



Screening of *Hibiscus* and *Cinnamomum* Plants and Identification of Major Phytometabolites in Potential Plant Extracts Responsible for Apoptosis Induction in Skin Melanoma and Lung Adenocarcinoma Cells

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Carcinogenesis is a major concern that severely affects the human population. Owing to persistent demand for novel therapies to treat and prohibit this lethal disease, research interest among scientists is drawing its huge focus toward natural products, as they have minimum toxicity comparable with existing treatment methods. The plants produce secondary metabolites, which are known to have the anticancer potential for clinical drug development. Furthermore, the use of nanocarriers could boost the solubility and stability of phytochemicals to obtain site-targeting delivery. The identification of potential phytochemicals in natural compounds would be beneficial for the synthesis of biocompatible nanoemulsions. The present study aimed to investigate the potential cytotoxicity of ethanol extracts of *Hibiscus syriacus* and *Cinnamomum loureirii* Nees plant parts on human skin melanoma (G361) and lung adenocarcinoma (A549) cells. Importantly, biochemical analysis results showed the presence of high phenol (50–55 µgGAE/mg) and flavonoids [42–45 µg quercetin equivalents (QE)/mg] contents with good antioxidant activity (40–58%) in *C. loureirii* Nees plants extracts. This plant possesses potent antiproliferative activity (60–90%) on the malignant G361 and A549 and cell lines correlated with the production of nitric oxide. Especially, *C. loureirii* plant extracts have major metabolites that exhibit cancer cell death associated with cell cycle arrest. These findings support the potential application of *Cinnamomum* for the development of therapeutic nanoemulsion in future cancer therapy.

Keywords: growth inhibition, *hibiscus syriacus*, *cinnamomum loureirii* nees, lung adenocarcinoma, nanoemulsions, skin melanoma cell line

INTRODUCTION

Despite advancements in cancer research, diagnosis, and therapy, cancer disease remains a serious health issue and a leading cause of death worldwide. Up to now, many studies of the potential health benefits of phytoconstituents and their feasible biomedical applications as food and pharmaceutical supplements have been discovered (Mishra et al., 2018; Sharifi-Rad et al., 2018). A comprehensive preclinical oncology study has indicated that plant-derived molecules could be administered as both isolated component and whole foods that undoubtedly affects carcinogenesis, including breast cancer (Kubatka et al., 2016; Kapinova et al., 2017; Kubatka et al., 2017). Phytochemicals have been proven effective as a strong antioxidant, immunomodulator, and anti-inflammatory agent detected by *in vitro* and *in vivo* experiments. In tumor-related studies, preclinical reports verified that several plant-derived substances (separated or as combinations) are capable of blocking carcinogenesis processes through inhibition of multiple targets in the tumor microenvironment, eventually suppressing anticancer molecular signaling pathways without undesirable adverse effects that usually occur with the traditional chemotherapies (Ham et al., 2015; Pistollato et al., 2015; Battino et al., 2019). Furthermore, numerous plant-derived molecules have been evidenced to control the angiogenesis, cell cycle, apoptosis, and activity of cancer stem cells in cancer tissue for malignant growth suppression in organisms (Kapinova et al., 2018; Kapinova et al., 2019). In contrast, it has been seen that the use of these plant products can improve therapeutic and radiotherapy efficiency and also reduce the toxicity of manufactured drugs when they were given concurrently (Chang et al., 2015; Szejka et al., 2016). Additionally, studies have been shown that nanotechnology-based drug delivery systems applied for delivering natural compounds have substantial advantages for cancer therapy (Navya et al., 2019). These reports suggest that nanoparticles can increase the uptake of water-insoluble phytochemicals and improve the route of these natural agents across cell membranes (Duan and Li, 2013), thereby improving selective cancer cell killing by sustained drug release (Rawal and Patel, 2019).

