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## Research Article

# Phytochemical Investigation of Egyptian Riverhemp: A Potential Source of Antileukemic Metabolites

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As part of our research group's continuous efforts to find alternative treatments for cancer, the aqueous ethanol extract of *Sesbania sesban* L. Merr. (SS, Egyptian riverhemp) demonstrated an antileukemic activity against K562 cell line. Bioguided fractionation of SS leaves hydroethanolic extract resulted in the isolation of one new compound (33) named as hederatriol 3-O- $\beta$ -D-glucuronic acid methyl ester as well as 34 known compounds. Seven compounds ((34), (22), (20), (24), (21), (19), and (35)) showed high antiproliferative effects ( $IC_{50}$  = 22.3, 30.8, 31.3, 33.7, 36.6, 37.5, and 41.5  $\mu$ M, respectively), while four compounds ((32), (5), (29), and (1)) showed milder activities ( $IC_{50}$  = 56.4, 67.6, 83.3, and 112.3  $\mu$ M, respectively). A mechanistic study was further carried out on a molecular genetics level against several transcription factors signaling pathways that are incorporated in the incidence of cancer. The results showed that compounds (22) and (21) demonstrated a specific inhibition of Wnt pathway ( $IC_{50}$  = 3.8 and 4.6  $\mu$ M, respectively), while compound (22) showed a specific inhibition of Smad pathway ( $IC_{50}$  = 3.8  $\mu$ M). Compound (34) strongly altered the signaling of Smad and E2F pathways ( $IC_{50}$  = 5  $\mu$ M). The bioactive metabolites were further investigated *in silico* by docking against several targets related to K562 cell line. The results showed that compounds (22) and (34) exhibited a strong binding affinity towards topoisomerase (docking score = -7.81 and -9.30 Kcal/Mole, respectively). Compounds (22) and (34) demonstrated a strong binding affinity towards EGFR-tyrosine kinase (docking score = -7.12 and -7.35 Kcal/Mole, respectively). Moreover, compound (34) showed a strong binding affinity towards Abl kinase (docking score = -7.05 Kcal/Mole).

## 1. Introduction

In our previous study, the antileukemic activities of 56 medicinal plants grown in Upper Egypt were checked, where *Sesbania sesban* (SS) leaves extract demonstrated a promising

antileukemic activity against the chronic myeloid leukemia cell line (K562) [1]. Considering the limited studies on the phytoconstituents of SS leaves, we carried out this research with the aim of bioguided isolation of the antileukemic metabolites from SS leaves.

Chronic myeloid leukemia (CML) is the third most regular mortality-causing neoplasm and the fourth predominant cancer among the Egyptian population [2, 3]. CML has a trademark and explicit chromosomal irregularity known as Philadelphia (Ph) chromosome (Bcr-Abl gene) that is produced from a reciprocal translocation between the Abl gene on chromosome 9 and the Bcr gene on chromosome 22 in the pluripotent hematopoietic stem cells [4].

The value of natural products in the arsenal of leukemia therapies is clearly correlated with several agents such as L-asparaginase, Daunorubicin, Anthracyclines, Vinca alkaloids (Vincristine and Vinblastine) [5, 6], Homoharringtonine, Indirubin, Flavopiridol, Maytansinoids, Meisoindigo [6], and the Podophyllotoxin derivatives (Etoposide and Teniposide) [7, 8].

*S. sesban* (Egyptian riverhemp or *Sesbania aegyptiaca* Pers.) is a perennial legume tree belonging to family Fabaceae (Figure 1). It has several names like *Aeschynomene aegyptiaca* (Pers.) Steud., *Aeschynomene sesban* L., *Sesbania confaloniana* Chiov., *Sesbania pubescens* sensu auct., *Sesbania punctata* DC., and *Emerus sesban* (L.) Kuntze [9].

Traditionally, SS leaves were used as a poultice for inflammatory conditions such as boils and abscesses as well as rheumatic swellings, and the juice of the fresh leaves was used as anthelmintic [10–12].

The methanolic extract of SS leaves exhibited several biological activities such as anti-inflammatory [13], antioxidant [14], molluscicidal [15, 16], antidiabetic [17], potent spermicidal [18], antimicrobial [16], and anthelmintic activities [10–12]. Phytochemical analyses of the leaves showed the presence of steroids such as  $\beta$ -sitosterol and campesterol [13], triterpenoids such as betulinic and oleanolic acids [13, 19, 20], saponins of oleanolic acid [15, 18], and flavonoids [21].

The aim of this study is to isolate the antileukemic metabolites of SS leaves through extensive biological and phytochemical investigations.

## 2. Materials and Methods

**2.1. General Experimental Procedures.** High-resolution electrospray ionization mass spectrometry (HRESIMS) data were acquired using a Bruker BioApex-FTMS with electrospray ionization (ESI). 1D ( $^1\text{H}$ NMR, DEPTQ) and 2D (HSQC, HMBC, and TOCSY) NMR spectra were recorded on a Bruker 400 and 500 MHz spectrometer. Optical activity was measured using AA-65 series automatic polarimeter (Cambridgeshire, PE26 1NF, England). HPLC analysis was conducted using an Agilent 1100 HPLC system (Supplementary Materials). GC-MS analysis was performed with an Agilent 7890B gas chromatograph (Supplementary Materials). Sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan) and silica gel (60–120  $\mu\text{m}$ -mesh, Merck, Darmstadt, Germany), reversed phase silica (40–63  $\mu\text{m}$ , Sorbent Technologies, 5955 Peachtree Corners East, Suite A, Norcross, GA 30071 USA), and Diaion<sup>®</sup> HP-20 (250  $\mu\text{m}$ , Supelco, Bellefonte, PA 16823-00048, USA) were used for column chromatography (CC). SPE cartridges silica gel and C18 (Supelco Inc., Bellefonte, PA, USA) were used in the fractionation



FIGURE 1: Entire *Sesbania sesban* branch.

work. Fractions from CC were monitored by TLC using precoated aluminum sheets (silica 60 F254, 0.25 mm (Merck, Darmstadt, Germany)). The fractions were dissolved in the appropriate solvents and spots were applied manually using the capillary micropipette, and the plates were then dried and developed in different mobile phase systems such as *n*-hexane: ethyl acetate (8:2), *n*-hexane: acetone (8:2), and DCM: methanol (9:1). The spots on the developed plates were detected using UV light (254 and 366 nm) and by spraying with 2% *p*-anisaldehyde- $\text{H}_2\text{SO}_4$  reagent followed by heating for 5–10 min (105°C). Number of spots, colors, and retardation factors (Rf values) for each of the spots were determined and recorded [22].

**2.2. Plant Material.** The leaves of *Sesbania sesban* were purchased from the medicinal, aromatic, and poisonous plants experimental station, Faculty of Pharmacy, Cairo University, Egypt, in April 2018. The plant material was authenticated by Professor Dr. Abdelhalim Mohamed, Flora and Phytotaxonomy Research Department, Horticulture Research Institute (HRI), Agricultural Research Center, Giza, Egypt. A voucher specimen (FUPD-45) was kept at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Fayoum University, Egypt.

**2.3. Extraction and Bioguided Isolation.** The shade-dried leaves (1 kg) were grounded and extracted with 75% ethanol at room temperature three times 24 h each. The total extract was concentrated to afford a crude residue (177 g). 83 g of the 75% ethanolic extract was suspended in water and exposed to successive fractionation using *n*-hexane and EtOAc to afford 9 g and 12 g, respectively. The remaining aqueous fraction was lyophilized to afford 61 g. All fractions were phytochemically investigated and tested against K562 cell line. The bioactive fractions were subjected to bioguided isolation techniques as described in the consequent text.

The *n*-hexane fraction (6 g) of SS leaves was saponified according to the published procedure [23] and the fatty acid

methyl ester (FAME) and unsaponifiable matter (USM) were then subjected to GC/MS analysis. USM (78.8 mg) was chromatographed on silica gel column by gradient elution with *n*-hexane/EtOAc (2.5% gradient) to give 9 subfractions (A1–A9). **Fr-A-6** (eluted by 7.5% EtOAc) was one spot on TLC and afforded compound 1 (11 mg). **Fr-A-8** (eluted by 10% EtOAc) was one spot on TLC and afforded compound 2 (25 mg).

