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# Alkenylated phenolics from *Syzygium lineatum* with antiproliferative activity against chronic myeloid leukemia cells

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**ABSTRACT**

*Syzygium lineatum* is traditionally used by Filipinos as anticancer regimen and food flavoring in the Philippines. However, its medicinal potential is yet to be validated. Herein, we report the antiproliferative and cytotoxic activities of *S. lineatum* extracts, sub-extracts, and alkenylated phenolic constituents gingkol (**1**) and bilobol (**2**) along with β-sitosterol (**3**) and a mixture of fatty acids (**4**–**6**) *in vitro* and *in silico*. Fractions 2 and 4 (SlPE4) from the biologically active petroleum ether sub-extract (SlPE), showed moderately strong antiproliferative activity against chronic myeloid leukemia (CML) cells (K-562). Chromatographic purification of fraction SlPE4 yielded antiproliferative compounds **1** and **2** against K-562 cells while SlPE2 afforded **3**–**6**. Bilobol (**2**), an alkenylated resorcinol, showed better selectivity *in vitro* compared to the phenolic congener, gingkol (**1**) highlighting the importance of increased hydroxylation in the aromatic structure of the compounds. To elucidate their putative mechanisms of action, molecular docking studies were performed versus establishing the CML targets BCR::ABL1 tyrosine kinase, c-Src kinase, and protein kinase B. *In silico* results showed moderate to good binding affinities of **1** and **2** in the active sites of the target kinases. Overall, the study validates the purported Philippine traditional anticancer use of *S. lineatum*, especially its constituents gingkol (**1**) and bilobol (**2**).

## INTRODUCTION

Among all diseases, cancer poses the highest economic, social, and clinical burden in terms of cause-specific disability-adjusted life years [1]. With a 20.2% lifetime risk of developing cancer and a mortality rate that is second worldwide after ischemic heart disease, the need to address cancer is paramount. In the Philippines, the latest statistics show that cancer is responsible for more than 60,000 deaths in the year 2017. Over the years, patients with cancer are treated with chemotherapy, radiotherapy, and surgery. However, it is common for cancer cells to develop resistance to treatment [2]. Furthermore, patients with late-stage diagnosis are often ineligible for surgery. Hence, there is a clear and urgent need for effective drugs for the treatment of patients with cancer. Recent advances in cancer treatment include the application of targeted therapies which have been successful in reducing mortality and minimizing toxicity [3]. Targeted therapy is an approach using designed drugs that specifically bind to aberrant proteins, such as those implicated in malignant transformation. The design of drugs used in targeted therapy is continuously influenced and inspired by natural products [4]. These natural products are unrivaled sources of anticancer compounds in this modern era of drug discovery due to applicability, accessibility,

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and diminished cytotoxicity. They act by modulating the cancer microenvironment and various signaling pathways [5].

Chronic myeloid leukemia (CML) is one of the many types of cancer. It is a clonal malignancy with a global incidence of around 2 per 100,000 persons. In the Philippines, CML along with other leukemias ranked fifth leading cause of cancer-related mortalities [6]. With this, the occurrence of leukemia and its changing trends were highly varied, and more preventive strategies must be adapted to each country [7]. A large majority of this disease is caused by chromosomal translocation between chromosomes 9 and 22, which results in the formation of the hybrid gene *BCR::ABL1*. The gene product BCR::ABL kinase is responsible for the impaired cellular signaling, thereby dysregulating normal cell proliferation, invasion, migration, and apoptosis which are hallmarks of cancer pathophysiology [8,9]. Thus, anti-CML agents have been continuously discovered and developed to target this hybrid protein [10,11]. The chemotherapeutics imatinib, nilotinib, and ponatinib being BCR-ABL-targetting tyrosine kinase inhibitors (TKIs) mitigate strongly CML progression. In the case of imatinib, which is an inhibitor of tyrosine kinase, it allowed CML patients to experience a life expectancy that is near-normal [12]. Due to concerns related to drug resistance brought by BCR::ABL mutations and overexpression, more chemical-based CML therapeutics are still warranted.

