Research Article

Bonellia albiflora: A Mayan Medicinal Plant That Induces Apoptosis in Cancer Cells

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Few studies have been carried out on the medical flora of Mexico’s Yucatan Peninsula in search for new therapeutic agents, in particular against cancer. In this paper, we evaluated the cytotoxic potential of the extract of Bonellia albiflora, a plant utilized in the traditional Mayan medicine for treatment of chronic injuries of the mouth. We carried out the methanolic extracts of different parts of the plant by means of extraction with the Soxhlet equipment. We conducted liquid-liquid fractions on each extract with solvents of increasing polarity. All extracts and fractions were evaluated for cytotoxic activity versus four human cancer cell lines and one normal cell line through a tetrazolium dye reduction (MTT) assay in 96-well cell culture plates. The methanolic root-bark extract possessed much greater cytotoxic activity in the human oropharyngeal cancer cell line (KB); its hexanic fraction concentrated the active metabolites and induced apoptosis with the activation of caspases 3 and 8. The results demonstrate the cytotoxic potential of the B. albiflora hexanic fraction and substantiate the importance of the study of the traditional Mayan medicinal plants.

1. Introduction

Traditional medicine is a practice that has been carried out from antiquity to our present time by inhabitants of the indigenous pueblos of Mexico, among which the Mayan population of the Yucatan Peninsula in Mexico is included. In the traditional Mayan medicine, plants are of great importance, which can be considered as evidence of their effectiveness for the control of many types of diseases. Likewise, they comprise one of the most important alternatives for health care, above all, in communities where primary health services are not accessible. In addition, they can be taken advantage of widely as a natural renewable resource. Together with what was previously described, the traditional medicine of the indigenous pueblos was recognized by the World Health Organization (WHO), which caused a powerful drive toward the research of medicinal plants [1].

The Mayan ethnomedical literature in its majority is composed of historic or descriptive studies, in whose contents there predominates a compendium of diseases and treatments known to Mayan healers of distinct eras [2, 3]. The Mayan people knew of and treated distinct diseases, including those of infectious origin (intestinal infections, infectious dermatitis, and respiratory infections), chronic diseases (asthma, fatigue, nephritis, and hypertension), and psychological-type diseases (insomnia, nervousness, and hysteria). In addition, they cured other illnesses such as the following: abscesses; calluses; corns, hard protuberances; polyps; tumors; and warts or sores, generally tangible or visible on the skin [1, 4].

In the traditional Mayan medicine of the Yucatan Peninsula, “cancer” is known as an illness or a set of illnesses that can manifest themselves as an affection of the skin or subadjacent muscle mass, or an affection in the form of pain in some internal organ. The term alludes to a difficult-to-cure illness or a one with a disagreeable aspect (when it affects the skin); if it is an internal cancer, the patient's semblance reveals the disease. The old inhabitants assigned names in the Mayan language to this set of symptoms; in the Mayan tongue, “cancer” is known as “tsunuz” or “tsunuztacan”, and hard protuberances or tumors are known as “chu’uchum” [3, 5].
Prior studies have demonstrated that the extracts of plants utilized in the traditional Mayan medicine for the treatment of the signs and symptoms suggestive of cancer possess cytotoxic activity [6]. Similarly, two studies conducted on two species of the genus Bonellia (Bonellia macrocarpa and Bonellia flammnea) from the Yucatan Peninsula reveal the presence of novel compounds, such as active agents with anti-carcinogenic activity [7, 8]. Within this context, the Yucatan Peninsula has five species of the genus Bonellia, among which the species B. macrocarpa, B. flammnea, and B. albiflora are employed in the traditional Mayan medicine for the treatment of the dermatological-type afflictions [5, 9, 10]. Of these three species, only B. albiflora has not been the object of any phytochemical or biological activity study. B. albiflora is denominated “Si’ik” in the traditional Mayan medicine and is used as an antitussive for the treatment of skin and mouth wounds and to relieve toothache pain [10]. In this work, we proposed an evaluation of the cytotoxic potential of the organic extracts of B. albiflora.

2. Materials and Methods

2.1. Plant Material. Bonellia albiflora (Lundell) B. Stål and Källersjö was collected from different Localities of the State of Yucatan, Mexico, during the summer of 2010. Plant material was identified and authenticated by taxonomists from the Department of Natural Resources of the Scientific Research Center of Yucatan (CICY).

2.2. Chemicals. Dulbecco’s modified Eagle’s medium (DMEM), heat-inactivated fetal bovine serum (FBS), and penicillin and streptomycin (PS) were purchased from Gibco, Carlsbad, CA, USA. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and etoposide were purchased from Sigma, St. Louis, MO, USA. Caspase assay kits and apoptotic DNA laddering kit were purchased from BioVision Research Products, Palo Alto, CA, USA.

