidation of four secondary metabolites, including one rare triterpene possessing E:B-friedo-hopane-type triterpenoid: simiarenol (**1**), two sterols: *β*-sitosterol (**2**), stigmasterol (**3**) and one sterol glycoside: stigmasterol glycoside (**4**). Their structures were identified by spectroscopic analyses. In addition, the structure of **1** was confirmed by X-ray crystallographic analysis. Furthermore, simiarenol (**1**) showed moderate cytotoxicity against three cancer cell lines such as Hep-G2, Lu-1, and MCF-7 with its IC_{50} values of 38.47 ± 2.68; 48.37 ± 3.05 and 46.85 ± 3.6 μg/mL, respectively. All four compounds were isolated from this plant for the first time.

Keywords: *Pterospermum truncatolobatum*, sterol, simiarenol, cytotoxicity.

1. Introduction

The plants of Sterculiaceae family are one of the most important plant families in the natural world. Some of them have been used in traditional medicines in Vietnam, China, etc. for the treatment of many common diseases such as cold, sore throat, diseases related to the digestive system, snakebite wound, and cancer treatment. Many secondary metabolites show antibacterial, anti-inflammatory, antioxidant, and inhibitory effects on cancer cells, etc. Therefore, plants of this family have been studied for their chemical composition and biological activity such as *Abroma augusta* Linn.F, *Cola cordifolia*, *Guazuma ulmifolia* Lam, etc. Thanks to the development of modern separation techniques, various alkaloids, phenylpropanoids, flavonoids, terpenoids and others have been purified, and identified from the Sterculiaceae plants [1]. The compounds isolated from plants of this family can be divided into five groups including the cyclopeptide

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alkaloid group, phenylpropanoid group, flavonoid, terpenoid, and other compounds [2-4]. *Pterospermum truncatolobatum* Gagnep. is a rare plant which grows wildly in several areas of Vietnam such as the provinces of Quang Binh, Quang Tri, Hue, and Lang Son. Its chemical constituents remain unknown. In the course of our investigation on the biologically active compounds from Vietnamese medicinal plants, we have collected *Pterospermum truncatolobatum* in Lang Son province that allowed us to study its chemical constituents and the result is reported in this paper.

2. Content

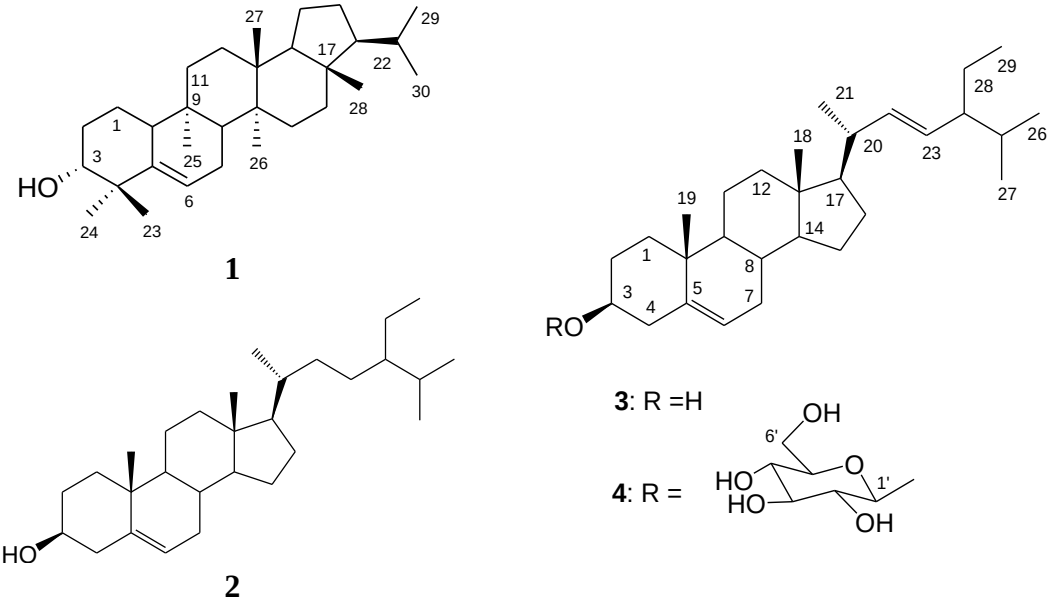
2.1. Material and methods

2.1.1. Plant material

The aerial parts of *Pterospermum truncatolobatum* were collected in Bac Son, Lang Son province in March 2018 and identified by Nghiem Duc Trong (Hanoi University of Pharmacy). A voucher specimen (LKL-02) has been deposited at the Faculty of Chemistry, Hanoi University of Education, Vietnam.