In search for new anticancer agents, natural products have long been tapped due to their purported efficacy and safety. Antiproliferative natural products comprised of alkaloids, flavonoids, glycosides, lignans, polyketides and oxidized terpenoids inhibit CML proliferation via induction of apoptosis. Apart from differentiating CML into monocyte/erythroid cell types, natural products also prevent the occurrence of multidrug resistance in CML cells. The Philippines being a megadiverse archipelagic country hosts native and endemic plants. Among them are medicinal plants reported for their anticancer activities [13–16]. The Philippines' Department of Health enlisted the "Ten scientifically validated" Philippine medicinal plants in 1992 (R.A. No. 8423—Philippine Institute of Traditional and Alternative Health Care), in which *Momordica charantia* (ampalaya) and *Quisqualis indica* (niyug-niyogan) were identified to have anticancer properties [17]. In the Northern Philippines, locals and natives from provinces such as Apayao and Cagayan utilize *Syzygium lineatum* (Roxb.) (DC.) Merr & Perry (locally known as "Malubeg" and "Alebadu"), a traditional medicine used to treat cancer and as a souring agent for cooking. *Syzygium lineatum* is a fruiting tree that grows 4–5 m in height and belongs to the family Myrtaceae [18,19]. Different species of the genus *Syzygium* have been reported to exhibit anticancer activities [20–22]. *Syzygium* natural products such as dimethyl cardamonins, phenolics, oleanolic, and betulinic acids have been reported to inhibit cell proliferation and induce apoptosis [23]. In this paper, we report the antiproliferative and cytotoxic activities of *S. lineatum* extracts, sub-extracts, and its alkenylated phenolic compounds 5-(8Z-pentadecenyl)resorcinol (gingkol, **1**) and 5-(8Z-pentadecenyl)resorcinol (bilobol, **2**) as well as β-sitosterol (**3**) and fatty acids **4**–**6** against HUVEC, K-562, and HeLa cell lines (Fig. 1). To understand the mechanism of action of the antiproliferative compounds against CML, *in silico* binding characteristics were interrogated against clinically approved target kinases in CML such as BCR::ABL kinase, c-Src kinase, and protein kinase B.

## MATERIALS AND METHODS

### Plant material

The leaves of *S. lineatum* were collected at Adams, Ilocos Norte, Northern Philippines (18.4602680°N; 120.9038960°E), in December 2016 and were identified by Mr. Michael A. Calaramo of Northwestern University Ecotourism Park and Botanic Gardens (NUEBG) and Dr. Cecilia Banag-Moran of University of Santo Tomas Herbarium (USTH). Voucher specimens were submitted to USTH (USTH015667) and the Herbarium of NUEBG (HNUL14950).

### Extraction and isolation

The air-dried and ground leaves of *S. lineatum* (2 kg) were percolated with 1:1 dichloromethane-methanol (16.0 l). The combined extracts were evaporated until dry under reduced pressure to afford a green syrup (313.0 g). A suspension of the crude extract in water was sequentially extracted with petroleum ether (4.7 l), followed by dichloromethane (6.9 l), and finally with n-butanol (2.0 l) yielding after evaporation 9[illegible] g of the petroleum ether sub-extract (SlP), 58.9 g of dichloromethane sub-extract (SlD), and 32.4 g of the n-butanol sub-extract (SlB). The petroleum ether sub-extract was subjected to silica gel vacuum liquid chromatography with increasing amounts of ethyl acetate (EtOAc) in petroleum ether as eluent (10%). Fractions were pooled according to their thin layer chromatography (TLC) profile. Iterative separation using silica gel column chromatography of the SlP fraction 2 (20.8 g) with 20:1 petroleum ether: EtOAc resulted in the isolation and purification of 3-(8Z-pentadecenyl)phenol or gingkol (**1**) along with the known compounds β-sitosterol (**3**) and a mixture of fatty acids (**4**–**6**). Meanwhile, repetitive separation of SlP fraction 4 (SlPE4) yielded compound **2** (bilobol) [24].