The EtOAc fraction (10 g) was chromatographed on a silica gel solid phase extraction (Si-SPE) column by gradient elution with *n*-hexane/EtOAc to give fractions (B1–B5). The subfraction **Fr-B-2** (520 mg) was first subjected to silica gel SPE column chromatography and eluted with *n*-hexane/EtOAc gradient (2% gradient) to afford 5 subfractions. Subfraction **Fr-B-2-2** was further purified using silica gel column and eluted with *n*-hexane/EtOAc (2.5% gradient) to afford compound (3) (5 mg) (eluted by 10% EtOAc). The subfraction **Fr-B-2-4** was further chromatographed on silica gel column and eluted with *n*-hexane/acetone (2.5% gradient) to afford compounds (4) (1 mg), (5) (15.6 mg), (6) (1 mg), and (7) (2.5 mg) (eluted by 7.5%, 10%, 12.5%, and 15% acetone, respectively). The subfraction **Fr-B-5** (4.5 g) was chromatographed on VLC silica gel column by gradient elution with DCM/MeOH (2.5% gradient) to afford 5 subfractions. **Fr-B-5-4** (614.8 mg) (eluted by 20% MeOH) was subjected to a column of Sephadex LH-20 and eluted with methanol to give 8 subfractions. **Fr-B-5-4-5** (164.2 mg) was chromatographed on a silica gel column and eluted with DCM/MeOH (2.5% gradient) to afford compound (8) (8 mg) (eluted by 25% MeOH). **Fr-B-5-4-6** (46 mg) was subjected to a column chromatography on Sephadex LH-20 and eluted with MeOH to give compound (9) (2 mg). **Fr-B-5-4-8** (34.3 mg) was subjected to column chromatography on a Sephadex LH-20 and eluted with MeOH to give compounds (10) (14.5 mg), (11) (11 mg), (12) (4 mg), and (13) (3 mg). **Fr-B-5-5** (2.35 g) was subjected to a column of Sephadex LH-20 and eluted with methanol to afford 8 subfractions. **Fr-B-5-5-4** (358 mg) was subjected to a column of Sephadex LH-20 and eluted with methanol to afford 5 subfractions. **Fr-B-5-5-4-3** (27 mg) was purified by HPLC RP column and eluted with water/methanol gradient to afford compounds (14) (20 mg) and (15) (2.36 mg) at retention times (RT) = 9.8 and 10.3 minutes, respectively (Fig. S1, Supplementary Materials). **Fr-B-5-5-4-5** (30 mg) was purified by HPLC RP column and eluted with water/methanol gradient to afford compounds (16) (15 mg) and (17) (3 mg) at RT = 8.0 and 8.29 minutes, respectively (Fig. S2, Supplementary Materials). **Fr-B-5-5-6** (80 mg) was chromatographed on a silica gel column by gradient elution with DCM/MeOH (2.5% gradient) to afford compound (18) (6 mg) (eluted by 25% MeOH).

The aqueous fraction (60.0 g) was subjected to a column chromatography using HP-20 ion exchange resin and eluted with MeOH/water (25% gradient) to afford five fractions (C1–C5). **Fr-C-5** (5 g) (eluted by 100% MeOH) was further chromatographed on a silica gel column by gradient elution with DCM/MeOH (2.5% gradient) to afford 9 subfractions. **Fr-C-5-2** (511 mg) (eluted by 2.5% MeOH) was further chromatographed on a silica gel column using gradient

elution with DCM/EtOAc (5% gradient) to afford compounds (19) (17.7 mg), (20) (33.3 mg), (21) (5 mg), (22) (8 mg), (23) (2 mg), (24) (52 mg), (25) (1.9 mg), and (26) (1.7 mg). **Fr-C-5-4** (511 mg) was chromatographed on RP-18-silica gel column by gradient elution with MeOH/water (5% gradient) to give four subfractions. **Fr-C-5-4-1** (7 mg) was purified by RP-18 preparative HPLC to give compounds (27) (20 mg) and (28) (6 mg) at retention times (RT) = 17.17 and 18.14 minutes, respectively (Fig. S3, Supplementary Materials). **Fr-C-5-4-3** (198 mg) was chromatographed on a silica gel column using DCM/MeOH (5% gradient) to afford compounds (29) (45 mg), (30) (2 mg), (31) (2 mg), (23) (3 mg), (33) (2 mg), and (34) (22.5 mg). **Fr-C-5-5** (672 mg) was chromatographed on reversed phase silica gel solid phase extraction (RP-SPE) column by gradient elution with MeOH/water (5% gradient) to give four subfractions. **Fr-C-5-5-2** (400 mg) was further chromatographed on a silica gel column by gradient elution with DCM/MeOH (5% gradient) to afford compound (35) (64.8 mg).

Compounds (1)–(35) were identified using <sup>1</sup>HNMR, DEPT-Q, HSQC, HMBC, and HRESIMS spectroscopic techniques as well as comparing these data with the literature.

**2.4. Hederatriol 3-O-β-D-Glucuronic Acid Methyl Ester (33).** White powder (MeOH),  $[\alpha]_{D}^{25} = +34.5$  ( $c = 0.15$  in MeOH). <sup>1</sup>H and DEPT-Q data (Table 1); HRESIMS  $m/z$  649.45082  $[M+H]^+$  (calcd. C<sub>37</sub>H<sub>61</sub>O<sub>9</sub>,  $m/z$  649.43156).

**2.5. Cytotoxicity Assay.** K562 cells from the American Type Culture Collection (ATCC) were plated in clear 384-well plates at an initial density of 2500 cells/well in 40 μL of growth medium (DMEM with 10% FBS and 1% Pen/step). Next day, the test agents were added in quadruplicates at the specified concentration and the treatment continued for 48 h and the cell viability was finally assessed using WST-8 assay Cell Counting Kit from Bimake, according to manufacturer's instructions. The results were calculated by measuring the absorbance at 450 nm using SpectraMax M5 plate reader (Molecular Devices). Cell viability was calculated in comparison to DMSO as a negative control, as well as Taxol and Doxorubicin as positive controls [24]. The extract and fractions were screened primarily at concentrations of 50 and 75 μg/mL and the percentage inhibitions were calculated. Compounds (1)–(35) were tested at six concentrations (5, 10, 25, 50, 75, and 100 μg/mL), where compounds (1), (5), (19), (20), (21), (22), (24), (29), (32), (34), and (35) were shown to be bioactive and their IC<sub>50</sub> values were calculated.

**2.6. Transfection and Luciferase Assays.** HeLa cells from ATCC were plated in white opaque 384-well plates at a density of 4300 cells/well in 30 μL of growth medium (DMEM with 10% FBS and 1% Pen/step). Next day, the medium was aspirated and replaced with DMEM containing 1% FBS. The cells were transfected with respective plasmids [25] using X-tremeGENE HP transfection reagent (Roche). After 24 h of transfection, the test agents were added in duplicates to the transfected cells, followed 30 min later by

TABLE 1: NMR data for compound (33) (500 MHz in DMSO- $d_6$ ).

Position	$\delta_C$ , type	$\delta_H$ ( $J$ in Hz)	HMBC	TOCSY
1	38.4, CH <sub>2</sub>	0.82		2, 3
2	25.5, CH <sub>2</sub>	1.59, 1.68		3
3	80.4, CH	3.50	1'	1
4	42.8, C			
5	46.4, CH	1.17	6	
6	17.6, CH <sub>2</sub>	1.39		
7	32.7, CH <sub>2</sub>	1.50		
8	39.2, C			
9	41.3, CH	2.77		
10	36.4, C			
11	23.3, CH <sub>2</sub>	1.80, 1.88		12
12	121.7, CH	5.76		11, 18
13	144.6, C			
14	41.8, C			
15	27.7, CH <sub>2</sub>	0.97, 1.61	27	
16	22.5, CH <sub>2</sub>	1.41		15
17	45.9, C	-		
18	47.5, CH	1.51		
19	46.3, CH <sub>2</sub>	1.59	12, 29, 30	28
20	30.9, C	-		
21	33.9, CH <sub>2</sub>	1.41		28
22	32.4, CH <sub>2</sub>	1.44		28
23	13.3, CH <sub>3</sub>	0.59	3, 4, 5, 24	
24	62.8, CH <sub>2</sub>	3.07, 3.44 (d, $J = 10.5$ Hz)	3	
25	16.0, CH <sub>3</sub>	0.87	10, 11	9
26	17.4, CH <sub>3</sub>	0.71	8, 14	
27	26.0, CH <sub>3</sub>	1.09	8, 13, 14, 15	
28	70.2, CH <sub>2</sub>	3.51 (S)	16, 18	18
29	23.9, CH <sub>3</sub>	0.87	20, 30	
30	33.3, CH <sub>3</sub>	0.86	18, 20	
1'	105.1, CH	4.33 (d, $J = 8$ Hz)	3	2', 3', 4', 5'
2'	74.1, CH	2.98 (t, $J = 8.5$ Hz)	1', 5'	1', 4'
3'	75.9, CH	3.16 (t, $J = 9$ Hz)	4'	1', 2', 5'
4'	72.1, CH	3.28 (t, $J = 9.5$ Hz)	2'	1', 2', 5'
5'	76.4, CH	3.65	1'	1', 2', 3'
6'	170.1, C			
7'	52.3, CH <sub>3</sub>	3.67 (s)	6'	2', 3', 4'

an inducing agent (IL-6 for Stat 3, TGF- $\beta$  for Smad, m-Wnt3a for Wnt, and PMA for AP-1, NFk-B, E2F, Myc, ETS, Notch, and Hedgehog). No inducer was added for pTK, FOXO, miR-2, K-Ras, and AhR. After 4 h or 6 h of induction, the cells were lysed by the addition of One-Glo luciferase assay system (Promega, Madison, WI, USA). The light output was detected in a GloMax-Multi+ Detection System with Instinct Software (Promega, Madison, WI, USA). This luciferase assay determines if the test agent was able to inhibit the activation of cancer-related signaling pathways. In the case of FOXO and mi-R21, the enhanced luciferase activity by the test agent was assessed [26].