2.1.2. General procedure

TLC was carried out on precoated Si gel GF_{254} (Merck). TLC spots were viewed at 254, 302, and 366 nm and visualized by spraying with 10% H_2SO_4 in methanol followed by heating until the spots appeared. Column chromatography was carried out on silica gel 60 (60 - 100 μM, Merck). Preparative HPLC was performed on a Jasco PU-2087 instrument with a UV-2070 (254 nm) and RI-2031 detectors using a Waters 5 SL-II column (10.0 × 250 mm), the flow rate of 1.0 mL/min. 1D and 2D NMR (^1H, ^{13}C NMR, HSQC, HMBC, and ROESY) spectra were recorded on a Bruker Avance 500 MHz Instrument. MS was measured on an Agilent 1260 series single quadrupole LC/MS system. X-ray reflection data was measured on a Bruker D8 Advance.



1 **2**

Figure 1. Structures of compounds 1-4

2.1.3. Extraction and Isolation

The fresh leaves and stems of *Pterospermum truncatolobatum* (4.0 kg) were dried and powdered. Then, it was extracted with methanol to afford the crude methanol extract (131 g), which was partitioned between *n*-hexane, CH_2Cl_2, EtOAc, butanol, and water. The CH_2Cl_2 fraction (8.1 g) was chromatographed on a silica gel column, eluting with *n*-hexane/EtOAc gradient (from 7/1 to 0/1, v/v) to give 11 sub-fractions. Sub-fr. 2 (36 mg) was further isolated by prep-HPLC with *n*-hexane/EtOAc (7/1) to yield compound **1** (9.1 mg), retention time 29.5 min. Sub-fr. 4 (40.5 mg) was purified by silica gel column, *n*-hexane/EtOAc (5/1) to give compound **2** (4.1 mg). Compound **3** (1.4 mg) was obtained from sub-fr. 5 (14.2 mg) by prep. HPLC with *n*-hexane/EtOAc (7/1), retention time 34.2 min. Finally, compound **4** (4.0 mg) was collected by washing the precipitate of the sub-fr. 11 (325 mg) in MeOH several times.

2.1.4. Spectral data for 1-4

Simiarenol (**1**): ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.61 (dd, J = 2.0, 4.0 Hz, H-6), 3.47 (t, J = 2.5 Hz, H-3), 1.14 (s, H-24), 1.05 (s, H-23), 0.99 (s, H-26), 0.93 (s, H-27), 0.90 (s, H-25), 0.89 (d, J = 6.5 Hz, H-29), 0.83 (d, J = 6.5 Hz, H-30), 0.78 (s, H-28). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 142.0 (C-5), 122.0 (C-6), 76.4 (C-3), 60.1 (C-21), 51.8 (C-18), 50.3 (C-10), 44.3 (C-8), 42.8 (C-17), 40.9 (C-4), 39.4 (C-14), 38.7 (C-13), 35.5 (C-16), 34.9 (C-9), 34.2 (C-11), 30.8 (C-22), 29.2 (C-23), 29.1 (C-15), 29.0 (C-12), 28.3 (C-20), 27.8 (C-2), 25.5 (C-24), 24.1 (C-7), 22.9 (C-30), 22.0 (C-29), 19.9 (C-19), 18.1 (C-1), 17.9 (C-25), 16.1 (C-28), 15.8 (C-26), 15.0 (C-27). ESI-MS: m/z 427.2 $[M+H]^+$.