2.3. Extraction and Fractionation. Each vegetal part was separated, dried, and pulverized. Dried powder of the separated plant material (100 g) was exhaustively extracted using a Soxhlet apparatus at 60°C of temperature with methanol (500 mL). The supernatants were filtered and evaporated under vacuum by means of a rotavaporator to obtain a dried extract. The methanol extract of each vegetal material (10 mg) was suspended in 20 mL methanol : water (1:3) and extracted successively using 50 mL of solvents of increasing polarity: hexane, dichloromethane, and ethyl acetate, such that the final residue extract was an aqueous fraction. The fingerprint of active hexane extract (5 mg) was obtained for gas chromatography-mass spectrometry (GC-MS).

2.4. Cell Lines and Culture. Cell lines of the oropharyngeal carcinoma (KB ATCC-CCL-17), laryngeal carcinoma (Hep-2), cervix adenocarcinoma (HeLa ATCC-CCL-2), and cervix squamous carcinoma (SiHa ATCC-CCL-35) as well as one normal cell line, canine cell kidney (MDCK ATCC-CCL-34), from the American Type Culture Collection (ATCC) were kindly provided by Veronica Vallejo-Ruiz from the East Biomedical Research Center-IMSS. The cells were cultured in DMEM medium, containing 10% SFB supplemented with 100 units/mL penicillin G and 100 μg/mL streptomycin in 5% CO₂-95% humidified air at 37°C.

2.5. Cytotoxicity Assay. The cytotoxicity was determined by the MTT assay according to the method described by Denizot and Lang [11] with some modifications. Briefly, 5 × 10⁴ viable cells from each cell line were seeded in a 96-well plate and incubated for 24–48 h. When cells reached >70% confluence, the medium was replaced and the cells were treated with the extract dissolved in DMSO (maximum concentration of 0.05%) at 2.34 to 300 g/mL. After 48 h of incubation, 10 μL MTT (5 mg/mL) was added to each well and incubated at 37°C for 4 h. The medium was removed and the formazan precipitate was dissolved in 100 μL of acidified isopropanol (0.4 N HCl). The optical density was determined with a spectrophotometer at 540 nm. Cells treated with 0.05% DMSO and docetaxel were used as negative and positive controls, respectively. The concentration of the extract that killed 50% of the cells (CC₅₀) was calculated by GraphPad Prism 4.00 software. All determinations were performed in triplicate. MDCK cell line was used to evaluate the selective index (SI) of extracts. SI is defined as the ratio of cytotoxic activity from normal cell and cancer cell lines.

2.6. GC-MS Analysis. The chromatographic separation was carried out by GC-MS analysis on an Agilent gas chromatograph, model 6890N, coupled to a mass selective detector, model 5975B. Compounds were separated on a DB-5 ms capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA). One microliter of the sample was injected into GC-MS using split mode (50:1). The injector temperature was 250°C. The column temperature was programmed as follows: initial temperature at 160°C for 3 min, 10°C/min to 240°C, 240°C for 2 min, 5°C/min to 250°C, 250°C for 10 min, 5°C/min to 300°C, and 300°C for 10 min. Mass detector conditions were the following: electronic impact (EI) mode at 70 eV; source temperature: 230°C; scanning rate: 1 scan/s; mass acquisition range: 20–600 amu; solvent delay, 4 min. Carrier gas was helium at 1 mL/min. Volatile components were tentatively identified by comparing their mass spectra using NIST Standard Reference Database Version NIST 05 for Windows. An authentic standard of bonediol compound was kindly provided by Dr. Peraza-Sánchez from CICY.

2.7. Analysis of DNA Fragmentation. DNA fragmentation was determined according to the method described by Tong et al. [12]. Briefly, the cells were treated with the extract at 10 and 50 μg/mL and incubated for 6, 12, and 24 h. After incubation, the cells were harvested by centrifugation and washed twice in ice-cold PBS. An apoptotic DNA laddering kit (BioVision apoptotic DNA ladder extraction kit) was used to isolate DNA according to the manufacturer’s protocol; the DNA in the samples was separated on 1.5% agarose gel containing 1 μg/mL of ethidium bromide. DNA bands were visualized under ultraviolet illumination and were photographed.
2.8. Assays of Caspases Activities. Caspases 3, 8, and 9 activities were performed using FLICE/Caspase Colorimetric assay kit, following the manufacturer’s protocols. Briefly, 5 × 10⁶ cells treated with 10 or 50 μg/mL extract for 6, 12, or 24 h were harvested, washed with PBS, and centrifuged at 800 × g for 10 min at 4°C. The cell pellets were resuspended in 50 μL lysis buffer and incubated on ice for 10 min before being centrifuged at 10,000 × g for 1 min. The supernatant was collected in a 1.5 mL tube and kept on ice. After measuring protein concentration, 200 μg of protein was dissolved in 50 μL cell lysis buffer. The reaction buffer with 10 mM DTT was added to each sample. Finally, a specific substrate for each caspase (DEVD-ρNA, IETD-ρNA, and IEHD-ρNA) was added to the samples, incubated at 37°C for 1 h, and read at 405 nm. The enzyme activity was expressed as fold over control sample.