Cinnamomum loureirii Nees (known as Vietnamese cinnamon) is a persistent plant of the family Lauraceae. *Cinnamomum* extracts, regardless of the species, have been accompanying a wide variety of health benefits. In ancient times, several edible plants were consumed as medicinal therapies in many countries due to the presence of various secondary metabolites, frequently called phytochemicals. Especially, *C. loureirii* is widely used as medicine in Korea. The inner bark of *C. loureirii* is acquired from the trees commonly used as a flavoring and spice agent (Li et al., 2010). It contains large quantities of bioactive components, such as tannins, essential oils, and coumarins. Many studies suggested that *Cinnamomum* has potent antioxidant activity (Mathew and Abraham, 2006) and antimicrobial activity (Ooi et al., 2012) and also plays a significant role in controlling lipid and glucose levels (Anderson, 2008; Anderson, 1997). Additionally, *C. loureirii* has been discovered to be applicable for the cure of inflammatory

diseases, dyspepsia, gastritis, and blood circulation disorders and could retain analgesic, antipyretic, anti-ulcerogenic, and anti-allergic effects (Kurokawa et al., 1998; Zhu et al., 1993; Yuan et al., 2017). Interestingly, *Cinnamomum*-synthesized gold nanoparticles can serve as outstanding computed tomography/photoacoustic agents for tumor recognition through nanopharmaeuticals (Chanda et al., 2010). To the best of our knowledge, no study has discovered the major components in *C. loureirii* Nees and its inhibitory effects on anticancer activity utilizing *in vitro* cell line models using different cell types. Another study showed that *Cinnamomum verum* essential oil had been proven against *Trypanosoma cruzi* that eventually interferes with the parasite differentiation process *in vitro* (Azeredo et al., 2014). Also, Wen et al. (2018) mentioned the use of *Cinnamomum osmophloeum* Kanehira leaf extracts for the treatment of hair loss and growth due to the presence of cinnamic acid and cinnamic aldehyde Wen et al. (2018).

Hibiscus syriacus belongs to the family Malvaceae that is known for its anthelmintic, antipyretic, and antifungal characteristics (Maganha et al., 2010). Previously, *H. syriacus* extracts have been shown to induce antioxidant activity (Kwon et al., 2003). Interestingly, all parts of *H. syriacus*, including fruit, stem, root, flower, and skin, show good therapeutics effect, thereby widely used as herbal medicinal in Asian countries. In 2008, Cheng et al. (2012) revealed that extracts prepared from *H. syriacus* skin could activate p53 for lung cancer cell apoptosis through activation of the apoptosis-inducing factor pathway. Besides, more recently, *H. syriacus*-synthesized gold nanoparticles were shown to act as a probable autophagy inducer for lipopolysaccharide-triggered macrophages inflammation, offering an innovative perception in the management of inflammation-related disorders (Xu et al., 2021).

This study aims to identify the activity of *H. syriacus* and *C. loureirii* for induction of cancer cell apoptosis. To investigate the cytotoxic effect of these plants, the ethanolic extracts were prepared and subjected to the treatment of cancer cells. To isolate the potential list of compounds from the selected candidate, liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) analysis was used in this study. The data obtained indicated that approximately 6,500 compounds were tentatively dispersed in the *C. loureirii* stem extracts, which have the strong presence of 11 major phytochemicals. Our data indicated the potential use of *C. loureirii* stem extracts as a natural anticancer product and offer evidence for the presence of vanilloloside and epicatechin as major potent agents for their use in health problems associated with cancer.

MATERIALS AND METHODS

Plant Materials

The plants used in this study are grown at the garden of Woori flowers at Gwacheon City, Gyeonggi-Do, Republic of Korea (Latitude-37.335224°N, longitude 126.822052°E). Experimental research on plants complies with relevant institutional, national, and international guidelines and legislation. The stem

TABLE 1 | Overview of plant extracts used in this study.