*Crystal data for **1***: Program used to solve structure ShelXT [6]. Refinement: on Least Squares minimisation. Crystal Data for $C_{30}H_{50}O$ (M = 426.70 g/mol): monoclinic, space group C2 (no. 5), a = 11.4354(13) Å, b = 7.3639(8) Å, c = 31.213(4) Å, β = 98.713(4)°, V = 2598.0(5) Å^3, Z = 4, T = 273.15 K, μ(MoKα) = 0.063 mm^{-1}, *Dcalc* = 1.091 g/cm^3, 17849 reflections measured (6.604° ≤ 2Θ ≤ 51.7°), 4705 unique (R_{int} = 0.0659, R_{sigma} = 0.0742) which were used in all calculations. The final R_1 was 0.0843 (I > 2σ(I)) and wR_2 was 0.2031 (all data).

β-sitosterol (**2**): ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.33 (t, J = 2.0 Hz, H-6), 3.52 (m, H-3), 1.01 (s, H-19), 0.92 (d, J = 6.5 Hz, H-21), 0.85 (t, J = 6.5 Hz, H-29), 0.83 (d, J = 7.0 Hz, H-26), 0.82 (d, J = 5.5 Hz, H-27), 0.68 (s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.2 (C-9), 45.9 (C-24), 42.3 (C-4, C-13), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.2 (C-20), 34.0 (C-22), 31.9 (C-7, C-8), 31.7 (C-2), 29.2 (C-25), 28.3 (C-16), 26.1 (C-23), 24.3 (C-15), 23.1 (C-28), 21.1 (C-11), 19.8 (C-26), 19.0 (C-19), 18.9 (C-21), 18.8 (C-27), 12.0 (C-29), 11.9 (C-18).

Stigmasterol (**3**): ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.35 (t, J = 2.0 Hz, H-6), 5.16 (dd, J = 9.0, 15.0 Hz, H-23), 5.02 (dd, J = 9.0, 15.0 Hz, H-22), 3.51 (m, H-3), 1.03 (d, J = 7.0 Hz, H-21), 1.02 (s, H-19), 0.85 (d, J = 7.0 Hz, H-26), 0.83 (t, J = 8.5 Hz, H-29), 0.80 (d, J = 7.5 Hz, H-27), 0.70 (s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 140.8 (C-5), 138.3 (C-22), 129.3 (C-23), 121.7 (C-6), 71.8 (C-3), 56.9 (C-14), 56.1 (C-17), 51.3 (C-24), 50.2 (C-9), 42.4 (C-13), 42.3 (C-4), 40.5 (C-20), 39.7 (C-12), 37.3 (C-1), 36.6 (C-10), 31.9 (C-2, C-7, C-8), 31.7 (C-25), 28.9 (C-16), 25.4 (C-28), 24.4 (C-15), 21.2 (C-21), 21.1 (C-11, C-26), 19.4 (C-27), 19.0 (C-19), 12.2 (C-18), 12.1 (C-29).

Stigmasterol glucoside (**4**): ^{1}H NMR (500 MHz, C_5D_5N): δ_H 5.16 (m, H-22), 5.02 (m, H-23), 4.99 (d, *J* = 8.0 Hz, H-1'), 3.95 (m, H-3), 1.21 (s, H-19), 1.04 (d, *J* = 6.5 Hz, H-21), 0.88 (t, *J* = 6.5 Hz, H-29), 0.87 (d, *J* = 6.5 Hz, H-26), 0.78 (d, *J* = 7.0 Hz, H-27), 0.64 (s, H-18). ^{13}C NMR (125 MHz, C_5D_5N): δ_C 140.8 (C-5), 138.6 (C-22), 129.4 (C-23), 121.8 (C-6), 102.3 (C-1'), 78.2 (C-3', C-5'), 78.1 (C-2'), 75.0 (C-4'), 71.4 (C-3), 62.5 (C-6'), 56.7 (C-14), 56.1 (C-17), 51.3 (C-24), 50.2 (C-9), 42.4 (C-13), 40.6 (C-20), 39.8 (C-12), 39.2 (C-4), 37.3 (C-1), 36.8 (C-10), 32.0 (C-2, C-25), 31.9 (C-7), 31.2 (C-8), 29.4 (C-16), 25.5 (C-28), 24.4 (C-15), 23.3 (C-11), 21.3 (C-21), 19.8 (C-19), 19.3 (C-26), 12.4 (C-27, C-29), 11.8 (C-18).