**2.7. Molecular Docking.** All docking simulations were conducted using MOE 2019 software (<https://www.chemcomp.com>). The receptors and the ligands were prepared using the standard structure optimization protocol of the software. The receptors were obtained from the protein data bank, PDB IDs: 3QX3, 3QRJ, 1M17, and 2SRC for topoisomerase, Abl kinase, EGFR-tyrosine kinase, and SRC

kinase, respectively. Then they were energy-minimized under AMBER12:EHT force field. The active sites were set as where the cocrystalized ligand was bound. The docking was performed using a molecular structure of compounds isolated from SS leaves using the general protocol of MOE DOCKTITE Wizard. The validation of docking experiments was achieved through the redocking of the cocrystalized ligands into their corresponding active sites and then the root means square deviation (RMSD) was calculated. The docking results were visualized and analyzed by DS Visualizer available from BIOVIA Inc. and the affinity of binding (docking scores) was calculated in Kcal/Mole for each compound against the selected targets.

### 3. Results and Discussion

Through our ongoing efforts to discover antileukemic natural compounds from Egyptian plants, SS leaves showed a potential promising result against K562 cell line *in vitro* [1]. Accordingly, SS leaves were selected for further phytochemical and biological investigations with the aim of

isolating the bioactive metabolites. The total extract and the successive fractions were screened against K562 cell line at a concentration of 20  $\mu\text{g}/\text{mL}$  and the percentage inhibitions were calculated. The extracts of 75% ethanol, *n*-hexane, ethyl acetate, and the aqueous fractions showed mild antileukemic activities with percent inhibitions of 13%, 16%, 23%, and 9%, respectively, at a concentration of 20  $\mu\text{g}/\text{mL}$ . All the fractions were phytochemically investigated, and their columns subfractions were screened for their antileukemic activities as described in the biological study results.

### 3.1. Phytochemical Study

**3.1.1. Bioguided Phytochemical Investigation of the *n*-Hexane Fraction.** The study resulted in the identification of 16 compounds in the saponifiable matter (Table S1, *Supplementary Materials*) with five majors identified as methyl palmitate, methyl elaidate, methyl linoleate, methyl linolenate, and ethyl linolenate at retention times = 33.1, 37.5, 37.8, 38.4, and 39.6 minutes, respectively. Moreover, 12 compounds were identified in the unsaponifiable matter (Table S2, *Supplementary Materials*) with five majors identified as phytol, stigmasta-5,22-dien-3-ol acetate (3, beta, 22 Z), stigmastan-3,5,22-trien, stigmasta-3,5-diene, and stigmasterol at retention times = 37.2, 47, 47.2, 52.6, and 66.1 minutes, respectively. Additionally, the unsaponifiable matter was purified and resulted in the isolation of two compounds identified as phytol (1) [27] and stigmasterol (2) [28] (Fig. S4, *Supplementary Materials*).

**3.1.2. Bioguided Phytochemical Investigation of the EtOAc Fraction.** The result was the isolation of 16 known compounds identified as liquidambaric lactone (3) [29], maslinic lactone (4) [30], oleanolic acid (5) [31], 11 $\alpha$ ,12 $\alpha$ -epoxy-oleanolic lactone (6) [32], oleanderolide (7) [33], stigmasterol glucoside (8) [34], *P*-aminophenol (9) [35], quercetin-3-*O*- $\alpha$ -L-rhamnopyranoside (10) [36], quercetin-3-*O*- $\beta$ -D-glucopyranoside (11) [36], quercetin-3-*O*- $\alpha$ -L-arabinoside (12) [37], quercetin-3-*O*- $\beta$ -D-xyloside (13) [37], mauritianin (14) [38], isorhamnetin 3-*O*-(2,6-di-*O*- $\alpha$ -rhamnosyl)- $\beta$ -D-galactopyranoside (15) [39], clitorin (16) [40], 3-[(*O*-6-deoxy- $\alpha$ -L-mannopyranosyl-(1 $\rightarrow$ 2)-*O*-[6-deoxy- $\alpha$ -L-mannopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranosyl) oxy]-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one (17) [41], and kaempferol-3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)]-(4-*O*-*E*-*p*-coumaroyl)- $\beta$ -D-galactopyranoside (18) [42] (Fig. S4 and Table S3, *Supplementary Materials*).

**3.1.3. Bioguided Phytochemical Investigation of the Aqueous Fraction.** The result was the isolation of one new compound (33) (Hederatriol 3-*O*- $\beta$ -D-glucuronic acid methyl ester), as well as 16 known compounds identified as betulinic acid (19) [31,43], ursolic acid (20) [44], 3 $\beta$ -*O*-(*cis*-*p*-coumaroyl)-2 $\alpha$ -hydroxyurs-12-en-28-oic acid (21) [45], 3 $\beta$ -*O*-(*trans*-*p*-coumaroyl)-2 $\alpha$ -hydroxyurs-12-en-28-oic acid (known as jacoumaric acid) (22) [45], obtuslin (23) [44], corosolic acid

(24) [45],  $\beta$ -sitosterol glucoside (25) [46], hederagenin-3-*O*- $\beta$ -D-glucuronopyranoside 6'-*O*-methyl ester (26) [47], kaempferol-3-*O*-rutinoside (27) [48], isorhamnetin-3-*O*-rutinoside (28) [49], hederagenin-3-*O*- $\beta$ -D-glucuronopyranoside (29) [47], 11-ketocorosolic acid (30) [50], hederagenin (31) [51], oleanolic acid 3-*O*- $\beta$ -D-glucuronopyranoside 6' methyl ester (32) [52], oleanolic acid 3-*O*- $\beta$ -D-glucuronopyranoside (34) [18], and chikusetsusaponin II (35) [53] (Fig. S4 and Table S3, *Supplementary Materials*).

Compounds (2), (5), (19), and (34) were previously reported in *SS* leaves [18,19], while this is the first report of compounds (1), (3)-(4), (6)-(18), (20)-(33), and (35) from this species.

**3.2. Structure Elucidation of Compound (33).** Compound (33) was isolated as a white powder (2 mg, soluble in MeOH),  $[\alpha]_D^{25} = +34.5$  ( $c = 0.15$  in MeOH). Its molecular formula,  $\text{C}_{37}\text{H}_{60}\text{O}_9$ , was derived from the  $^1\text{D}$  NMR data (Table 1) and the supportive HRESIMS ion at  $m/z$  649.45082  $[\text{M}+\text{H}]^+$  (calcd. for  $\text{C}_{37}\text{H}_{61}\text{O}_9$ , 649.43156). The molecular formula revealed the hydrogen deficiency index to be 29.6 ppm. From the  $^1\text{H}$  NMR spectrum, the seven distinct methyls [ $\delta_{\text{H}}$  3.67 (3H, s), 1.09 (3H, s), 0.87 (6H, s), 0.86 (3H, s), 0.78 (3H, s), 0.71 (3H, s), and 0.59 (3H, s)] were observed alongside with the signal assignable to one olefinic proton [ $\delta_{\text{H}}$  5.76 (1H, t)]. Two oxygenated methylenes were detected ( $\delta_{\text{H}}$  3.51 (2H, s), 3.07 (1H, d,  $J = 10.5$  Hz), and 3.44 (1H, d,  $J = 11$  Hz)). Five oxygenated methines ( $\delta_{\text{H}}$  3.67 (1H), 3.50 (1H), 3.28 (1H, t), 3.16 (1H, t), 2.98 (1H, t)) alongside the one anomeric proton ( $\delta_{\text{H}}$  4.33 (d,  $J = 8$  Hz)) were detected. The DEPTQ-135 spectra of (33) showed 37 carbon signals (Table 1) of which 30 signals were assigned to the aglycone moiety (hederatriol) and 7 to a glucuronic acid methyl ester moiety. The anomeric carbon resonances ( $\delta_{\text{C}/\text{H}}$  105.1/4.33) as well as four oxygenated methine signals at  $\delta_{\text{C}/\text{H}}$  74.1/2.98, 75.9/3.16, 72.1/3.28, and 76.4/3.67 alongside the carbonyl ester ( $\delta_{\text{C}}$  170.1) and the attached methoxy signal ( $\delta_{\text{C}/\text{H}}$  52.3/3.67) disclosed the presence of glucuronic acid 6' methyl ester moiety. The anomeric configuration of the glucuronoid unit was determined to be based on the coupling constant ( $J_{\text{H1}} = 8$  Hz). There were 30 carbons left for the skeleton, indicating a triterpenoid core of (33). The skeletal carbons, particularly the six methyl carbons ( $\delta_{\text{C}}$  33.3, 26.0, 23.9, 17.4, 16.0, and 13.3), alongside the two oxygenated methylenes [ $\delta_{\text{C}}$  62.8 (C-24) and 70.2 (C-28)] implied an olean-type triterpenes alcohol derivative (hederatriol) scaffold for (33) [54]. Free alcoholic methylene at position C28 was confirmed by 2D HMBC & TOCSY correlations between the OH-proton at C28 ( $\delta_{\text{H}}$  3.5) and C18 in HMBC spectrum. Moreover, protons H16, H19, and H21 showed TOCSY correlations to H28. Free alcohol OH signal ( $\delta_{\text{H}}$  3.79) showed TOCSY correlation with H28 methylene. All of the previous data supported the presence of hederatriol. The HMBC cross-peaks enabled the substituent groups to be positioned. The glucuronoid moiety was connected to C-3 as suggested by the HMBC correlation of H-1' with  $\delta_{\text{C}}$  80.4 (C-3) and H-3 to the anomeric carbon at  $\delta_{\text{C}}$  105.1 (C-1') of the glucuronoid moiety (Figure 2). The structure of compound