3. Results and Discussion

3.1. Cytotoxic Activity of Methanolic Extracts. The cytotoxicity results of the methanolic extracts from different parts of B. albiflora are summarized in Table 1. The root bark's methanolic extract exhibited the most interesting cytotoxic activity compared to extracts of B. albiflora leaves and stem bark, with a CC₅₀ of 12–31 μg/mL on the four human cancer cell lines. KB cell line showed a greater sensitivity to the extract with a CC₅₀ of 12.64 μg/mL. The nontumor canine kidney cell line MDCK was less sensitive to the effects of the extract with an SI of 5.63. The cell pellets were resuspended in 200 μg/mL of 12.64 g/mL for 6, 12, or 24 h. The cell pellets were resuspended in 50 μL lysis buffer and incubated on ice for 10 min before being centrifuged at 10,000 × g for 1 min. The supernatant was collected in a 1.5 mL tube and kept on ice. After measuring protein concentration, 200 μg of protein was dissolved in 50 μL cell lysis buffer. The reaction buffer with 10 mM DTT was added to each sample. Finally, a specific substrate for each caspase (DEVD-ρNA, IETD-ρNA, and IEHD-ρNA) was added to the samples, incubated at 37°C for 1 h, and read at 405 nm. The enzyme activity was expressed as fold over control sample.

| Extract      | MDCK   | KB      | HeLa   | Hep-2 | SiHa   |
|--------------|--------|---------|--------|-------|--------|
| Leaves       | 91.39  | 23.85 (3.83) | 47.05 (1.94) | 35.20 (2.59) | 47.45 (1.92) |
| Stem bark    | 249.40 | 62.30 (4.00) | NA     | 72.30 (3.45) | NA     |
| Root bark    | 173.52 | 12.64 (13.72) | 31.85 (5.44) | 35.34 (4.91) | 31.50 (5.50) |
| Docetaxel    | 1.10   | 0.23 (4.78)  | 0.20 (5.50)  | 0.08 (13.75) | 0.32 (3.43)  |

NA: no activity > 200 μg/mL.

3.2. Cytotoxic Activity of Fractions. The methanolic extracts of different parts of the plant were fractionated with solvents of increasing polarity for later cytotoxicity studies in the cell lines. The hexanic fraction obtained from the liquid-liquid partitioning of the methanolic extract of root bark (HFBa) presented superior cytotoxic effects compared to the original extract, with a CC₅₀ between 2 and 27 μg/mL in the distinct cell lines (Table 2). The hexanic fraction's SI also improved compared to the original extract in the cell lines evaluated (SI = 5–54). The methanolic fractions of the bark and leaves extracts were not active at concentrations of >200 μg/mL (data not shown).

Previously, we conducted a bio-guided study to evaluate the antiproliferative activity of B. macrocarpa, yielding the isolation of the compound bonediol, which showed moderate activity in cancer cell lines [8]. However, the present study did not show cytotoxic effects with HFBa comparable to the original methanolic extract in the cell lines evaluated (Table 2). An explanation to these results may be that bonediol inhibits some point of cellular proliferation (cycle cell or replication of DNA), while the effects that are observed in the cytotoxic assay are damage or general toxicity (apoptosis or necrosis) [14].

HFBa presented better cytotoxic effects compared to bonediol and was more selective toward the tumor than toward normal cells; SI is considered an indicator of biological activity and is not related to cytotoxicity if the SI is >10 [15]. In this regard, only HFBa satisfied these criteria and was more potent in the KB cell line with a CC₅₀ of 2.73 μg/mL; this cell line is related to oral cancer and is in agreement with the plant's use in the traditional Mayan medicine for chronic oral lesions [10], a term that could be related with cancer.