### Antiproliferative and cytotoxicity assay

To measure the antiproliferative and cytotoxic activities of *S. lineatum* extracts, sub-extracts, and compounds, inhibitory and cytotoxic concentrations against CML (K-562), human umbilical vein endothelial cord (HUVEC), and HeLa cell lines were measured using the CellTiter-Blue® viability assay based on the method developed by Krauth *et al.* [25]. The $GI_{50}$ and $CC_{50}$ values were determined as the value at the intersection of the dose curve with the 50% line, compared to the untreated control. Imatinib and doxorubicin, which are standard anticancer drugs, were used as positive controls [26].

### Molecular docking studies

*Ligand and protein preparation*

To elucidate potential binding propensities and modalities of the compounds, they were formatted as ligands and were prepared and optimized in Avogadro (1.20). Target proteins were prepared and minimized in UCSF Chimera

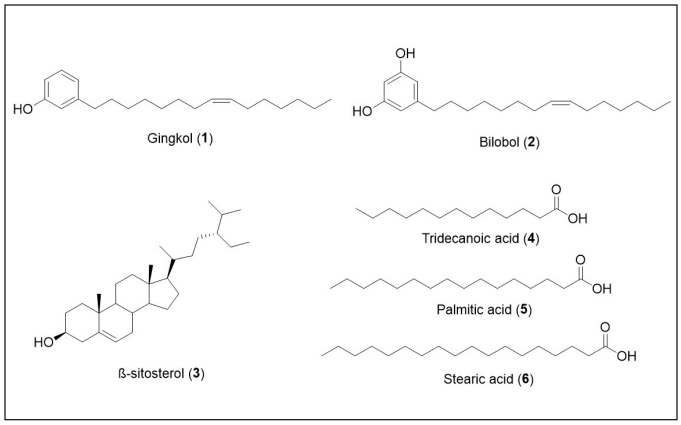


**Figure 1.** Structures of 5-(8*Z*-pentadecenyl)phenol (gingkol, **1**), 5-(8Z-pentadecenyl)resorcinol (bilobol, **2**), β-sitosterol (**3**), and fatty acids **4**–**6** from *S. lineatum*.

(1.16) based on previously reported methodologies using the Gasteiger charge method [27]. SMILES formats of compounds were fetched from PubChem while proteins were obtained from the Protein Data Bank (PDB) through their PDB IDs. (PDB ID: 2GQG), inactive (PDB ID: 2HYY), and T334I_D382N imatinib-resistant (PDB ID: 5MO4) BCR::ABL kinase, c-Src kinase (2SRC), and protein kinase B (4EJN) were selected as potential therapeutic targets due to their established roles in CML pathophysiology [28–30].

*Docking simulation*

Molecular docking, an *in silico* tool to assess binding affinities of compounds, especially natural products, to target receptors or proteins, was performed using UCSF Chimera (1.16) [31]. A grid-based "flexible ligand into flexible active site" protocol was followed in the entire simulation [32]. To visualize and enumerate the amino acid residues involved in the binding as well as evaluate the kind of intermolecular interactions, BIOVIA Discovery Studio (4.1) was utilized.

**ADME properties and BOILED-Egg prediction**

To predict the drug-likeness of the compounds, their absorption, distribution, metabolism, and excretion (ADME) properties were assessed based on the number of violations in Lipinski's rule of five (LRo5). Meanwhile, pharmacokinetic behaviors [gastrointestinal (GI) absorption and brain penetration] were predicted using the Brain Or IntestinaL EstimateD permeation (BOILED)-Egg methodological model [33,34]. The SMILES notations of the ligands were inputted in SWISSADME (ttp://www.swissadme.ch/index.php) and parameters such as molecular weight, lipophilicity, and number of H-acceptors and donors were recorded [35].