2.2. Results and discussion

Compound **1** was isolated as white crystals. Its ESI-MS has a *quasi*-molecular ion peak at *m/z* 427.2 $[M+H]^+$, corresponding to the molecular formula of $C_{30}H_{50}O$ with 6 degrees of unsaturation. Its ^{1}H NMR spectrum showed the presence of one olefinic proton, one carbinol, eighth methyls. Analysis of the ^{13}C NMR spectrum indicated that this compound contained 30 carbon atoms, including two olefinic carbons (142.0 and 122.0 ppm), one carbinol carbon (76.4 ppm), and the other as shown in the experimental section. Its 1D NMR suggested that compound **1** is a triterpenoid [7]. The hydroxyl group was located at C-3 due to HMBC correlations between i) H-3 and C-1, C-5; ii) H-23, H-24, and C-3. Furthermore, the double bond was deduced at C-5 and C-6 since H-6 was coupled to C-4, C-7, C-8, and C-10 in its HMBC spectrum. Interestingly, the methyl group (C-25) was attached to C-11 since it was correlated with C-8, C-10, and C-11. Two methyl doublets (C-29 and C-30) were linked with C-22 since they showed long-range correlations with C-21 and C-22 (Fig. 2) suggesting the presence of an isopropyl partial structure of **1** which was bonded with C-21 of the E-ring. Consequently, compound **1** is a pentacyclic triterpenoid with a very rare skeleton, which contained variations in B and E rings as compared to the hopane-type triperpenoid. Therefore, compound **1** was E:B-friedo-hopane-type triterpenoid [7]. The relative configuration of **1** was deduced from its ROESY spectrum. There were NOE correlations between H-26 and H-18, H-18, and H-21 indicating that all H-26, H-18 and H-21 were α-face. Furthermore, H-28 showed NOE correlation with H-27 and H-22 suggesting that they are β-face. Fortunately, the crystals of compound **1** were successfully obtained by recrystallization in propanol that allowed us to measure its X-ray crystallographic analysis (Fig. 3) to confirm its structure. Finally, the structure of compound **1** was determined as shown in Fig. 1 and named simiarenol [7].

Compound **2** was isolated as a white powder. Analysis of its ^{1}H and ^{13}C NMR spectra indicated that it contained one double bond (δ_H 5.33 ppm and δ_C 140.8, 121.7 ppm) and one carbinol group (δ_H 3.52 and δ_C 71.8 ppm) together with six methyl groups, including two methyl singlets, three methyl doublets, and one methyl triplet. These NMR spectral data were identical to those of β-sitosterol [8]. Thus, compound **2** is β-sitosterol.

Compound **3** was also obtained as a white powder. The NMR spectral data of compound **3** resembled those of compound **2** except for the presence of two more olefinic protons at 5.16 (dd, *J* = 9.0, 15.0 Hz, H-23) and 5.02 (dd, *J* = 9.0, 15.0 Hz, H-22) instead of signals of two methylenes of **2**. The double bond $C_{22\text{-}23}$ was established as *trans* due to

their big coupling constants (J = 15 Hz) were observed. Therefore, compound **3** was identified as stigmasterol [9].