3.3. GC-MS Analysis. Identification and chemical analysis of bioactive hexane fraction by GC-MS is displayed in Table 3. The chromatogram revealed a total of eight peaks, six of which were identified by the database: dodecanoic acid; tridecanoic acid; 2-nonyl-malonic acid, dimethyl ester; stigmasta-7,16-dien-3-ol; 9,19-Cyclo-lanost-24-en-3-ol; and bonediol. This last one was identified by retention time and which were identified by the database: dodecanoic acid; tridecanoic acid; 2-nonyl-malonic acid, dimethyl ester; stigmasta-7,16-dien-3-ol; 9,19-Cyclo-lanost-24-en-3-ol; and bonediol. This last one was identified by retention time and mass spectrum of an authentic standard [6].

The extract of leaves was the second in greatest activity, only on KB cell line with a CC₅₀ of 23.85 μg/mL according to NCI criteria, followed by that of the stem bark's extract, which was less cytotoxic to KB and Hep-2 cell lines.

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Bonediol was isolated from the methanolic extract of B. macrocarpa roots as a bioactive component. In this work, we detected the presence of this compound at a low concentration; thus, it could be referred to as a possible bonediol was isolated from the methanolic extract of B. macrocarpa roots as a bioactive component. In this work, we detected the presence of this compound at a low concentration; thus, it could be referred to as a possible
Table 2: Cytotoxicity of organic fractions from methanolic extract of *B. albiflora* root bark and bonediol.

| Extract          | MDCK  | KB   | HeLa  | Hep-2  | SiHa  |
|------------------|-------|------|-------|--------|-------|
| Hexane           | 148.48| 2.73 | 14.29 | 15.48  | 27.02 |
| Dichloromethane  | NA    | NA   | NA    | NA     | NA    |
| Ethyl acetate    | NA    | NA   | NA    | NA     | NA    |
| Aqueous          | NA    | NA   | NA    | NA     | NA    |
| Bonediol         | 139.71| 80.60| 115.45| 92.50  | 54.40 |
| Docetaxel        | 1.10  | 0.23 | 0.20  | 0.08   | 0.32  |

NA: no activity > 200 μg/mL.

Table 3: Chemical composition of hexane fraction of *B. albiflora*.

| Peak no. | Retention time (min) | Peak relative (%) | m/z (relative abundance %) | Component                                      |
|----------|----------------------|-------------------|-----------------------------|------------------------------------------------|
| 1        | 5.802                | 13.221            | 200 (10), 171 (10), 157 (30), 143 (10), 129 (40), 115 (20), 101 (15), 85 (30), 73 (100), 60 (85), 43 (70), 29 (40). | Dodecanoic acid                                |
| 2        | 7.012                | 5.256             | 214 (10), 185 (10), 171 (35), 157 (5), 143 (5), 129 (40), 115 (25), 97 (15), 85 (20), 73 (95), 60 (85), 43 (70), 29 (35). | Tridecanoic acid                               |
| 3        | 8.091                | 6.222             | 208 (19), 166 (13), 152 (100), 137 (18), 121 (6), 107 (5), 91 (13), 77 (13), 55 (5), 41 (13), 28 (19). | Unidentified                                   |
| 4        | 9.826                | 37.396            | 259 (5), 156 (7), 145 (70), 132 (100), 113 (13), 100 (20), 87 (18), 69 (16), 55 (33), 41 (31), 29 (13). | 2-Nonyl-malonic acid, dimethyl ester           |
| 5        | 14.192               | 5.381             | 350 (100), 209 (80), 195 (24), 179 (48), 164 (12), 151 (16), 136 (2), 75 (8), 57 (8), 43 (20), 28 (26). | Unidentified                                   |
| 6        | 16.389               | 8.983             | 294 (70), 209 (13), 179 (10), 153 (100), 139 (5), 123 (20), 77 (9), 41 (20). | Bonediol                                       |
| 7        | 37.548               | 13.634            | 412 (22), 369 (10), 341 (10), 300 (15), 271 (80), 246 (20), 207 (90), 173 (10), 147 (40), 107 (43), 81 (75), 55 (80), 43 (100), 28 (40). | Stigmasta-7,16-dien-3-ol                      |
| 8        | 39.632               | 9.907             | 426 (25), 411 (100), 393 (45), 259 (10), 215 (10), 187 (15), 173 (15), 161 (15), 135 (25), 109 (40), 69 (90), 55 (40), 41 (45). | 9,19-Cyclo-lanost-24-en-3-ol                   |