## RESULTS

***In vitro* antiproliferative and cytotoxic activities of *S. lineatum* leaf extracts and fractions, and compounds 1–6**

Antiproliferative and cytotoxicity assessments of the crude extract (SlC), petroleum ether (SlPE), dichloromethane (SlD), and butanol sub-extracts (SlB) were carried out using a CellTiter-Blue assay. The antiproliferative activity versus normal HUVEC and human leukemia cells (K-562) is reported as $GI_{50}$, while cytotoxicity versus cervical cancer cells (HeLa) as $CC_{50}$. The petroleum ether sub-extract (SlPE) showed the strongest anti-proliferative and cytotoxic activity among the three sub-extracts against the three cell lines (Table 1). In addition, fraction 4 of the petroleum sub-extract (SlPE4) exhibited antiproliferative activity against K-562 at 12.6 (±1.1) ug/ml and cytotoxicity against HeLa at 22.2 (±0.9) ug/ml. Chromatographic purifications of fractions SlPE4 and SlPE2 (the next antiproliferative and cytotoxic fraction) afforded six compounds and were spectroscopically identified as 3-(8Z-pentadecenyl)phenol or gingkol (**1**), 3-(8Z-pentadecenyl)resorcinol or bilobol (**2**), β-sitosterol (**3**), and mixture of fatty acids (**4**–**6**). Compounds **1**–**6** were subjected to CellTiter-Blue assay wherein **1** and **2** exhibited potent cytotoxic activity against K-562 cells. Bilobol (**2**), an alkenylated resorcinol, showed better selective antiproliferative activity compared to gingkol (**1**), a phenolic congener (Table 2). For comparison, imatinib was used as a positive drug control.

***In silico* binding propensities and interactions between test compounds 1–2 and target CML-associated proteins**

To further elucidate the potential mechanism of action of compounds 1 and 2 against CML (K-562), these were docked onto the adenosine triphosphate (ATP)-binding site of an active, inactive, and T334I_D382N imatinib-resistant BCR::ABL kinase; c-Src kinase; and protein kinase B (Table 3; Fig. 2). These proteins are well-established promising pharmaceutical targets of small molecules [28–30]. Interestingly, the findings from the *in silico* docking simulations corroborated with the results from the *in vitro* antiproliferative assessment. Both gingkol (1) and bilobol (2) which exhibited antiproliferative activity versus K-562 showed moderate to favorable antagonistic binding activities onto the active sites of selected CML-associated protein targets (Table 3; Fig. 2). Gingkol (**1**) showed its best binding propensity to imatinib-resistant BCR::ABL kinase (BE = −8.1 kcal/mol) with H-bonds formed with M337 and E335; *pi*-sigma interactions with L267, L389, and Y272; *pi-pi* bonding with F336; and *pi*-alkyl bonds with F401, I334, K290, V318, A399, M309, and V275 (Fig. 2a).

Meanwhile, bilobol (**2**) showed the best binding energy (BE) against the active and imatinib-resistant BCR::ABL kinases with BE of −8.0 kcal/mol. Congruent to the result of *in vitro* assay versus K-562, bilobol (**2**) showed a slightly better BE of −8.0 kcal/mol against active BCR::ABL kinase compared to that of gingkol (**1**) (BE = −7.9 kcal/mol) (Table 3). For active BCR::ABL kinase, compound **2** established *pi*-sigma interactions with Y253; *pi-pi* bonding with Y253; and *pi*-alkyl or alkyl bond with several other residues (V299, A380, K271, V256, A269, L370, and L248) (Fig. 2b). For the resistant structure, **2** exhibited *pi*-sigma interactions with L267, Y272, and L389; *pi*-alkyl or alkyl bridges with V318, M309, A399, K290, I334, V275, I332, F401, and A288; and *pi-pi* bond with F336 (Fig. 2c). Interestingly, both compounds **1** and **2** showed either higher or similar binding propensity to

**Table 1.** Growth inhibitory and cytotoxic concentration at 50% (µg/ml) of *S. lineatum* leaf extracts and fractions against HUVEC, K-562, and HeLa cells based on triplicates.

| | $GI_{50}$ | | $CC_{50}$ |
|---|---|---|---|
| **Extract** | **HUVEC** | **K-562** | **HeLa** |
| SlC | >50 | >50 | >50 |
| SlPE | 31.8 ± 1.2 | 22.9 ± 0.8 | 25.8 ± 3.8 |
| SlD | >50 | >50 | >50 |
| n-butanol | >50 | >50 | >50 |
| SlPE1 | >50 | 43.4 ± 0.5 | 40.4 ± 1.0 |
| SlPE2 | 43.5 ± 1.5 | >50 | 42.4 ± 1.2 |
| SlPE3 | 40.8 ± 2.2 | >50 | 46.8 ± 1.0 |
| SlPE4 | 20.2 ± 0.8 | 12.6 ± 1.1 | 22.2 ± 0.9 |
| SlPE5 | >50 | >50 | >50 |
| SlPE6 | 46.9 ± 3.9 | >50 | 47.4 ± 0.9 |