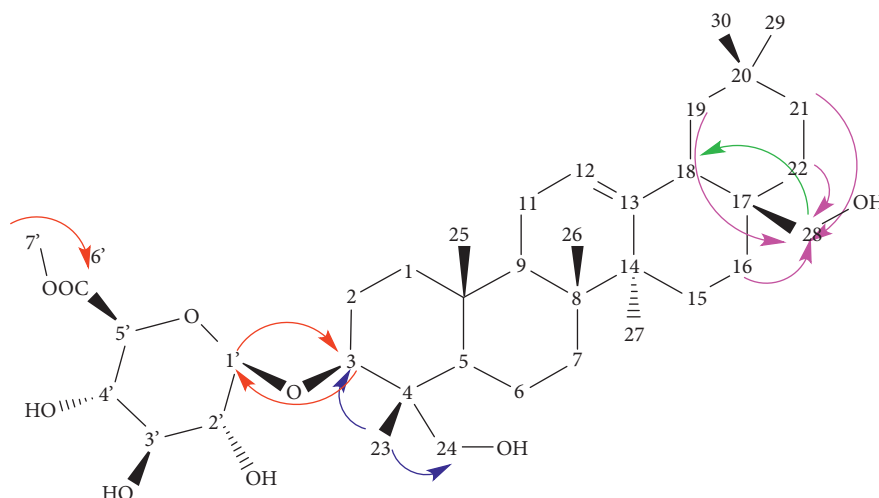


FIGURE 2: Structure and HMBC and TOCSY correlations of compound (33).

(33) is considered to be new due to the 3-*O*-glycosylation of hederatriol with glucuronic acid methyl ester. Thus, compound (33) was elucidated as hederatriol 3-*O*- $\beta$ -D-glucuronic acid methyl ester (NMR data, Figures S5–S11, *Supplementary Materials*).

### 3.3. Biological Study

**3.3.1. Cytotoxicity against Leukemia K562 Cell Line.** The *n*-hexane fraction was fractionated by saponification with KOH, where the yielded saponifiable and unsaponifiable matters were tested against K562 cells and showed inhibitions of  $IC_{50} = 75.7$  and  $73.9 \mu\text{g/mL}$ , respectively. Accordingly, the phytoconstituents in both fractions were investigated using GC-MS analysis.

The EtOAc fraction (Fr-B) was fractionated using Si-SPE column and the five resulted subfractions were tested against K562 cells at concentrations of 50 and  $75 \mu\text{g/mL}$ . The results showed that subfractions Fr-B-2 and Fr-B-5 were the bioactive fractions with percent inhibitions of 93.7% and 47.3%, respectively, at a concentration of  $75 \mu\text{g/mL}$  (Figure S12, *Supplementary Materials*). The subfraction Fr-B-2 was chromatographed using silica gel SPE column and the resulting subfractions were tested against K562 cells. Subfractions Fr-B-2-2 and Fr-B-2-4 were the most cytotoxic with percent inhibitions of 45% and 47%, respectively, at a concentration of  $75 \mu\text{g/mL}$  (Fig. S13, *Supplementary Materials*). The subfraction Fr-B-5 was chromatographed using VLC silica gel column and the resulting subfractions were tested against K562 cells. Subfractions Fr-B-5-4 and Fr-B-5-5 were the most cytotoxic with percent inhibitions of 28% and 85%, respectively, at a concentration of  $75 \mu\text{g/mL}$  (Fig. S13, *Supplementary Materials*).

The aqueous fraction (Fr-C) was chromatographed using HP-20 ion exchange resin and the five resulting subfractions that were screened for their activity against K562 cells at concentrations of 50 and  $75 \mu\text{g/mL}$ . The results showed that subfraction Fr-C-5 was the bioactive fraction with 94.4% percent of inhibition at concentrations of 50 and  $75 \mu\text{g/mL}$

(Fig. S14, *Supplementary Materials*). Subfraction Fr-C-5 was chromatographed on silica gel column and the eight produced subfractions that were screened for their activity against K562 cells at concentrations of 50 and  $75 \mu\text{g/mL}$ . The results showed that subfractions Fr-C-5-2 and Fr-C-5-4 were the most active ones with 65% and 63% percent of inhibitions, respectively, at a concentration of  $75 \mu\text{g/mL}$  (Fig. S15, *Supplementary Materials*).

All the detected bioactive subfractions were subjected to several chromatographic techniques as mentioned in the Materials and Methods section with the aim of isolating and elucidating the structures of the bioactive constituents.

Compounds (1)–(35) were screened against K562 cell line at concentrations of 50 and  $75 \mu\text{g/mL}$  and  $IC_{50}$  values were calculated. Oleanolic acid 3-*O*- $\beta$ -D-glucuronopyranoside (34) was identified as the most antiproliferative compound isolated from SS extract with  $IC_{50}$  value of  $22.3 \mu\text{M}$  compared to Taxol ( $\sim 2 \mu\text{M}$ ) and Doxorubicin ( $\sim 4 \mu\text{M}$ ). Other active triterpenes were betulinic acid (19), ursolic acid (20), 3 $\beta$ -*O*-(*cis-p*-coumaroyl)-2 $\alpha$ -hydroxyurs-12-en-28-oic acid (21), jacoumaric acid (22), corosolic acid (24), and chikusetsusaponin II (35) with  $IC_{50}$  values of 37.5, 31.3, 36.6, 30.8, 33.7, and  $41.5 \mu\text{M}$ , respectively. Phytol (1), oleanolic acid (5), hederagenin 3-*O*- $\beta$ -D-glucuronopyranoside (29), and oleanolic acid 3-*O*- $\beta$ -D-glucuronopyranoside methyl ester (32) showed mild activity against K562 cell line with  $IC_{50}$  values of 112.3, 67.6, 83.3, and  $56.4 \mu\text{M}$ , respectively (Figure 3). Out of the 35 isolated compounds, the triterpene glycosides were shown to be the most active antileukemic compounds in this plant. Antileukemic activities of betulinic acid (19) [55], ursolic acid (20) [56], oleanolic acid (5) [57], and phytol (1) [58] were previously reported in literature but this is the first report of antileukemic activities of compounds (21), (22), (24), (29), (32), (34), and (35). Moreover, methyl linolenate, the major constituent of the saponifiable matter of the *n*-hexane fraction of SS leaves, was

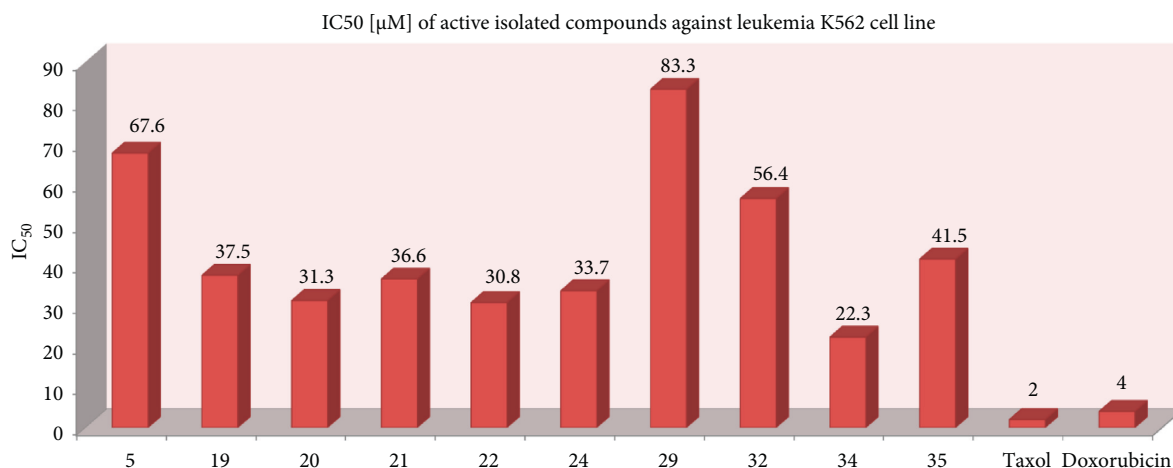


FIGURE 3: IC<sub>50</sub> [μM] of the active isolated compounds against leukemia K562 cell line.