3.4. DNA Fragmentation. We observed that the methanol extract of the roots of *B. albiflora* showed typical morphology of apoptosis (data not shown) on KB cell lines. Similarly, HFBA was shown to induce apoptotic morphology on KB cell lines. These results led us to evaluate whether the hexanic fraction that demonstrated the greatest cytotoxicity and apoptosis morphological characteristics in the KB cell line could induce this process; thus, we evaluated the fragmentation of DNA, typical of the process of apoptosis. DNA fragmentation was registered from lesser to greater magnitude within a treatment concentration range of 10 or 50 μg/mL and an incubation-time range of 6–24 h. Figure 2 shows typical DNA fragmentation in KB cells after treatment with 50 μg/mL HFBA and an 18 h incubation period. Several studies have shown the apoptotic effect of certain plant methanolic extracts [20–24]. However, few studies have
investigated the chemical characteristics of the compounds that may possess this activity. In those few studies, it was generally found that the low-polarity organic fractions are responsible for the apoptotic effect on the cell lines, coinciding with the results obtained in this study [25, 26].

It is unknown whether the compounds in HFBa are responsible for apoptosis induction, but it cannot be attributed to a single compound such as bonediol that, although present in the extract, requires high concentrations to induce apoptosis (data not shown), unlike HFBa, which induces DNA fragmentation at 10 μg/mL. With regard to the above, in addition to bonediol, we report the presence of dodecanoic acid and a derivative of stigmasterol as components of HFBa. These compounds have been associated with cytotoxic activity observed for the hexane fraction of Crocus sativus [27]. Furthermore, some authors have demonstrated that derivatives of stigmasterol showed significant cytotoxic activity in cancer cell lines that depended on apoptosis [28–31]. In particular, spinasterol (stigmastera-7, 22-dien-3beta-ol) has demonstrated decreased incidence of skin tumors in vivo [32]. In fact, spinasterol and the derivative reported in this study differ in double enlace at position 22 in spinasterol and position 16 for the stigmasterol derivative. Perhaps, stigmastera-7, 22-dien-3beta-ol could contribute to cytotoxic activity observed in this study. In addition, it is known that lanostanes are a group of tetracyclic triterpenoids derived from lanosterol, which have multiple activities against cancer cells including the induction of apoptosis [33, 34]. Possibly, the lanostane- and stigmasterol-type compounds reported in the active hexane fraction have a degree of cytotoxic activity and an effect of inducing apoptosis. It is likely that several compounds present in the hexane fraction act synergistically to induce cytotoxicity and apoptosis.

3.5. Analysis of the Activity of Caspases. To know whether the mechanism of activation of DNA fragmentation was induced by activation of apoptosis via the intrinsic or extrinsic pathway, we evaluated the activity of caspases that is characteristic
of each. The incubation periods were of 2, 4, 6, and 12 h to obtain an activation profile. Caspase 8 was activated after 6 h of treatment with 50 μg/mL of HFBa; the increase was three times greater compared to control cells without treatment (negative control) (Figure 3). No increase was observed in the activation of caspase 8 in the 2, 4, and 12 h incubation periods. Caspase 9 was not activated in KB cells after treatment with 50 μg/mL of HFBa during 2–12 h, which suggests a lack of apoptosis activation by the intrinsic pathway (Figure 4). Caspase 3 activity increased four folds compared to that of the control in HFBa-treated cells, which is in agreement with caspase 8 activation (Figure 5). The increase of caspase 8 activity is typical of extrinsic-pathway activation of apoptosis, which in turn activates other procaspases, among these is caspase 3, which in turn leads to the degradation of nuclear proteins such as laminin A, fodrin, actin, and gelsolin. It also leads to the release of the caspase-activated DNase protein inhibitor (ICAD), converting it into the caspase-activated DNase (CAD) enzyme, whose objective is DNA degradation [35], as depicted in Figure 2. Given that caspase 9 was not activated, we conclude that B. albiflora induces apoptosis by the extrinsic pathway. This evidences the great potential that this fraction possesses as an alternative or adjunct therapy in the treatment of cancer. In the literature are several studies of extracts from plants and their effect on the induction of apoptosis by activation of extrinsic signaling mediated by natural and synthetic triterpenoids [46–48]. Additionally, the lanostane-type triterpenoid, polyporenic acid C isolated from Poriacocos, induces caspase-8-mediated apoptosis in human lung cancer [49]. Notably, β-sitosterol (structural isomer of stigmasterol) has been shown to induce apoptosis by activation of the extrinsic pathway, activating Fas signaling in human breast cancer cells [50]. This might suggest that both the triterpene and sterol components of the extract used in this study would have a major role in the induction of apoptosis mediated by the activation of the extrinsic pathway.

To our knowledge, this is the first time it has been demonstrated that the extract of a plant employed in the traditional Mayan medicine possesses an effect on apoptosis. Future studies will be directed toward standardizing the extract and evaluating the latter with in vivo models. Additional studies are necessary for elucidating the compounds responsible for the observed cytotoxic activity and their exact mechanism of apoptosis.