**Table 2.** Growth inhibitory and cytotoxic concentration at 50% (µg/ml) of gingkol, bilobol, and the reference anti-CML drug imatinib against HUVEC, K-562, and HeLa cells based on triplicates.

| | $GI_{50}$ | | $CC_{50}$ |
|---|---|---|---|
| **Compound** | **HUVEC** | **K-562** | **HeLa** |
| Gingkol (1) | 18.2 ± 1.1 | 17.9 ± 0.2 | 36.6 ± 3.1 |
| Bilobol (2) | 17.2 ± 0.3 | 3.6 ± 0.6 | 26.2 ± 0.6 |
| β-sitosterol (3) | >50.0 | >50.0 | >50.0 |
| Tridecanoic acid (C13) | >50.0 | >50.0 | >50.0 |
| Palmitic acid (C16) | >50.0 | >50.0 | >50.0 |
| Stearic acid (C18) | >50.0 | >50.0 | >50.0 |
| Imatinib | 10.9 ± 1.2 | $0.1 \pm 6.7 \times 10^{-3}$ | 8.8 ± 1.4 |

the imatinib-resistant BCR::ABL kinase while imatinib showed decreased binding affinity to the imatinib-resistant structure (BE = −9.7 kcal/mol) compared to its binding activities to the nonresistant targets. Residues M337, I332, F336, Y272, A288, V275, L267, and L389 were common targets in the imatinib-resistant structure of the two test compounds and the positive control. Finally, although the positive control imatinib showed the highest binding propensity to BCR::ABL kinase (active conformation) ATP-binding site (BE = −9.8 kcal/mol), notable residues targeted by imatinib have also been bound to either compounds **1** or **2**, or both such as Y253, A269, V256, L370, and L248.

### ADME properties and BOILED-Egg predictions on compounds 1 and 2

Due to the favorable *in vitro* and *in silico* antiproliferative activities of gingkol (**1**) and bilobol (**2**) against CML cell line (K-562) and target kinases (BCR::ABL, c-Src, and protein kinase B), the drug-likeness and pharmacokinetic behaviors of both compounds were predicted using database-driven analysis in SWISSADME (Table 4). Compounds **1** and **2** showed only one LRo5 violation and thus are considered highly druggable pharmacophores. However, only compound

**Table 3.** Binding energies (BEs) and interactions between test compounds 1–2 and target proteins. Imatinib served as positive control.