Plant	Family	Location	Part used
<i>Hibiscus syriacus</i> "Columbine"	Malvaceae	Korea	Twig
<i>Hibiscus syriacus</i> "Hanol-tanshim"	Malvaceae	Korea	Twig
<i>Hibiscus syriacus</i> Champion	Malvaceae	Korea	Twig
<i>Hibiscus syriacus</i> Taehwagang	Malvaceae	Korea	Twig
<i>Cinnamomum loureirii</i> Nees	Lauraceae	Korea	Twig, Leaves

of the *Hibiscus* plants used in the study was collected under the permission of the Research Institute of Woori Flowers. At the same time, *Cinnamomum* plants were collected under permission from the Korea Tropical Plant Research Center, Cheju Island, Republic of Korea. These plants were identical to a specimen deposited to the National Wild Plant bank, National Institute of Biological Resources, Ministry of Environment, Republic of Korea. These plant families and parts used are briefly outlined in **Table 1**.

Extract Preparation

For extract preparation, each plant portion was collected and powdered with a blender and then placed in 70% EtOH in a shaking incubator for the next 2 days. Next, all the procedures were performed similarly as described in our earlier report (Kaushik et al., 2020). Cell experiments were performed using various obtained extracts dilutions to analyze anticancer properties (**Figure 1**).

Cell Culture and Extract Treatments

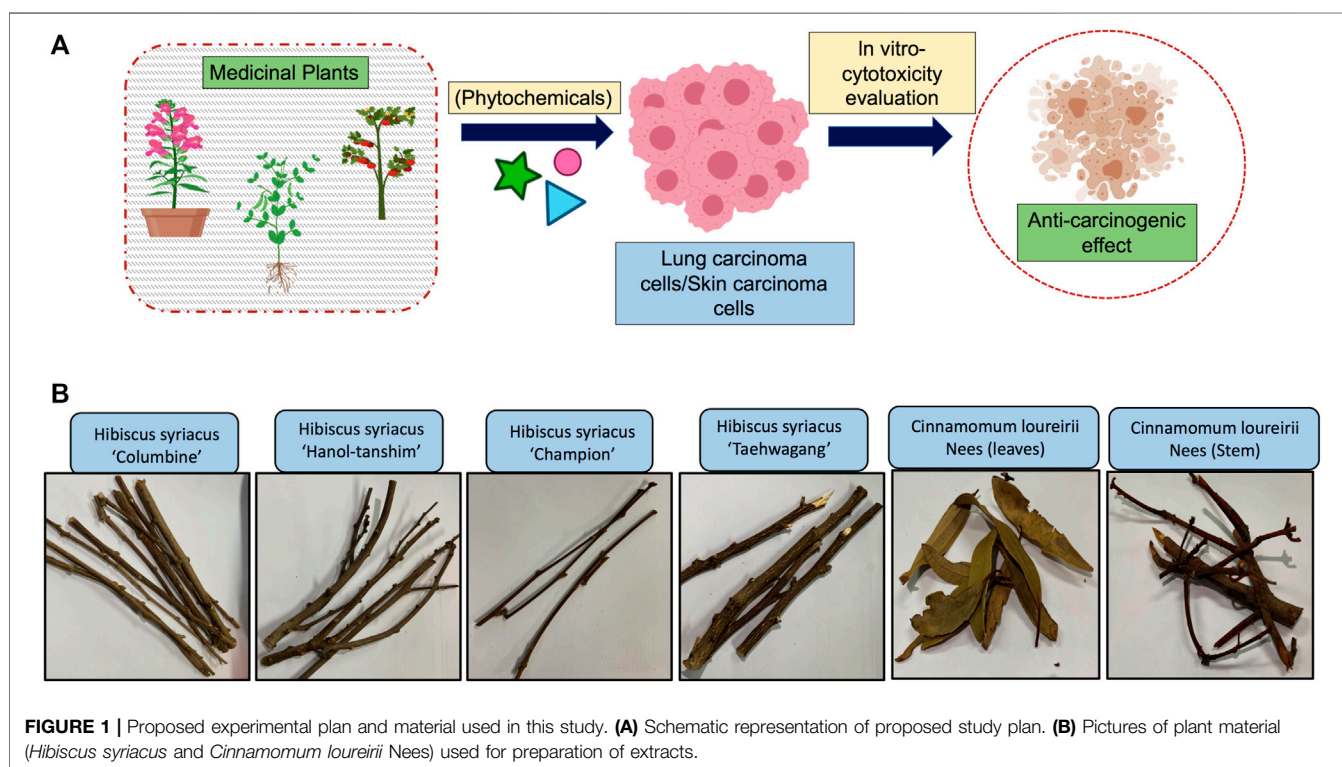
Human lung carcinoma A549 and skin melanoma G361 cells were bought from the Korean Cell Line Bank (Korea). Both the A549 and G361 cancer cells were cultured in Dulbecco's modified Eagle medium and Roswell Park Memorial Institute-1640 media, supplemented with 10% fetal bovine serum, 100 µg/ml of streptomycin, and 100 U/mL of penicillin (Gibco, Waltham, MA, United States). These cells were kept in a humidified incubator at 37°C containing 5% CO₂ and regularly passaged each 2–3 days. Stock concentrations were prepared in DI water for desired cell experiments.