TABLE 2: IC<sub>50</sub> [μM] of active isolated compounds against selected transcription factor signaling pathways in HeLa cells

Compound no.	Stat 3/IL-6	Smad/TGF-β	AP-1/PMA	NF-kB/PMA	E2F/PMA	Myc/PMA	ETS/PMA	Notch/PMA	FOXO	Wnt/m-Wnt-3a	Hedgehog/PMA	pTK	miR-21	K-Ras	AhR
21	13.9	9.5	20.9	21.3	10.5	15.2	11.4	11.3	ND	4.6	14.4	ND	21.4	23.1	ND
22	17.5	3.8	22.2	32.3	ND	18.3	18.9	21.0	ND	3.8	20.1	ND	25.8	26.3	ND
24	15.9	13.9	16.3	18.8	ND	14.4	16.5	17.9	ND	13.5	16.9	ND	18.2	15.5	17.5
34	6.9	5.8	7.0	6.3	5.3	5.9	6.8	6.5	ND	6.70	7.40	7.30	8.00	NA	ND

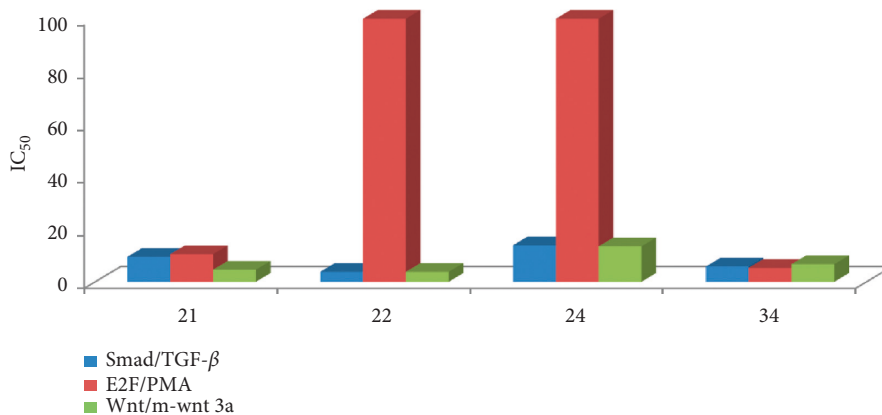


FIGURE 4: IC<sub>50</sub> [μM] of active isolated compounds against selected transcription factor signaling pathways.

previously reported for its cytotoxicity against K562 cell line [59]. Other isolated compounds showed no cytotoxicity against K562 cell line.

**3.3.2. Triterpenes Selectively Target Cancer Signaling Pathways.** The most active isolated compounds (21), (22), (24), and (34) against K562 cell line were further evaluated for targeting the cancer signaling pathways. These compounds were tested for their influence on the battery of 15-plex transcription factor assay. The results (Table 2 and Figure 4) showed that compounds (22) and (21) showed

specific inhibition of Wnt pathway (IC<sub>50</sub> = 3.8 and 4.6 μM, respectively) while compound (22) showed specific inhibition of Smad (IC<sub>50</sub> = 3.8 μM). Compound (34) can strongly alter the signaling pathways of Smad and E2F (IC<sub>50</sub> = 5 μM). From these results, we can conclude the correlation between the inhibition of Smad, E2F, and Wnt signaling pathways and the leukemia K562 cell line growth inhibition (Figure 5).

As cancer signaling plays a complex role in CML, the observed results from this study could be helpful in finding new therapeutic agents and molecular genetics-based treatment targeting these metabolic pathways. Despite the fortune



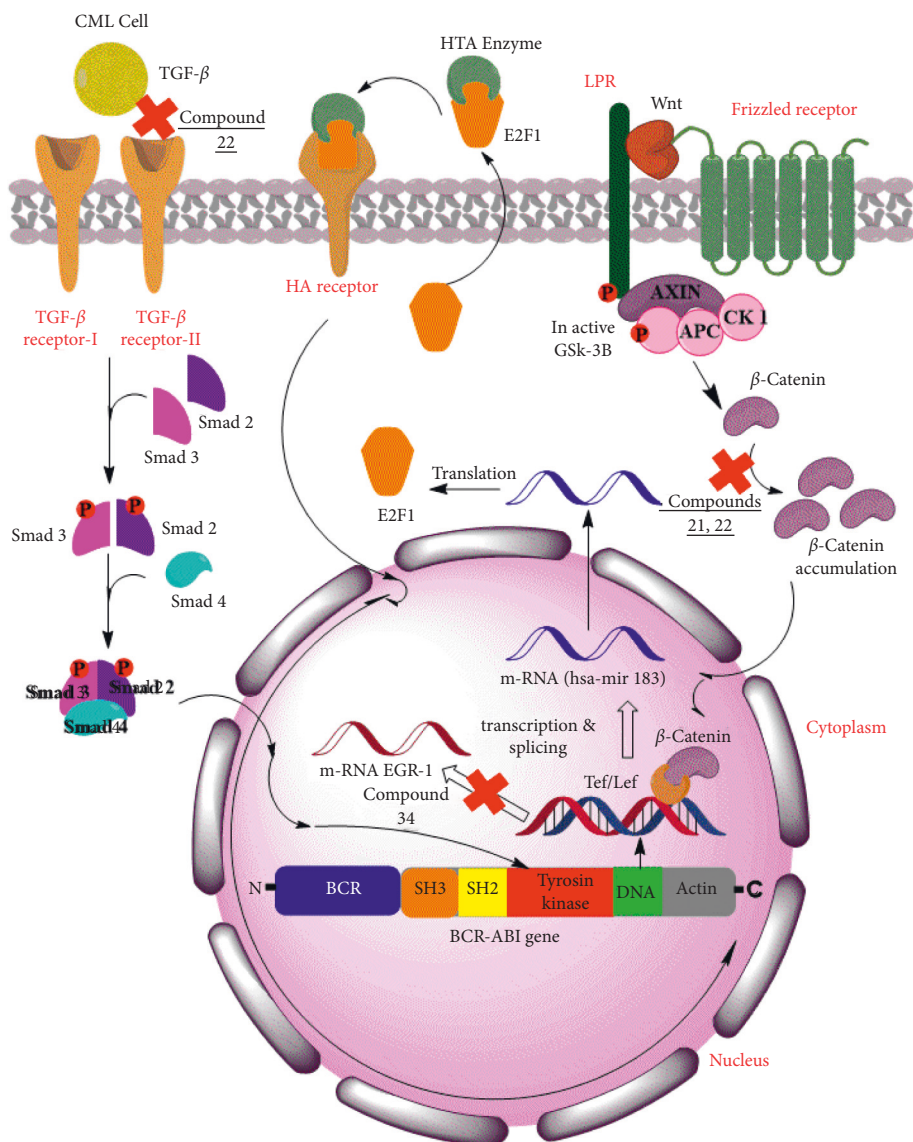


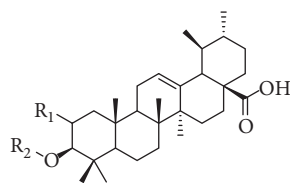
FIGURE 5: Mode of action of active isolated compounds against Smad, Wnt, and E2F signaling pathways.

available research on the molecular details of TGF- $\beta$  signaling, little is known regarding how TGF- $\beta$  and Smad influence CML [60]. One of these realized relationships is that the constitutively active tyrosine kinase produced by the specific Bcr-Abl fusion gene on the Philadelphia chromosome can enhance the resistance of malignant cells to TGF- $\beta$ 1-induced growth inhibition and apoptosis [61]. Previous studies reported ursolic acid and oleanolic acid as antagonists of TGF- $\beta$ 1 binding to its receptor in Balb/c 3T3 cells [62]. A recent study revealed that inhibition of E2F1 reduced CML cell proliferation, leading to p53-mediated apoptosis [63]. The BCR-ABL1 protein kinase-dependent pathway intervened by the upregulation of *hsa-mir183*, the downregulation of its direct target early growth response 1 (EGR1), and, as a consequence, upregulation of E2F1 [58]. Wnt signaling assumes a significant part in the malignancies of the hematopoietic system [64]. Several compounds that are natural in origin were reported in literature for targeting Wnt pathway as

a mechanism for anticancer activity such as ursolic acid and corosolic acid that were reported as antagonists of the Wnt/ $\beta$ -catenin pathway [65–67]. Correlating the previous literature to the results, a mechanism of action of the active compounds could be supposed. The active compounds were mainly oleanolic acid and ursolic acid derivatives which exhibited their antileukemic activities by targeting Smad, E2F, and Wnt signaling pathways (Figure 5).

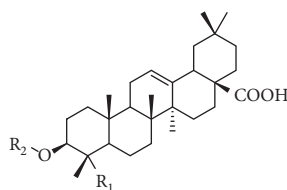
**3.3.3. Molecular Docking.** Multitarget therapies are crucial in the field of complex diseases such as cancers due to activation of compensatory mechanisms and consecutive cellular pathways [68]. The recent research showed a correlation between CML cell line K562 and several proteins which are considered as targets whose inhibition leads to antiproliferative effect in this cell line. Four target proteins, human topoisomerase II beta in complex with DNA and





R <sub>1</sub>	R <sub>2</sub>	Compound name	K562 IC <sub>50</sub> μM	Smad/ TGF-β	E2F /PMA	Wnt/m- wnt 3a
H	H	Ursolic acid (20)	31.3			
OH	H	Corosolic acid (24)	33.7	13.9	NA	13.5
OH	<i>Z-p</i> - coumaric acid	3β- <i>O</i> -( <i>cis-p</i> -Coumaroyl)-2α- hydroxyurs-12-en-28-oic acid (21)	36.6	9.5	10.5	4.6
OH	<i>E-p</i> - coumaric acid	Jacoumaric acid (22)	30.8	3.8	NA	3.8

FIGURE 8: Antileukemic activities of different types of ursane triterpenes isolated from SS leaves.