| Compounds | Target proteins | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Active BCR::ABL kinase | | Inactive BCR::ABL kinase | | Imatinib-resistant BCR::ABL kinase | | c-Src kinase | | Protein kinase B | |
| | BE (kcal/mol) | Interactions | BE (kcal/mol) | Interactions | BE (kcal/mol) | Interactions | BE (kcal/mol) | Interactions | BE (kcal/mol) | Interactions |
| Gingkol (1) | −7.9 | G249, Y253 (H-bond), L370, A269, L248, V256, I313, K271, A380, V399 (alkyl/*pi*-alkyl), Y253 (*pi-pi*) | −7.4 | L248, L370 (*pi*-sigma), Y253 (*pi-pi*), A269, F382, V256, K271, A380, M290, V289, V299 (alkyl/*pi*-alkyl) | −8.1 | M337 E335 (H-bond), L267, L389, Y272 (*pi*-sigma), F336 (*pi-pi*), F401, I334, K290, V318, , A399, M309, V275 (alkyl/*pi*-alkyl) | −7.1 | M341 (H-bond), L273 (*pi*-sigma), V281, A403, V323, L407, M314, L325, F405, L393, A293 (alkyl/*pi*-alkyl) | −7.7 | D292, D81 (H-bond), W80, K268, V270, L264, L210, Y272 (alkyl/*pi*-alkyl), W80 (C-H bond) |
| Bilobol (2) | −8.0 | Y253 (*pi*-sigma), V299, A380, K271, V256, A269, L370, L248 (alkyl/*pi*-alkyl), Y253 (*pi-pi*) | −7.9 | M318 (H-bond), F317, Y253, L370, L248 (*pi*-sigma), F317, Y253 (*pi-pi*), F382, A269, V256, K271, I313, M290, V299 (alkyl/*pi*-alkyl) | −8.0 | L267, Y272, L389 (*pi*-sigma), V318, M309, A399, K290, I334, V275, I332, F401, A288 (alkyl/*pi*-alkyl), F336 (*pi-pi*) | −7.0 | E339 (H-bond), L273 (*pi*-sigma), A293, F405, A403, L325, M314, I336, L407, V323, V281, L393 (alkyl/*pi*-alkyl) | −7.7 | D292, T81, T82, Y272 (H-bond), L210, L264, Y268, V270 (alkyl/*pi*-alkyl), W80 (C-H bond) |
| **Imatinib** | −9.8 | M318 (H-bond), Y253, E286 (attractive charge/*pi*-cation), A269, V256, L370, L248 (alkyl/*pi*-alkyl), N322 (C-H bond) | −10.2 | Y253, F382, D381, E286 (*pi*-cation/*pi*-anion), V289, L354, I293, M290, K271, V299 (alkyl/*pi*-alkyl), T315 (C-H bond) | −9.7 | Q271, T338, M337 (H-bond), D344 (*pi*-anion), I332 (C-H bond), F336, Y272 (*pi-pi*), A288, V275, L267 (*pi*-alkyl), L389 (*pi*-sigma) | −10.0 | D348, D386, D404 (attractive charge/*pi*-anion), M341, Y340, A293 (alkyl/*pi*-alkyl), L393, V281, L273 (*pi*-sigma), L273 (C-H bond) | −12.4 | N54, Q79 (H-bond), Y272, L210 (*pi*-alkyl), D292 (*pi*-anion), W80 (C-H bond, *pi-pi*), L264, V 270 (*pi*-sigma) |