Radical Scavenging Activity

The plant extracts effect on DPPH (Sarikurkcu et al., 2016) radicals were assessed in *Hibiscus* and *Cinnamomum* species (Kim J. H. et al., 2016). Briefly, 1 ml of sample solution was added to 4 ml of a 0.004% DPPH prepared in methanol. Ascorbic acid (Sigma-Aldrich) was included as the standard to determine DPPH activity in the test samples. The developed color in the test sample was read at 517 nm using absorbance at room temperature after 30 min of incubation using an enzyme-linked immunosorbent assay reader (Epoch; BioTek Instruments, United States).

Determination of Total Bioactive Components

Total phenolic and flavonoid constituents were detected using the methods as previously described (Zengin et al., 2014). To measure the phenolic contents, gallic acid (0.04–200 µg/ml) was utilized as



the standard in the experiment. The concentrates of phenolic constituents that exist in *H. syriacus* and *C. loureirii* Nees were shown in milligrams of gallic acid equivalents per gram of individual extract. Nevertheless, quercetin (00.4–200 µg/ml) was used as the standard for flavonoids, and the entire flavonoid contents were shown in milligrams of QE per gram of individual extract.

Cell Viability Assay

To detect cancer cell viability, cells were exposed with various extract concentrations (1,000, 500, 250, 125, and 62.5 µg/ml) and incubated for 48 h. After desired time point, viability was measured using alamarBlue (AB; Thermo Fisher Scientific, United States) dye. Concisely, an AB solution was added to every well, incubated for the next 2 h, and measured as described in our earlier work (Nguyen et al., 2021). Observed fluorescence was reflected as a measure of AB dye conversion in the untreated and treated cell samples.

Cell Cycle Arrest

Briefly, 48 h of post-extract treatments, the cells were collected and rinsed with ice-cold phosphate-buffered saline continued by fixation with 70% cold EtOH at 4°C for 10–12 h. Subsequently, the cells were washed further, resuspended in a cell cycle staining solution including 1 µg/ml RNase A with 5 µg/ml propidium iodide (PI), and incubated for 25–30 min in the dark place (Bhartiya et al., 2021). Immediately, the stained cells were examined using a flow cytometer (BD FACSVers; United States) with FACSuite software. In every test sample, 10,000 events/samples were identified.

Nitric Oxide Measurement (Griess Assay)

The amount of nitric oxide (NO) formed was calculated from the aggregation of the nitrite (NO₂⁻, steady NO metabolite) by Griess reagent assay. In this experiment, the 100-µl culture supernatant was collected from untreated and treated samples and mixed with 100-µl Griess reagent, and the absorbance was accessed at 540 nm (Green et al., 1982). The amount of nitrite was calculated using the standard curve.

Phytoconstituents Characterization Fourier-Transform Infrared and Ultraviolet-Visible Spectroscopy Analysis

Ultraviolet-Visible (UV-Vis) absorption spectroscopy was carried out using a J-815 spectrophotometer (JASCO, Japan). Fourier transformed infrared (FTIR) spectroscopy was performed using a Shimadzu QATR-S spectrometer (Kyoto, Japan). For both UV-Vis and FTIR measurements, the *C. loureirii* Nees extracts were dissolved in methanol.

Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry Analysis

The *C. loureirii* Nees stem extracts were analyzed at the ideal conditions using LC-QTOF-MS supplied with PDA detector (Waters, United States) and asymmetry C18 column of 100 × 2.1 mm, 1.8 mm particle size (Waters, United States). The *C. loureirii* Nees stem extracts were prepared in high-performance

liquid chromatography grade pure methanol to produce 20 ppm. For analysis, the mobile phases used as A (water with 0.1% formic acid) and B (100% acetonitrile). The gradients elution were used as earlier (Alara et al., 2018), using the injection volume of 20 ml and flow rate of 0.5 ml/min. The bioactive constituents in the *C. loureirii* Nees stem extracts were tentatively designated with Waters UNIFY Software 1.0.0 (Alara et al., 2018).

Statistical Analysis

All results are expressed as the means ± standard deviation of triplicate assessments from three independent experiment sets. Significant differences between groups were examined using the Student's t-test in PRISM 9 software. Multiple group comparisons were evaluated using analysis of variance. Levels of significance are mentioned by **p* < 0.05, ***p* < 0.01, and ****p* < 0.001.

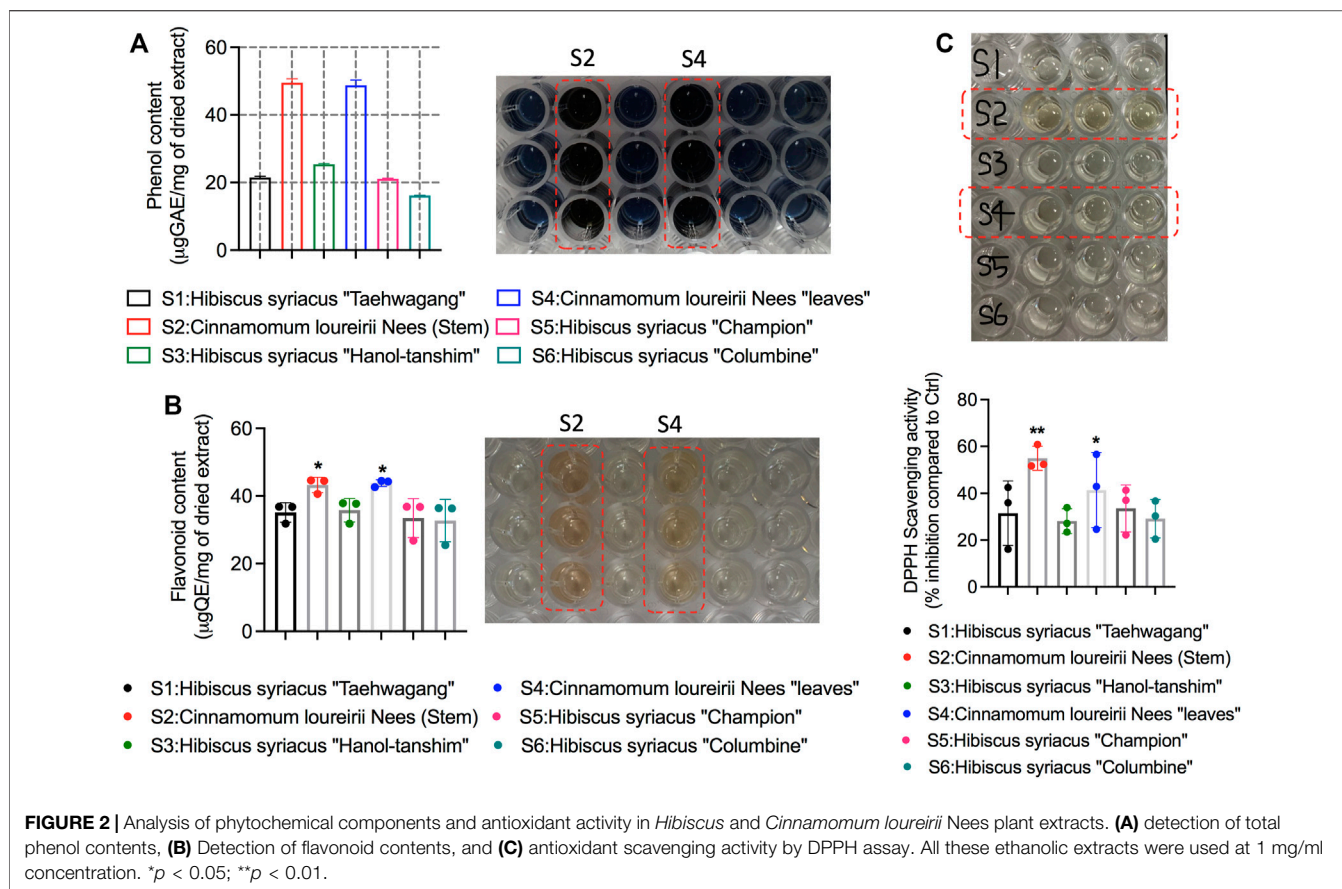
RESULTS

Determination of Phytoconstituents Levels in *Hibiscus syriacus* and *Cinnamomum loureirii* Nees Plant Extracts

Before performing cell experiments, the first objective of this study was to examine all the plant ethanolic extracts for their phytoconstituent levels. Primary screening of all plant species of *H. syriacus* and *C. loureirii* Nees extracts (described in **Table 1**) showed the existence of phytochemical constituents at different levels. These extracts were subjected to qualitative biochemical assessments to identify secondary metabolites such as total phenols and flavonoids. Higher levels of phenols and flavonoids were detected in *Cinnamomum* plant extracts over *Hibiscus* plant species extracts, as shown in **Figure 2A**. Colorimetric visualization demonstrated the high intensity of color production in *Cinnamomum* extracts only (**Figure 2B**). It has been shown that plants possess antioxidant activity mainly because of the presence of phenolic compounds, including flavonoids and polyphenols. Flavonoids are believed to be key antioxidants in traditional herbal medicine (Dragland et al., 2003) (Pietta, 2000). Interestingly, *Cinnamomum* ethanolic extracts have shown augmented antioxidant activity (55%) as observed by DPPH free radical inhibition, which was well correlated with their increased phytoconstituents levels. Notably, *Cinnamomum* stem ethanolic extracts have higher antioxidants activity as compared with leaf ethanolic extracts (**Figure 2C**).

Chemical Profiling of *Cinnamomum loureirii* Nees Stem and Leaves Using Fourier Transformed Infrared, Ultraviolet-Visible, and Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry Analysis

Plant extracts are complex mixtures of multiple phytochemicals that are difficult to identify specifically. Further to study the chemical composition of the *C. loureirii* Nees extracts, we have performed several characterizations using different chemical analysis techniques. **Figure 3A** shows the UV-Vis spectrum of