R <sub>1</sub>	R <sub>2</sub>	Compound name	K562 IC <sub>50</sub> μM
CH <sub>3</sub>	H	Oleanolic acid (5)	67.6
CH <sub>3</sub>	Glucuronic acid	Oleanolic acid 3- <i>O</i> -β- <i>D</i> - glucuronopyranoside (34)	22.3
CH <sub>3</sub>	Glucuronic acid methyl ester	Oleanolic acid 3- <i>O</i> -β- <i>D</i> - glucuronopyranoside methyl ester (32)	56.4
CH <sub>3</sub>	β- <i>D</i> - glucuronopyranoside (6→1)-β- <i>D</i> - glucopyranoside	Chikusetsusaponin II (35)	41.5
CH <sub>2</sub> OH	Glucuronic acid	Hederagenin 3- <i>O</i> -β- <i>D</i> -glucuronopyranoside (21)	83.3

FIGURE 9: Antileukemic activities of different types of oleanane triterpenes isolated from SS leaves.

respectively) compared to etoposide (docking score = -7.10 Kcal/Mole). Compounds (22) and (34) demonstrated a strong binding affinity towards EGFR-tyrosine kinase (docking score = -7.12 and -7.35 Kcal/Mole, respectively) compared to erlotinib (docking score = -6.99 Kcal/Mole). Moreover, compound (34) showed a strong binding affinity towards Abl kinase (docking score = -7.05 Kcal/Mole)

compared to rebastinib (docking score = -7.86 Kcal/Mole) (Table 3 and Figure 6).

3.3.4. *Structure Activity Relationship (SAR) Study.* The structures of the isolated compounds could be classified as oleanane triterpenoids, oleanane lactones triterpenoids, ursane

triterpenoids, lupane triterpenes, steroids, and flavonoid glycosides (Fig. S3 and Table S4, Supplementary Materials).

Among the different classes of isolated compounds, triterpenes were shown as the bioactive antileukemic molecules present in the SS leaves. The potency of the isolated triterpenoids prompted us to investigate the structure activity relationships underlying their antileukemic activities.

In this study, three types of triterpenoids aglycones were isolated and identified: lupane triterpenoids represented by betulinic acid, oleanone type represented by oleanolic acid, and ursane type represented by ursolic acid. The results showed that the most active antileukemic triterpenoid is ursane type followed by lupane and the least active type is oleanone triterpenoids (Figure 7).

From ursane type triterpenoids, six compounds (20)–(24) and (30) were isolated. Comparing their structures and antileukemic activities, two positions, C-2 and C-3, were believed to affect the activity. Hydroxylation at C-2 position slightly decreases the antileukemic activity. Also, the esterification at C-3 position by coumaric acid can increase or decrease the activity depending on the configuration of coumaric acid moiety. The *trans* coumaroyl derivative, jacoumaric acid (22), showed higher antileukemic activity than corosolic acid (24), while *cis* derivative, 3 $\beta$ -*O*-(*cis-p*-coumaroyl)-2 $\alpha$ -hydroxyurs-12-en-28-oic acid (21), showed lower activity and these results support the idea of the higher activity of *trans* conformers than the *cis* one (Figure 8).

The SAR study showed that the activity of oleanolic acid can be greatly increased when esterified at C-3 position by glucuronic acid. Compound (34) showed strong antileukemic activity compared to compound (5) due to the presence of glucuronic acid moiety attached at C-3. In addition, the free OH group in glucuronic acid is crucial for the activity and, as the results show, when it is methylated as in compound (32) or glycosylated as in compound (35), the activity decreased. Meanwhile, for oleanone triterpenes, the methylation at C-23 as in compounds (5), (32), and (34) increases the activity compared to compound (29) (Figure 9).

#### 4. Conclusion

One new compound (33) and 34 known compounds were isolated from the leaves of *Sesbania sesban* encompassing 19 triterpenoids ((3)–(7), (19)–(24), (26), and (29)–(35)), three steroids ((2), (8), and (25)), 11 flavonoid glycosides ((10)–(18) and (27)–(28)), one fatty alcohol (1), and one phenolic compound (9). The antileukemic activity of the isolated compounds was evaluated against leukemia K562 cell line *in vitro* and 11 triterpenoids exhibited remarkable antileukemic activities against this cell line. Compounds (34) (oleanolic acid 3-*O*- $\beta$ -D-glucuronopyranoside) and (22) (jacoumaric acid) exhibited the strongest activities with IC<sub>50</sub> values of 22.3 and 30.8  $\mu$ M, respectively. The mechanism of the antileukemic activity of these compounds could be attributed to the attenuation of Smad, Wnt, and E2F signaling pathways. Compound (22) demonstrated a specific inhibition of Wnt and Smad signaling with an IC<sub>50</sub> value of 3.8  $\mu$ M for both pathways. Meanwhile compound (34) showed an inhibition of E2F with an IC<sub>50</sub> value of 5  $\mu$ M.

Moreover, the binding patterns of the bioactive molecules against leukemia cell related targets were explored by docking them against topoisomerase, EGFR-tyrosine kinase, and Abl kinase. Compounds (22) and (34) exhibited a strong binding affinity towards topoisomerase (docking score = -7.81 and -9.30 Kcal/Mole, respectively). Furthermore, SAR study reveals that the presence of a carboxyl group either in the triterpene or in the sugar moiety was identified as contributing to the cytotoxic activity.

#### Data Availability

Data are available in the Supplementary Materials.

#### Conflicts of Interest

The authors declare no conflicts of interest.

#### Acknowledgments

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#### Supplementary Materials

Table S1: results of GC/MS analysis of fatty acid methyl ester (FAME) of the n-hexane fraction of SS leaves. Table S2: results of GC/MS analysis of unsaponifiable matter of the n-hexane fraction of SS leaves. Table S3: list of compounds isolated from *Sesbania sesban* L. leaves. Figure S1: HPLC (RP column) purification of compounds (14) and (15) using MeOH/water gradient. Figure S2: HPLC (RP column) purification of compounds (16) and (17) using MeOH/water gradient. Figure S3: HPLC (RP column) purification of compounds (27) and (28) using MeOH/water gradient. Figure S4: chemical structures of compounds isolated from SS leaves. Figure S5: <sup>1</sup>H NMR spectrum of compound (33). Figure S6: deptq135 spectrum of compound (33). Figure S7: dept spectrum of compound (33). Figure S8: HSQC spectrum of compound (33). Figure S9: TOCSY spectrum of compound (33). Figure S10: TOCSY spectrum of compound (33). Figure S11: ESI(-) MS spectrum of compound (33). Figure S12: bioguided screening of EtOAc (Fr-B) column subfractions against leukemia K562 cell line. Figure S13: bioguided screening of Fr-B-2 column subfractions and Fr-B-5 column subfractions against leukemia K562 cell line. Figure S14: bioguided screening of aqueous (Fr-C) column subfractions against leukemia K562 cell line. Figure S15: bioguided screening of Fr-C-5 column subfractions against leukemia K562 cell line. (*Supplementary Materials*)

#### References

- [1] M. H. H. Shima Mohammed Abdelgawad, G. Ahmed Fawzy, and H. I. El-Askary, "In vitro antileukemic activity of extracts of some medicinal plants from upper Egypt in human chronic leukemia K562 cell line," *Tropical Journal of Natural Products Research*, vol. 5, no. 12, pp. 2115–2122, 2021.