4. Conclusions

The hexanic fraction of B. albiflora roots exerts cytotoxic effects and induces apoptosis via the extrinsic pathway, which suggests its potential for the treatment of cancer. We suggest the complete isolation of the components present in the hexanic fraction of B. albiflora for evaluation in the cytotoxic assay and induction of apoptosis, to elucidate which are the active compounds as well as to understand the mechanism of action.

Conflict of Interests

The authors declare that they have no conflict of interests and no financial connection to any commercial entity mentioned in the paper.

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References

[1] J. M. Spieler, “World Health Organization, the special programme of research, development and research training in human reproduction task force on indigenous plants for fertility regulation,” Korean Journal of Pharmacognosy, vol. 12, pp. 94–97, 1981.
[2] R. M. Mendieta, Plantas Medicinales Del Estado De Yucatán, CECSA, Yucatán, México, 1st edition, 1981.
[3] G. Balam, Cosmogonia Y Uso Actual De Las Plantas Medicinales De Yucatán, Universidad Autónoma de Yucatán, Yucatán, México, 1992.
[4] G. M. Cragg and D. J. Newman, “Plants as a source of anti-cancer agents,” Journal of Ethnopharmacology, vol. 100, no. 1-2, pp. 72–79, 2005.

[5] R. Osadao, El Libro del Judío o Medicina Doméstica, Descripción de las Virtudes de las Verbas Medicinales de Yucatán, Mérida, Yucatan Mexico, 1834.

[6] E. Caamañ-Fuentes, L. W. Torres-Tapia, P. Simá-Polanco, S. R. Peraza-Sánchez, and R. Moo-Puc, “Screening of plants used in Mayan traditional medicine to treat cancer-like symptoms,” Journal of Ethnopharmacology, vol. 135, no. 3, pp. 719–724, 2011.

[7] A. Sánchez-Medina, L. M. Peña-Rodríguez, F. May-Pat et al., “Identification of sakurosaosaponin as a cytotoxic principle from Jacquinia flammea,” Natural Product Communications, vol. 5, no. 3, pp. 365–368, 2010.

[8] E. Caamañ-Fuentes, L. W. Torres-Tapia, R. Cedillo-Rivera, R. Moo-Puc, and S. R. Peraza-Sánchez, “Bonediol, a new alkyl catechol from Bonellia macrocarpa,” Phytochemistry Letters, vol. 4, no. 3, pp. 345–347, 2011.

[9] B. Ståhl and M. Källersjö, “Reinstatement of Berberis koreana”, Nomenclature, Forma de Vida, Uso, Manejo y Distribucion de las Especies Vegetales de la Peninsula de Yucatan, pp. 312–380, Universidad Autónoma de Yucatán, Yucatán, México, 2003.

[10] F. Denizot and R. Lang, “Rapid colorimetric assay for cell growth and survival - Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability,” Journal of Immunological Methods, vol. 89, no. 2, pp. 271–277, 1986.

[11] X. Tong, S. Lin, M. Fujii, and D.-X. Hou, “Echinocystic acid induces apoptosis in HL-60 cells through mitochondria-mediated death pathway,” Cancer Letters, vol. 212, no. 1, pp. 21–32, 2004.

[12] R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, “Protocols for screening chemical agents and natural products against animal tumors and other biological systems,” Cancer Chemotherapy Reports, vol. 3, pp. 59–61, 1972.

[13] R. Moo-Puc, D. Robledo, and Y. Freile-Pelegri, “In vitro cytotoxic and anti-proliferative activities of marine macroalgae from Yucatán, Mexico,” Ciencias Marinas, vol. 35, no. 4, pp. 345–358, 2009.

[14] C. Vonthron-Sénécheau, B. Weniger, M. Ouatata et al., “In vitro antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants,” Journal of Ethnopharmacology, vol. 87, no. 2-3, pp. 225–231, 2003.

[15] A. L. Okunade and D. F. Wiemer, “Jacquinonic acid, an antitumor triterpenoid from Jacquinia pungens,” Phytochemistry, vol. 24, no. 6, pp. 1203–1205, 1985.

[16] K. Bhattacharyya, T. Kar, P. K. Duttta, G. Bocelli, and L. Righi, “Dimethyl olea-18(13),15(16)-diene-3β,28-diacetate,” Acta Crystallographica Section C, vol. 55, no. 6, pp. 992–994, 1999.

[17] K. Bhattacharyya, T. Kar, P. Kumar, B. Achari, and G. Bocelli, “Oleana-12(13),15(16)-diene-3β,28-diyldiacetate,” Acta Crystallographica, vol. 56, no. 2, pp. 60–61, 2000.