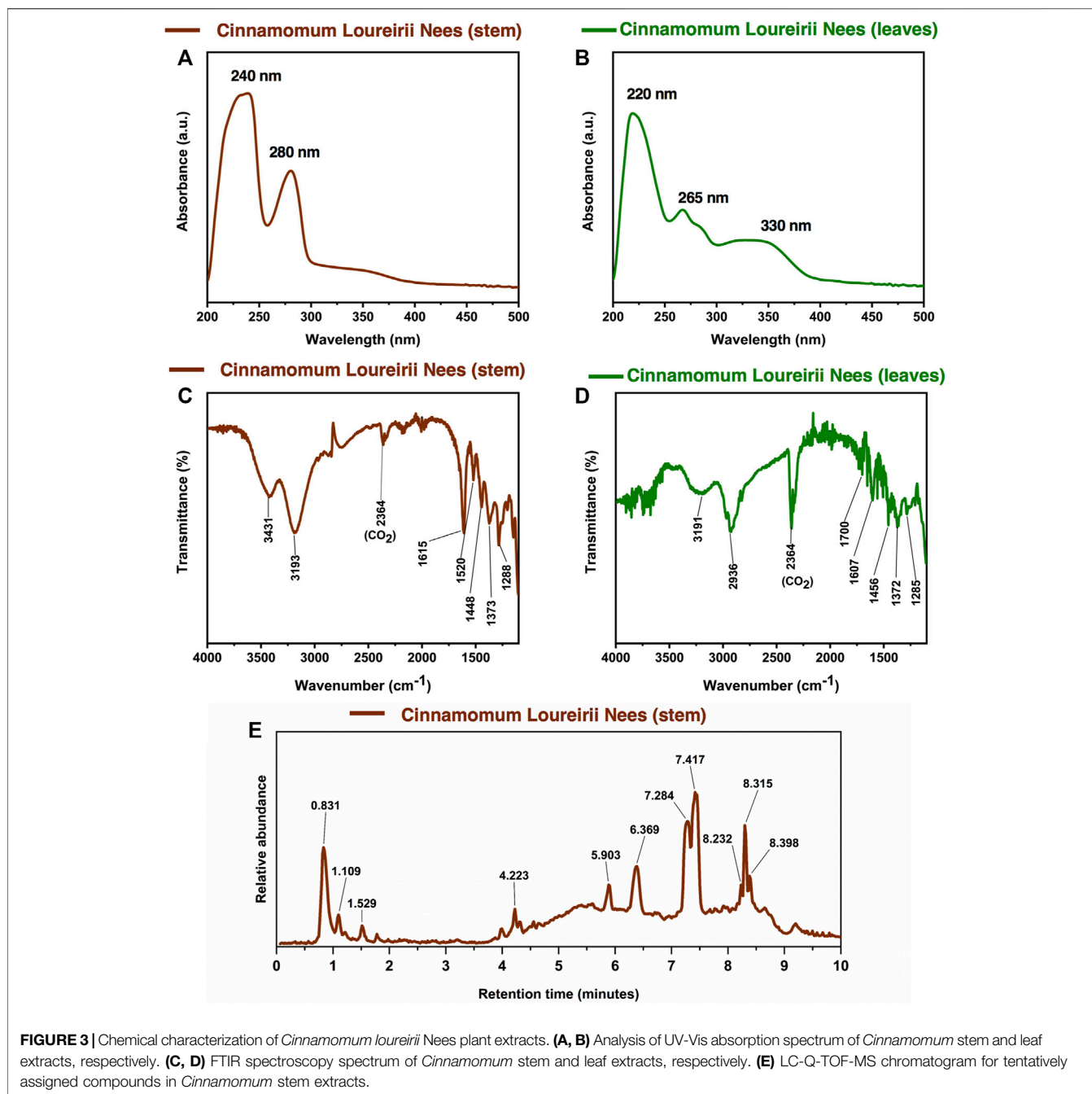
Cinnamomum stem ethanolic extracts, revealing two absorption peaks located at 240 and 280 nm (**Figure 3A**). On the other hand, the leaf ethanolic extracts demonstrate the absorption peaks at 220 and 266 nm, together with a broad band at 330 nm (**Figure 3B**).

Both *Cinnamomum* stem and leaf extracts possess strong absorption in the range of 200–400 nm, indicating the existence of flavonoids and their derivatives in the extracts (Jurasekova et al., 2006). Afterward, FTIR spectroscopy was carried out to identify the functional groups present in the *C. loureirii* extracts. **Figure 3C** shows the vibration absorption bands observed in the *Cinnamomum* stem extracts, i.e., OH stretching ($3,431, 3,190 \text{ cm}^{-1}$), C=C stretching ($1,615 \text{ cm}^{-1}$), N-O stretching ($1,520 \text{ cm}^{-1}$), C-H bending ($1,448 \text{ cm}^{-1}$), S=O stretching ($1,373 \text{ cm}^{-1}$), and C-O stretching ($1,288 \text{ cm}^{-1}$) groups. The FTIR spectrum of the *Cinnamomum* stem extracts is shown in **Figure 3D**, with the presence of OH stretching ($3,191 \text{ cm}^{-1}$), C-H stretching ($2,936 \text{ cm}^{-1}$), C=O stretching ($1,700 \text{ cm}^{-1}$), C=C stretching ($1,615 \text{ cm}^{-1}$), C-H bending ($1,456 \text{ cm}^{-1}$), S=O stretching ($1,372 \text{ cm}^{-1}$), and C-O stretching ($1,285 \text{ cm}^{-1}$) groups. **Table 2** summarizes the details of functional groups in *Cinnamomum* stem and leaf extracts detected by FTIR spectroscopy. **Figure 3E** shows the LC-QTOF-MS analysis of the *C. loureirii* Nees stem extracts. The details of identified phytochemicals are listed in **Table 2**. We noted that the extract of *Cinnamomum loureirii* Nees contains an enormous number of phytochemicals; thus, it is difficult to list all the