- [2] M. A. Aboul-Soud, H. A. El-Shemy, K. M. Aboul-Enein, A. M. Mahmoud, A. M. Al-Abd, and D. A. Lightfoot, "Effects of plant-derived anti-leukemic drugs on individualized leukemic cell population profiles in Egyptian patients," *Oncology letters*, vol. 11, no. 1, pp. 642–648, 2016.
- [3] A. S. Ibrahim, H. M. Khaled, N. N. Mikhail, H. Baraka, and H. Kamel, "Cancer incidence in Egypt: results of the national population-based cancer registry program," *Journal of cancer epidemiology*, vol. 2014, Article ID 437971, 18 pages, 2014.
- [4] S. Eskandari and R. Yazdanparast, "Bcl6 gene-silencing facilitates PMA-induced megakaryocyte differentiation in K562 cells," *Journal of Cell Communication and Signaling*, vol. 11, no. 4, pp. 357–367, 2017.
- [5] M. Karon, E. J. Freireich, E. Frei et al., "The role of vincristine in the treatment of childhood acute leukemia," *Clinical Pharmacology & Therapeutics*, vol. 7, no. 3, pp. 332–339, 1966.
- [6] D. M. Lucas, P. C. Still, L. Bueno Perez, M. R. Grever, and A. Douglas Kinghorn, "Potential of plant-derived natural products in the treatment of leukemia and lymphoma," *Current Drug Targets*, vol. 11, no. 7, pp. 812–822, 2010.
- [7] J. M. S. van Maanen, J. Retel, J. De Vries, and H. M. Pinedo, "Mechanism of action of antitumor drug etoposide: a review," *JNCI Journal of the National Cancer Institute*, vol. 80, no. 19, pp. 1526–1533, 1988.
- [8] K. Hande, "Etoposide: four decades of development of a topoisomerase II inhibitor," *European Journal of Cancer*, vol. 34, no. 10, pp. 1514–1521, 1998.
- [9] T. G. Heuzé, D. Bastianelli, and F. Lebas, "Sesban (Sesbania sesban)," 2015, <https://www.feedipedia.org/node/253>.
- [10] E. Debela, A. Tolera, L. O. Eik, and R. Salte, "Condensed tannins from Sesbania sesban and Desmodium intortum as a means of *Haemonchus contortus* control in goats," *Tropical Animal Health and Production*, vol. 44, no. 8, 2012.
- [11] E. G. Kamel, M. A. El-Emam, S. S. Mahmoud, F. M. Fouda, and F. E. Bayaomy, "Parasitological and biochemical parameters in Schistosoma mansoni-infected mice treated with methanol extract from the plants Chenopodium ambrosioides, Conyza dioscorides and Sesbania sesban," *Parasitology International*, vol. 60, no. 4, pp. 388–392, 2011.
- [12] A. D. Soren, R. P. Chen, and A. K. Yadav, "In vitro and in vivo anthelmintic study of Sesbania sesban var. bicolor, a traditionally used medicinal plant of Santhal tribe in Assam, India," *Journal of Parasitic Diseases*, vol. 45, no. 1, pp. 1–9, 2021.
- [13] A. U. Tatiya, P. R. Dande, R. E. Mutha, and S. J. Surana, "Effect of saponins from of Sesbania sesban L.(Merr) on acute and chronic inflammation in experimental induced animals," *Journal of Biological Sciences*, vol. 13, no. 3, pp. 123–130, 2013.
- [14] S. N. Fitrianyah, I. Fidrianny, and K. Ruslan, "Correlation of total phenolic, flavonoid and carotenoid content of Sesbania sesban (L. Merr) leaves extract with DPPH scavenging activities," *International Journal of Pharmacognosy and Phytochemical Research*, vol. 9, no. 1, pp. 89–94, 2017.
- [15] A.-C. Dorsaz, M. Hostettmann, and K. Hostettmann, "Molluscicidal saponins from Sesbania sesban," *Planta Medica*, vol. 54, no. 03, pp. 225–227, 1988.
- [16] M. N. Walekhwa, T. K. Ogeto, M. K. Murithi, and Z. L. Malago, "Pharmacological effects of Sesbania sesban: a systematic review," *International Journal of Research in Medical Sciences*, vol. 8, no. 3, p. 1185, 2020.
- [17] R. B. Pandhare, B. Sangameswaran, P. B. Mohite, and S. G. Khanage, "Antidiabetic activity of aqueous leaves extract of Sesbania sesban (L) Merr. in streptozotocin induced diabetic rats," *Avicenna Journal of Medical Biotechnology*, vol. 3, no. 1, pp. 37–43, 2011.
- [18] N. Das, P. Chandran, and S. Chakraborty, "Potent spermicidal effect of oleanolic acid 3-beta-D-glucuronide, an active principle isolated from the plant Sesbania sesban Merrill," *Contraception*, vol. 83, no. 2, pp. 167–175, 2011.
- [19] M. Farooq, I. Varshney, and M. S. Khan, "Saponins and sapogenins IV," *Journal of the American Pharmaceutical Association*, vol. 48, no. 8, pp. 466–468, 1959.
- [20] S. Manoharan and J. Kaur, "Anticancer, antiviral, antidiabetic, antifungal and phytochemical constituents of medicinal plants," *American Journal of PharmTech Research*, vol. 3, pp. 149–169, 2013.
- [21] H. Dianhar, Y. M. Syah, D. Mujahidin, E. H. Hakim, and L. D. Juliawaty, "A flavone derivative from Sesbania sesban leaves and its cytotoxicity against murine leukemia P-388 cells," *AIP Conference Proceedings*, vol. 1589, no. 1, pp. 411–413, 2014.
- [22] M. Namadina, S. Yahuza, H. Haruna et al., "Pharmacognostic and antioxidant activity of bryophyllum pinnatum leaves," *International Journal Of Science for Global Sustainability*, vol. 6, no. 2, 2020.
- [23] I. Finar, *Organic Chemistry*, ELBS, Ghitorni, India, 6 edition, 1973.
- [24] M. Kageyama, K. Li, S. Sun et al., "Anti-tumor and anti-metastasis activities of honey bee larvae powder by suppressing the expression of EZH2," *Biomedicine & Pharmacotherapy*, vol. 105, pp. 690–696, 2018.
- [25] P. Balachandran, M. A. Ibrahim, J. Zhang, M. Wang, D. S. Pasco, and I. Muhammad, "Crosstalk of cancer signaling pathways by cyclic hexapeptides and anthraquinones from rubia cordifolia," *Molecules*, vol. 26, no. 3, p. 735, 2021.
- [26] M. A. Zaki, P. Balachandran, S. Khan et al., "Cytotoxicity and modulation of cancer-related signaling by (Z)- and (E)-3, 4, 3', 5'-tetramethoxystilbene isolated from Eugenia rigida," *Journal of Natural Products*, vol. 76, no. 4, pp. 679–684, 2013.
- [27] D. Arigoni, W. Eisenreich, C. Latzel et al., "Dimethylallyl pyrophosphate is not the committed precursor of isopentenyl pyrophosphate during terpenoid biosynthesis from 1-deoxyxylulose in higher plants," *Proceedings of the National Academy of Sciences*, vol. 96, no. 4, pp. 1309–1314, 1999.
- [28] P. Jain, S. Bari, and S. Surana, "Isolation of stigmasterol and  $\gamma$ -sitosterol from petroleum ether extract of woody stem of *Abelmoschus manihot*," *Asian journal of biological sciences*, vol. 2, no. 4, pp. 112–117, 2009.
- [29] Y. Zhu, Y. J. Guan, Q. Z. Chen et al., "Pentacyclic Triterpenes from the resin of Liquidambar formosana have anti-angiogenic properties," *Phytochemistry*, vol. 184, Article ID 112676, 2021.
- [30] G. R. Mallavarapu and E. Muralikrishna, "Maslinic lactone from the heartwood of Terminalia alata," *Journal of Natural Products*, vol. 46, no. 6, pp. 930–931, 1983.
- [31] M. A. Hossain and Z. Ismail, "Isolation and characterization of triterpenes from the leaves of Orthosiphon stamineus," *Arabian Journal of Chemistry*, vol. 6, no. 3, pp. 295–298, 2013.
- [32] Q. Fu, L. Qiu, H.-M. Yuan, T. Yu, L. Zou, and D. and E. Paeonenoides, "Two new nortriterpenoids from *Paeonia lactiflora* and their inhibitory activities on NO production," *Helvetica Chimica Acta*, vol. 99, no. 1, pp. 46–49, 2016.
- [33] L. Fu, S. Zhang, N. Li et al., "Three new triterpenes from nerium oleander and biological activity of the isolated compounds," *Journal of Natural Products*, vol. 68, no. 2, pp. 198–206, 2005.