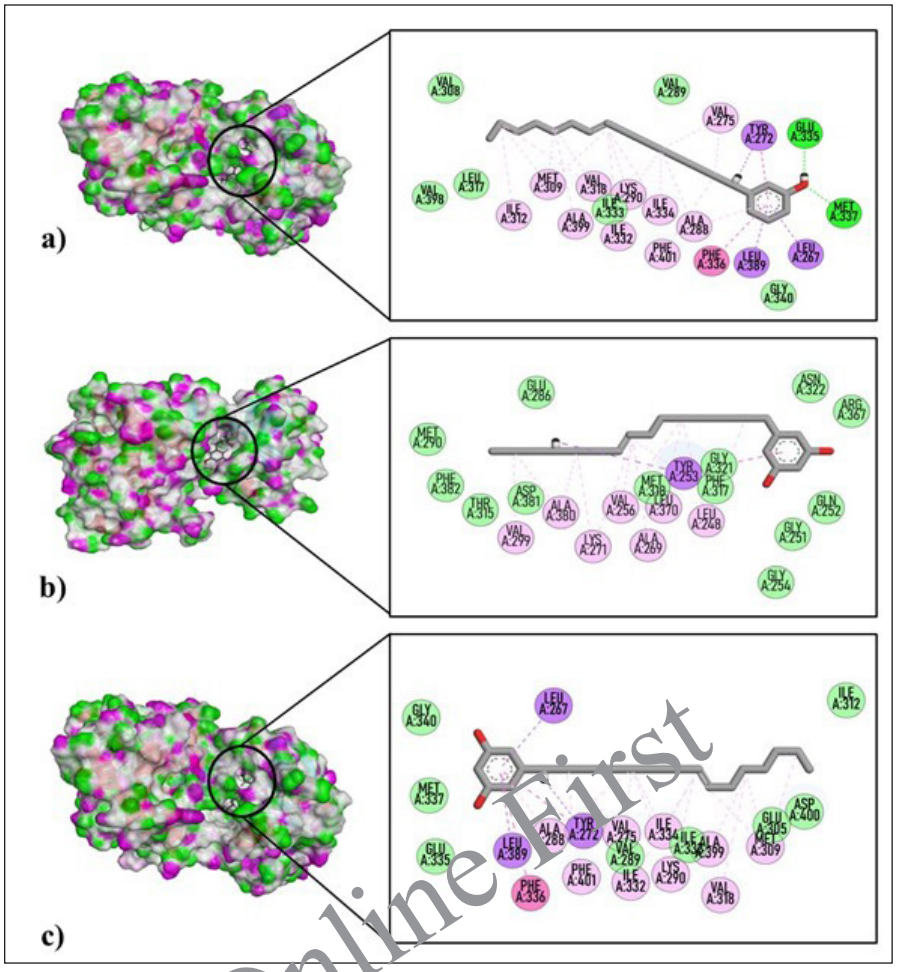




**Figure 2.** Dock poses and interacting residues of (**a**) gingkol (**1**) versus imatinib-resistant BCR::ABL kinase, (**b**) bilobol (**2**) versus active BCR::ABL1 kinase structures, and (**c**) bilobol (**2**) versus imatinib-resistant BCR::ABL kinase.

**Table 4.** *In silico* predictions of ADME properties and pharmacokinetic behaviors of compounds **1** and **2**.

| Compound | Drug-likeness (ADME) predictions | | | | | | Pharmacokinetic behavior (BOILED-Egg) predictions | |
|---|---|---|---|---|---|---|---|---|
| | MW < 500 | No. of H-bond acceptors < 10 | No. of H-bond donors < 5 | Lipophilicity MLogP < 4.15) | No. of Violations based on LRo5 | Drug-likeness | BBB permeant | Passively absorbed by the GI tract |
| Gingkol (1) | 302.49 g/mol | 1 | 1 | 5.45 | 1 | Yes | No | No |
| Bilobol (2) | 318.49 g/mol | 2 | 2 | 4.79 | 1 | Yes | No | Yes |

**2** was predicted to be passively absorbed by the GI tract based on BOILED-Egg.

## DISCUSSION

In this study, extracts, sub-extracts, and alkenylated phenolic constituents gingkol (**1**) and bilobol (**2**) from the leaves of *S. lineatum* exhibited promising anticancer activities against HUVEC, K-562, and HeLa cell lines *in vitro*. In general, extracts from several members of the genus *Syzygium* have been reported to exhibit anticancer properties based on *in vitro* cell viability biological assay evidence. For example, *S. aromaticum* extracts showed cytotoxic activity versus breast cancer cell lines MCF-7 and MDA-MB-231 in a dose-dependent manner by inducing cell cycle arrest at the S phase and promoting apoptotic activities [36,37]. Interestingly, *S. aromaticum* conferred anticancer properties against colon, cervical, liver,

ovarian, and pancreatic cancer cell lines. Water and ethanolic extracts of *S. aqueum* also conferred anticancer properties against MCF-7 cells. In addition, other *Syzygium* species such as *S. samarangense* and *S. jambo* extracts exhibited cytotoxic activities versus colon cancer and liver cancer cells, respectively [38–40]. In the Philippines, *S. lineatum* is among the traditional medicinal plants used by Ilocano natives and locals against cancer and other diseases [18,19]. This study contributes to the growing interest in *Syzygium* species as a bioresource of novel potential anticancer agents. More significantly, our results confirm the anticancer potentials and use of *S. lineatum*.

With the high morbidity associated with CML in the present and in the next 20 years which is expected to increase by 60% by then, as well as the continuous occurrence of chemotherapeutic resistance in CML treatment, the discovery and development of new-generation drugs are warranted, thus, ushering the potentials of compounds **1** and **2** as anticancer agents and/or structural drug scaffolds [41,42]. Herein, the study reports the *in vitro* and *in silico* antiproliferative potentials of these two fatty phenolics, especially against K-562 (chronic myelogenous leukemic cell lines), of *S. lineatum* for the first time. We also report for the first time the isolation of alkenylated phenolics gingkol (**1**) and bilobol (**2**), and **3**–**6** from *S. lineatum*. Interestingly, both phenolic compounds also exhibited promising inhibitory activity and selectivity to CML. Comparing the activities of **1** and **2**, the alkenylated resorcinol (bilobol, **2**) conferred better cytotoxicity against the three cell lines (HUVEC, HeLa, and K-562). This could be attributed to the increase of hydroxylic functionalization in **2**. In general, hydroxylation of compounds including alkenylated phenolic natural products has been widely reported to increase chemical stability, bioactivity including inhibitory effects on key enzymes in disease pathogenesis, and binding interactions to specific molecular disease targets [43–47].