[18] H. J. Lee, H. Y. Kang, C. H. Kim et al., “Effect of new rotenoid glycoside from the fruits of Amorpha fruticosa LINNE on the growth of human immune cells,” Cytotechnology, vol. 52, no. 3, pp. 219–226, 2006.

[19] P. Bontempo, D. Rigano, A. Doto et al., “Genista sessilifolia DC extracts induce apoptosis across a range of cancer cell lines,” Cell Proliferation, vol. 46, no. 2, pp. 183–192, 2013.

[20] J.-W. Chon, J.-H. Sung, E.-J. Hwang, and Y.-K. Park, “Chlorella methanol extract reduces lipid accumulation in and increases the number of apoptotic 3T3-L1 cells,” Annals of the New York Academy of Sciences, vol. 1171, pp. 183–189, 2009.

[21] K.-J. Jo, M.-R. Cha, M.-R. Lee, M.-Y. Yoon, and H.-R. Park, “Methanolic extracts of Uncaria rhynchophylla induce cytotoxicity and apoptosis in HT-29 human colon carcinoma cells,” Plant Foods for Human Nutrition, vol. 63, no. 2, pp. 77–82, 2008.

[22] S. Nadova, E. Miadokova, L. Alfoldiova et al., “Potential antioxidant activity, cytotoxicity and apoptosis-inducing effects of Chelidonium majus L. extract on leukemia cells,” Neuroendocrinology Letters, vol. 29, no. 5, pp. 649–652, 2008.

[23] T. N. Hasan, G. Shafi, N. A. Syed et al., “Methanolic extract of Nigella sativa seed inhibits SiHa human cervical cancer cell proliferation through apoptosis,” Natural Product Communications, vol. 8, no. 2, pp. 213–216, 2013.

[24] C. P. Preetthy, R. Padmapriya, V. S. Periasamy et al., “Antiproliferative property of n-hexane and chloroform extracts of Anisomeles malabarica (L.). R. Br. in HPV16-positive human cervical cancer cells,” Journal of Pharmacology and Pharma- therapeutics, vol. 3, no. 1, pp. 26–34, 2012.

[25] A. Chicca, B. Adinolfi, E. Martinotti et al., “Cytotoxic effects of Echinacea root hexanic extracts on human cancer cell lines,” Journal of Ethnopharmacology, vol. 110, no. 1, pp. 148–153, 2007.

[26] C.-J. Zheng, L. Li, W.-H. Ma, T. Han, and L.-P. Qin, “Chemical constituents and bioactivities of the liposoluble fraction from different medicinal parts of Crocus sativus,” Pharmaceutical Biology, vol. 49, no. 7, pp. 756–763, 2011.

[27] H. H. Kim, S. U. Choi, C. S. Kim, and K. R. Lee, “Cytotoxic activity of Hovenia dulcis,” Journal of Ethnopharmacology, vol. 87, no. 2, pp. 129–133, 2003.

[28] R. Chowdhury, R. B. Rashid, M. H. Sohrab, and C. M. Hasan, “2α,3α-hydroxystigmaster-4-ene-3-one: a new bioactive steroid from Toona ciliata (Melaceae),” Pharmazie, vol. 58, no. 4, pp. 272–273, 2003.

[29] K. H. Kim, S. U. Choi, C. S. Kim, and K. R. Lee, “Cytotoxic steroids from the trunk of Berberis koreana,” Bioscience, Biotechnology and Biochemistry, vol. 76, no. 4, pp. 825–827, 2012.

[30] A. B. Awad and C. S. Fink, “Phytotherapies as anitcancer dietary components: evidence and mechanism of action,” Journal of Nutrition, vol. 130, no. 9, pp. 2127–2130, 2000.

[31] I. V. Musharvaya and A. P. Domingo, “Anticarcinogenetic potential of spinasterol isolated from squash flowers,” Teratology, Carcinogenesis, and Mutagenesis, vol. 20, pp. 99–105, 2000.

[32] J. L. Rios, I. Andújar, M. C. Recio, and R. M. Giner, “Lanostane-type triterpenoids from the number of apoptotic 3T3-L1 cells,” Journal of Natural Products, vol. 75, no. 11, pp. 2016–20144, 2012.

[33] L. Zhou, Y. Zhang, L. A. Gapter, H. Ling, R. Agarwal, and K.-Y. Ng, “Cytotoxic and anti-oxidant activities of lanostane-type triterpenes isolated from Porio cocos,” Chemical and Pharmaceutical Bulletin, vol. 56, no. 10, pp. 1459–1462, 2008.