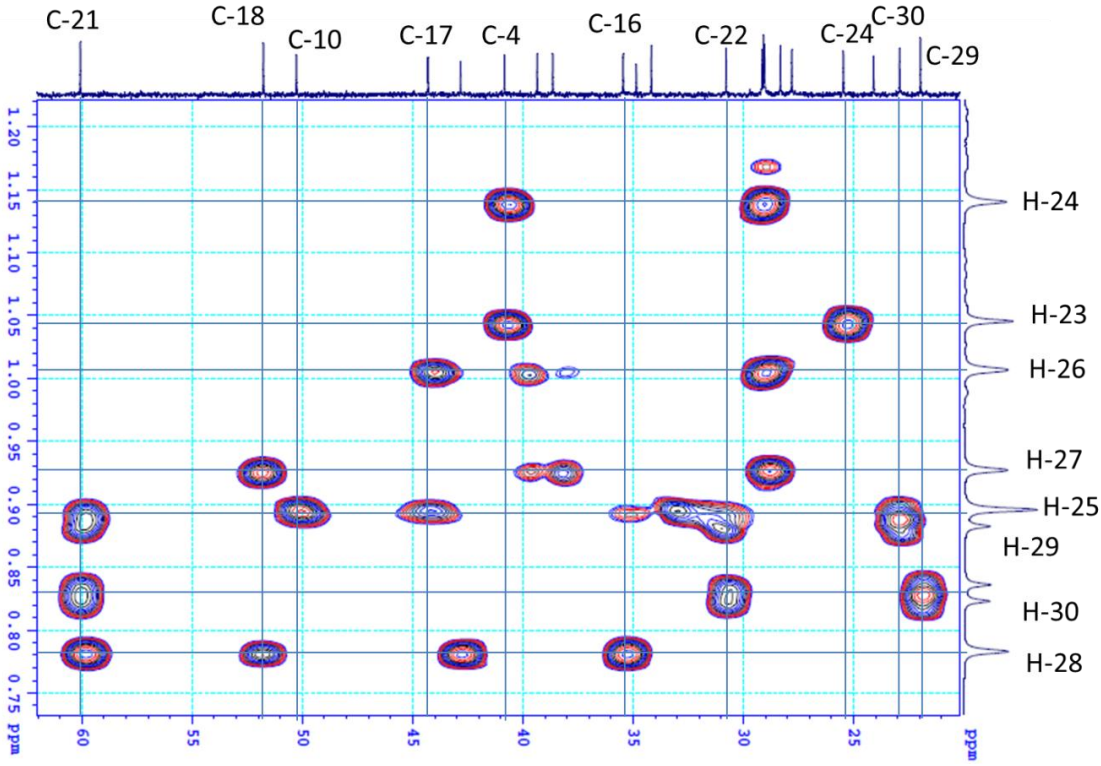


Figure 2. Important HMBC correlations of compound 1

Analysis of the NMR spectra of compound **4** suggested that it possessed a stigmasterol partial structure. However, the signals for *β*-D-glucose were also observed in its NMR spectra. An anomeric proton and carbon were resonanced at 4.99 (d, J = 8.0 Hz) in its ^{1}H NMR spectrum and at 102.3 ppm in its ^{13}C NMR spectrum, respectively. Consequently, compound **4** is characterized as stigmasterol glucoside [10].

Previously, E:B-Friedo-Hopane type triterpenoids showed several interesting biological activities, such as cytotoxicity against several cancer cells [11], the antinociceptive effect [12]. Thus, the anticancer property of simiarenol (**1**) against KB (a human epidermal carcinoma), HepG2 (hepatocellular carcinoma), LU-1 (human lung carcinoma), and MCF7 (human breast carcinoma) cell lines were investigated by the method described in [13]. The result showed that compound **1** could inhibit the growth of three cancer cells such as Hep-G2, Lu-1, and MCF-7 with its IC_{50} values of 38.47 ± 2.68; 48.37 ± 3.05 and 46.85 ± 3.6 μg/mL, respectively. However, compound **1** did not show activity toward the KB cell line (IC_{50} > 64 μg/mL). This is the first report on the chemical constituents and cytotoxicity of the Vietnamese *Pterospermum truncatolobatum*.

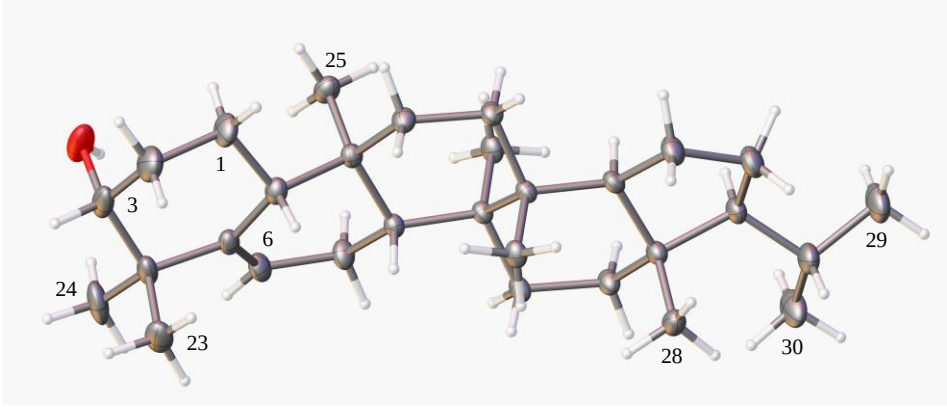


Figure 3. X-ray crystallographic analysis of compound 1

3. Conclusions

Four secondary metabolites, simiarenol (**1**), *β*-sitosterol (**2**), stigmasterol (**3**), and stigmasterol glycoside (**4**) were isolated from the aerial parts of Vietnamese *Pterospermum truncatolobatum*. In addition, simiarenol (**1**) possessing a very rare skeleton showed moderate cytotoxic activity against Hep-G2, Lu-1, and MCF-7 with its IC_{50} values of 38.47 ± 2.68; 48.37 ± 3.05 and 46.85 ± 3.6 μg/mL, respectively. This investigation suggests the possible application of this plant for anti-cancer treatment in Vietnam.