In addition, three pharmaceutically important kinases (BCR::ABL, c-Src, and protein kinase B) have been targeted by compounds **1** and **2** as shown by the results of the docking simulations. BCR::ABL1 is a well-established target as it is a chimeric constitutively active tyrosine kinase that is associated with CML cell differentiation and survival. It is the protein product of the chromosomal translocation between chromosomes 9 and 22 [48]. Many anticancer drugs like imatinib have been developed as tyrosine kinase inhibitors [49]. In this paper, three BCR:ABL tyrosine kinases were targeted—active, inactive, and imatinib-resistant. Although BCR::ABL possesses both ATP-binding and allosteric sites, the earlier was chosen as a target as this is the usual mechanism of action of clinically approved TKIs. Competitive inhibition in the ATP-binding domain yields impaired protein phosphorylation, thereby decreasing intracellularly mediated cell proliferation, growth, and survival necessary in CML cellular pathophysiology [50,51]. Comparing the results of the docking studies to the three ABL1 kinases, the alkenylated phenolics **1** and **2** from *S. lineatum* conferred the best binding affinity to the imatinib-resistant structure that harbors T315I mutation. Meanwhile, the drug control imatinib showed a weaker propensity to this structure, although this is given due to the associated resistance. Furthermore, several residues such as M337, I332, F336, Y272, A288, V275, L267, and L389 were identified as common targets between imatinib, and the two test compounds and these could serve as initial target hotspots of the compounds. Thus, both compounds **1** and **2** may serve as prodrugs or templates in developing new-generation TKIs against cancer cells harboring chemotherapeutic resistance.

Other than the three ABL1 kinases, c-Src, and protein kinase B also served as CML targets. c-Src is associated with cellular invasion and metastasis brought about by decreased cell-to-cell adhesion when c-Src is overexpressed [26,28,52]. Meanwhile, protein kinase B (or AKT) is a downstream target in the $PI_3K$/AKT signaling pathway which is involved in cell proliferation, apoptosis, and development of cancer chemotherapeutic resistance. Thus, inhibition of AKT negatively affects the $PI_3K$/AKT pathway leading to apoptosis and inhibition of cell growth in many types of cancer [29,53,54]. In our results, both compounds **1** and **2** showed moderate binding to both c-Src and AKT kinases, thereby highlighting their multitargeting potentials as anticancer agents against cell proliferation and drug resistance while promoting apoptosis and cell growth arrest.

## CONCLUSION

Our study validated the traditional medicinal use of *S. lineatum* against cancer as we report the antiproliferative and cytotoxic activities of its extracts, sub-extracts, and alkenylated phenolics gingkol (**1**) and bilobol (**2**) against HUVEC, HeLa, and K-562 cell lines for the first time. *In vitro* results further suggested the sensitivity of K-562 (CML) cells to gingkol (**1**) and bilobol (**2**) with the latter showing better selectivity to K-562 cells. Molecular docking experiments supported putative multitargeting binding mechanisms of **1** and **2** in the active pockets of associated target kinases BCR::ABL1, c-Src, and protein kinase. In-depth studies on **1** and **2** are, therefore, warranted to explore further their *in vitro* and *in vivo* mechanisms of action against CML.

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## AUTHOR CONTRIBUTIONS

FVI, VNODL, JAHM: conceptualization, investigation, data collection and analysis, manuscript preparation, and manuscript editing. ALC, APGM: conceptualization, design, manuscript preparation, editing, and review. All authors have read and approved the present version of the manuscript.

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## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest in the publication.

## ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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