[34] J. F. R. Kerr, C. M. Winterford, and B. V. Harmon, “Apoptosis: its significance in cancer and cancer therapy,” Cancer, vol. 73, no. 8, pp. 2003–2026, 1994.

[35] R. R. Somasagara, M. Hegde, K. K. Chiruvella, A. Musini, B. Choudhary, and S. C. Raghavan, “Extracts of strawberry fruits induce intrinsic pathway of apoptosis in breast cancer cells and inhibits tumor progression in mice,” PLoS ONE, vol. 7, no. 10, Article ID e47021, 2012.
[37] J.-Q. Yu, Y. Yin, J.-C. Lei et al., “Activation of apoptosis by ethyl acetate fraction of ethanol extract of Dianthus superbus in HepG2 cell line,” Cancer Epidemiology, vol. 36, no. 1, pp. e40–e45, 2012.

[38] A. C. Tan, I. Konczak, I. Ramzan, and D. M.-Y. Sze, “Native Australian fruit polyphenols inhibit cell viability and induce apoptosis in human cancer cell lines,” Nutrition and Cancer, vol. 63, no. 3, pp. 444–455, 2011.

[39] H. C. Pal, I. Sehar, S. Bhushan, B. D. Gupta, and A. K. Saxena, “Activation of caspases and poly (ADP-ribose) polymerase cleavage to induce apoptosis in leukemia HL-60 cells by Inula racemosa,” Toxicology in Vitro, vol. 24, no. 6, pp. 1599–1609, 2010.

[40] Y.-C. Tien, J.-Y. Lin, C.-H. Lai et al., “Cardamom tinctoria L. prevents LPS-induced TNFα signaling activation and cell apoptosis through JNK1/2-NFκB pathway inhibition in H9c2 cardiomyoblast cells,” Journal of Ethnopharmacology, vol. 130, no. 3, pp. 505–513, 2010.

[41] M. E. Juan, U. Wenzel, V. Ruiz-Gutierrez, H. Daniel, and J. M. Planas, “Olive fruit extracts inhibit proliferation and induce apoptosis in HT-29 human colon cancer cells,” Journal of Nutrition, vol. 136, no. 10, pp. 2553–2557, 2006.

[42] M.-J. Liu, Z. Wang, H.-X. Li, R.-C. Wu, Y.-Z. Liu, and Q.-Y. Wu, “Mitochondrial dysfunction as an early event in the process of apoptosis induced by woodforin I in human leukemia K562 cells,” Toxicology and Applied Pharmacology, vol. 194, no. 2, pp. 141–155, 2004.

[43] H. S. Choi, H. S. Seo, J. H. Kim, J. Y. Um, Y. C. Shin, and S. G. Ko, “Ethanol extract of Paeonia suffruticosa Andrews (PSE) induced AGS human gastric cancer cell apoptosis via fas-dependent apoptosis and MDM2-p53 pathways,” Journal of Biomedical Science, vol. 19, article 82, 2012.

[44] S. Pandey, S. J. Chatterjee, P. Ovadje, M. Mousa, and C. Hamm, "The efficacy of dandelion root extract in inducing apoptosis in drug-resistant human melanoma cells," Evidence-Based Complementary and Alternative Medicine, vol. 2011, Article ID 129045, 11 pages, 2011.

[45] P. Ovadje, C. Hamm, and S. Pandey, "Efficient induction of extrinsic cell death by dandelion root extract in human chronic Myelomonocytic Leukemia (CMML) cells," PLoS ONE, vol. 7, no. 2, Article ID e30604, 2012.

[46] W.-S. Suh, Y. S. Kim, A. D. Schimmer et al., "Synthetic triterpenoids activate a pathway for apoptosis in AML cells involving downregulation of FLIP and sensitization to TRAIL," Leukemia, vol. 17, no. 11, pp. 2122–2129, 2003.

[47] Z. D. Nassar, A. F. A. Aisha, N. Idris et al., "Koetjapic acid, a natural triterpenoid, induces apoptosis in colon cancer cells," Oncology Reports, vol. 27, no. 3, pp. 727–733, 2012.

[48] H. Ling, L. Zhou, X. Jia, L. A. Gapter, R. Agarwal, and K.-Y. Ng, "Polyporinic acid C induces caspase-8-mediated apoptosis in human lung cancer A549 cells," Molecular Carcinogenesis, vol. 48, no. 6, pp. 498–507, 2009.

[49] A. B. Awad, M. Chinnam, C. S. Fink, and P. G. Bradford, "β-Sitosterol activates Fas signaling in human breast cancer cells," Phytomedicine, vol. 14, no. 11, pp. 747–754, 2007.