- [34] S. K. Nigam, G. Misra, and C. R. Mitra, "Stigmasterol glucoside a constituent of *Adenanthera pavonina* seed and leaf," *Planta Medica*, vol. 23, no. 2, pp. 145–148, 1973.
- [35] R. Eyanagi, Y. Hisanari, and H. Shigematsu, "Studies of paracetamol/phenacetin toxicity: isolation and characterization of p-aminophenol-glutathione conjugate," *Xenobiotica*, vol. 21, no. 6, pp. 793–803, Jun 1991.
- [36] K. R. Markham and B. Ternai, "<sup>13</sup>C NMR of flavonoids—II," *Tetrahedron*, vol. 32, no. 21, pp. 2607–2612, 1976.
- [37] M. Kalegari, M. D. Miguel, JdF. G. Dias et al., "Phytochemical constituents and preliminary toxicity evaluation of leaves from *Rourea induta* Planch.(Connaraceae)," *Brazilian Journal of Pharmaceutical Sciences*, vol. 47, no. 3, pp. 635–642, 2011.
- [38] I. Dini, G. Carlo Tenore, and A. Dini, "Phenolic constituents of *Kancolla* seeds," *Food Chemistry*, vol. 84, no. 2, pp. 163–168, 2004/02/01/2004.
- [39] F. Ferreres, D. M. Pereira, P. Valentão, P. B. Andrade, R. M. Seabra, and M. Sottomayor, "New phenolic compounds and antioxidant potential of *catharanthus roseus*," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 21, pp. 9967–9974, 2008/11/12 2008.
- [40] N. Morita, M. Arisawa, M. Nagase, H. Hsu, and Y. Chen, "Studies on the constituents of formosan leguminosae. I. The constituents in the leaves of *Clitoria ternatea* L.," *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan*, 1977.
- [41] R. S. Mohammed, A. H. Abou Zeid, E. A. El-Kashoury, A. A. Sleem, and D. A. Waly, "A new flavonol glycoside and biological activities of *Adenanthera pavonina* L. leaves," *Natural Product Research*, vol. 28, no. 5, pp. 282–289, 2014.
- [42] G. C. Kite, E. R. Rowe, G. P. Lewis, and N. C. Veitch, "Acylated flavonol tri- and tetraglycosides in the flavonoid metabolome of *Cladrastis kentukea* (Leguminosae)," *Phytochemistry*, vol. 72, no. 4-5, pp. 372–384, 2011/04/01/2011.
- [43] H. H. F. Koolen, E. R. Soares, F. M. A. da Silva, A. Q. L. de Souza, E. Rodrigues Filho, and A. D. L. de Souza, "Triterpenes and flavonoids from the roots of *Mauritia flexuosa*," *Revista Brasileira de Farmacognosia*, vol. 22, no. 1, pp. 189–192, 2012.
- [44] W. Seebacher, N. Simic, R. Weis, R. Saf, and O. Kunert, "Complete assignments of <sup>1</sup>H and <sup>13</sup>C NMR resonances of oleanolic acid, 18 $\alpha$ -oleanolic acid, ursolic acid and their 11-oxo derivatives," *Magnetic Resonance in Chemistry*, vol. 41, no. 8, pp. 636–638, 2003.
- [45] C.-K. Lee, "Ursane triterpenoids from leaves of *Melaleuca leucadendron*," *Phytochemistry*, vol. 49, no. 4, pp. 1119–1122, 1998.
- [46] A. Hamed, M. Ismail, M. M. El-Metwally et al., "Diverse polyketides and alkaloids from *Penicillium* sp. KHMM: structural elucidation, biological and molecular docking studies," *Zeitschrift fuer Naturforschung, C: Journal of Biosciences*, vol. 74, no. 5-6, pp. 131–137, 2019.
- [47] S. Srivastava and D. Jain, "Triterpenoid saponins from plants of *Araliaceae*," *Phytochemistry*, vol. 28, no. 2, pp. 644–647, 1989.
- [48] R. González-Laredo, M. Flores De La Hoya, M. Quintero-Ramos, and J. Karchesy, "Flavonoid and cyanogenic contents of *chaya* (spinach tree)," *Plant Foods for Human Nutrition*, vol. 58, no. 3, pp. 1–8, 2003.
- [49] A. M. El-Hawiet, S. M. Toaima, A. M. Asaad, M. M. Radwan, and N. A. El-Sebakhy, "Chemical constituents from *Astragalus annularis* forssk. and *A. trimestris* L., Fabaceae," *Revista Brasileira de Farmacognosia*, vol. 20, no. 6, pp. 860–865, 2010.
- [50] A. Ikuta, H. Tomiyasu, Y. Morita, and K. Yoshimura, "Ursane-and oleanane-type triterpenes from *ternstroemia gymnanthera* callus tissues," *Journal of Natural Products*, vol. 66, no. 8, pp. 1051–1054, 2003.
- [51] B. S. Joshi, K. L. Singh, and R. Roy, "Complete assignments of <sup>1</sup>H and <sup>13</sup>C NMR spectra of the pentacyclic triterpene hederagenin from *Nigella sativa* Linn.," *Magnetic Resonance in Chemistry*, vol. 37, no. 4, pp. 295–298, 1999.
- [52] D. Zhang, Y. Fu, J. Yang et al., "Triterpenoids and their glycosides from *Glinus oppositifolius* with antifungal activities against *Microsporum gypseum* and *Trichophyton rubrum*," *Molecules*, vol. 24, no. 12, p. 2206, 2019.
- [53] T.-D. Lin and J. Shoji, "Constituents of *Panacis Japonici* Rhizoma. VI the structure of *chikusetsu-saponin* II, IVC," *Journal of the Chinese Chemical Society*, vol. 26, no. 1, pp. 29–35, 1979.
- [54] K. Tori, S. Seo, A. Shimaoka, and Y. Tomita, "Carbon-13 NMR spectra of olean-12-enes. Full signal assignments including quaternary carbon signals assigned by use of indirect <sup>13</sup>C, <sup>1</sup>H spin couplings," *Tetrahedron Letters*, vol. 15, no. 48, pp. 4227–4230, 1974.
- [55] W. Qiuling, H. Jing, F. Jun, and H. Mei, "Antitumor effect of betulinic acid on human acute leukemia K562 cells in vitro," *Journal of Huazhong University of Science and Technology - Medical sciences*, vol. 30, no. 4, pp. 453–457, 2010.
- [56] B. Wu, X. Wang, Zf Chi et al., "Ursolic acid-induced apoptosis in K562 cells involving upregulation of PTEN gene expression and inactivation of the PI3K/Akt pathway," *Archives of Pharmacal Research*, vol. 35, no. 3, pp. 543–548, 2012.
- [57] S. Pan, J. Hu, T. Zheng, X. Liu, Y. Ju, and C. J. C. Xu, "Oleanolic acid derivatives induce apoptosis in human leukemia K562 cell involved in inhibition of both Akt1 translocation and pAkt1 expression," *Cytotechnology*, vol. 67, no. 5, pp. 821–829, 2015.
- [58] S. Anuchapreeda et al., "Antileukemic cell proliferation of active compounds from kaffir lime (*citrus hystrix*) leaves," in *Molecules*, eng, Ed., vol. 25, no. 6, p. 1300, 2020.
- [59] H. Ge, X. Kong, L. Shi, L. Hou, Z. Liu, and P. Li, "Gamma-linolenic acid induces apoptosis and lipid peroxidation in human chronic myelogenous leukemia K562 cells," *Cell Biology International*, vol. 33, no. 3, pp. 402–410, 2009.
- [60] G. M. Møller, V. Frost, J. V. Melo, and A. Chantry, "Upregulation of the TGF $\beta$  signalling pathway by bcr-abl: implications for haemopoietic cell growth and chronic myeloid leukaemia," *FEBS Letters*, vol. 581, no. 7, pp. 1329–1334, 2007.
- [61] S. Mohan, A. B. Abdul, S. I. Abdelwahab et al., "Typhonium flagelliforme inhibits the proliferation of murine leukemia WEHI-3 cells in vitro and induces apoptosis in vivo," *Leukemia Research*, vol. 34, no. 11, pp. 1483–1492, 2010.
- [62] S. Murakami, H. Takashima, M. Sato-Watanabe et al., "Ursolic acid, an antagonist for transforming growth factor (TGF)- $\beta$ 1," *FEBS Letters*, vol. 566, no. 1-3, pp. 55–59, 2004.
- [63] F. Pellicano, L. Park, L. E. M. Hopcroft et al., "hsa-mir183/EGR1-mediated regulation of E2F1 is required for CML stem/progenitor cell survival," *Blood*, vol. 131, no. 14, pp. 1532–1544, 2018.
- [64] F. J. Staal, F. Famili, L. Garcia Perez, and K. Pike-Overzet, "Aberrant Wnt signaling in leukemia," *Cancers*, vol. 8, no. 9, p. 78, 2016.
- [65] D. Liu, L. Chen, H. Zhao, N. D. Vaziri, S.-C. Ma, and Y.-Y. Zhao, "Small molecules from natural products targeting the Wnt/ $\beta$ -catenin pathway as a therapeutic strategy," *Bio-medicine & Pharmacotherapy*, vol. 117, Article ID 108990, 2019.
- [66] M. Sheremet, S. Kapoor, P. Schröder, K. Kumar, S. Ziegler, and H. Waldmann, "Small molecules inspired by the natural



- product withanolides as potent inhibitors of wnt signaling,” *ChemBioChem*, vol. 18, no. 18, pp. 1797–1806, 2017.
- [67] M. Ishibashi, “Screening for natural products that affect Wnt signaling activity,” *Journal of Natural Medicines*, vol. 73, no. 4, pp. 697–705, 2019.
- [68] Z. i. Skok, N. Zidar, D. Kikelj, and J. Ilaš, “Dual inhibitors of human DNA topoisomerase II and other cancer-related targets,” *Journal of Medicinal Chemistry*, vol. 63, no. 3, pp. 884–904, 2019.
- [69] A. R. James, B. Unnikrishnan, R. Priya et al., “Computational and mechanistic studies on the effect of galactoxyloglucan: imatinib nanoconjugate in imatinib resistant K562 cells,” *Tumor Biology*, vol. 39, no. 3, Article ID 101042831769594, 2017.
- [70] N. H. Zamakshshari, G. C. L. Ee, I. S. Ismail, Z. Ibrahim, and S. H. Mah, “Cytotoxic xanthenes isolated from *Calophyllum depressinervosum* and *Calophyllum buxifolium* with antioxidant and cytotoxic activities,” *Food and Chemical Toxicology*, vol. 133, Article ID 110800, 2019.