REFERENCES

[1] L.M.R. Al Muqarrabun, N. Ahmat, 2015. Medicinal uses, phytochemistry, and pharmacology of family Sterculiaceae: A review. *European Journal of Medicinal Chemistry*, Vol. 92, pp. 514-530.

[2] S. Li, Y. Shi, X.-Y. Shang, B.-S. Cui, Y. Yuan, X.- G. Chen, Y.-C. Yang, J.-G. Shi, 2009. Triterpenoids from the roots of *Pterospermum heterophyllum* Hance. *Journal of Asian Natural Products Research*, Vol. 11, pp. 652-657.

[3] W. Chen, W.Tang, L.Lou, W.Zhao, 2006. Pregnane, coumarin, and lupane derivatives and cytotoxic constituents from *Helicteres angustifolia*. *Phytochemistry*, Vol. 67, pp. 1041-1047.

[4] P. Dixit, M.P. Khan, G. Swarnkar, N. Chattopadhyay, R. Maurya, 2011. Osteogenic constituents from *Pterospermum acerifolium* Wild. Flowers. *Bioorganic & Medicinal Chemistry Letters*, Vol. 21, pp. 4617-4621.

[5] N. T. Uyen, 2020. Studies on the chemical constituents of the *n-hexane extract of Pterospermum truncatolobatum*. Graduation thesis, HNUE.

[6] G.M. Sheldrick, 2015. SHELXT–Integrated space-group and crystal-structure determination. *Acta Cryst.*, Vol. A71, pp. 3-8.

[7] A. K. Chakravarty, K. Masuda, H. Suzuki, H. Ageta, 1994. Unambiguous assignment of ^{13}C chemical shifts of some hopane and migrated hopane derivatives by 2D NMR. *Tetrahedron*, Vol. 50, pp. 2865-2876.

[8] P. Arjun, S. Jha, P. N. Murthy, A.S. Manik, 2010. Isolation and characterization of Stigmast-5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa* T. Anders. *In. J. Pharm. Sci. Res.,* Vol. 1, pp. 95-100.

[9] E. M. Moko, D. Rahardiyan, 2020. Structure of stigmasterols in Bran of Red Rice from Minahasa Regency, North Sulawesi, Indonesia. *Fullerene Journ. of Chem.,* Vol. 5, pp. 16-22.

[10] A. O. Oladimeji, I. A. Oladosu, A. Jabeen, A. Faheem, M. A. Mesaik, M. S. Ali, 2017. Immunomodulatory activities of isolated compounds from the root-bark of Cussonia arborea. *Pharmaceutical Biology,* Vol. 55, pp. 2240-2247.

[11] J. H. Yu, D. X. Wu, Z. P. Yu, Y. P. Li, Y. Y. Wang, S. J. Yu, H. Zhang, 2019. New E:B-Friedo-Hopane type triterpenoids from *Euphorbia peplus* with simiarendiol possessing significant cytostatic activity against HeLa cells by induction of apoptosis and S/G2 cell cycle arrest. *Molecules,* Vol. 24, pp. 3106.

[12] K.N. Kuroshima, F. Campos-Buzzi, R.A. Yunes, F. Delle Monache, V. Cechinel Filho, 2005. Chemical composition and antinociceptive properties of Hyeronima alchorneoides leaves. *Pharmaceutical Biology*, Vol. 43, pp. 573-578.

[13] D.A. Scudiero, R.H. Shoemaker, D.P. Kenneth, A. Monks, S. Tierney, T.H. Nofziger, M.J. Currens, D. Seniff, M.R. Boyd, 1988. Evaluation of a soluable tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Can. Res.,* Vol. 48, pp. 4827-4833.