existing compounds. Herein, 11 significantly separated compounds from the chromatography were mentioned. For instance, several sugar molecules were detected, such as D-1-[(3-carboxypropyl)amino]-1-deoxyfructose (RT: 0.831 min), vanilloside (RT: 1.529 min), and (1x, 2x)-guaiacyl glyceryl 3-glucoside (RT: 0.831 min). Flavonoids and polyphenols and are also presented in the extract, i.e. epicatechin (RT: 4.223 min), (7R, 8R,E)-8-methyl-6-(2-methylpropylidene)octahydroindolizine-7,8-diol (RT: 5.903 min) (1R,2S,4R, 8R)-p-menthane-1,2,8,9-tetrol. The MS detection of flavonoids and polyphenols agrees with the FTIR and UV-Vis results.

Evaluation of Antiproliferative Activity *Cinnamomum loureirii* Nees Plant Extracts Against A549 Human Lung Adenocarcinoma and G361 Skin Melanoma Cell Lines

To investigate the antiproliferative potential of *Cinnamomum* plant extracts prepared from both stem and leaves, we have tested different concentrations of these extracts against G361 skin cancer and A549 lung cancer cells to check their effect on different tissue types. After 24 h of cell attachment, both the cells were exposed with 0, 62.5, 125, 250, 500, and 1,000 µg/ml concentrations of plant extracts and incubated for the further 48 h to check their effect on cell survival. The result of the



alamarBlue assay revealed that both *Cinnamomum* stem and leaf extracts gradually declined the survival percent of G361 and A549 cancer cells as the concentration increased (**Figures 4A,B**). *Cinnamomum* stem and leaf ethanolic extracts exhibited IC_{50} values of 188.45 and 504.08 $\mu\text{g/ml}$, respectively, in G361 skin cancer cells. In the case of A549 lung cancer cells, *Cinnamomum* stem and leaf ethanolic extracts exhibited IC_{50} values of 102.40 and 295.10 $\mu\text{g/ml}$, respectively, in G361 skin cancer cells. Remarkably, *Cinnamomum* stem extracts have a higher potential to suppress cancer cell viability over leaf extracts regardless of tissue-specific cell type. Taken together, these

alamarBlue results suggest that *Cinnamomum* plant extracts were able to inhibit the growth of cancer cells in a concentration-dependent fashion.

NO Production by *Cinnamomum loureirii* Nees Plant Extracts Against Cancer Cell Lines

·NO has been shown to play important roles in cancer biology, including the innate immune response, neovascularization, cancer metastasis, and cell death (Wink et al., 1998); we next

TABLE 2 | FTIR functional groups present in *Cinnamomum loureirii* stem and leaf extracts.

Functional groups	Absorption (cm ⁻¹)- stem	Absorption (cm ⁻¹)- leaves
OH stretching	3,431, 3,190	3,191
C-H stretching	-	2,936
C=O stretching	-	1,700
C=C stretching	1,615	1,607
N-O stretching	1,520	-
C-H bending	1,448	1,456
S=O stretching	1,373	1,372
C-O stretching	1,288	1,285

questioned where our *Cinnamomum* stem and leaf plant ethanolic extracts were able to generate NO in cancer cells. To this end, cell supernatant was collected from G361 and A549 cancer cells after treatment at various concentrations, and all samples were subjected to Griess assay for NO detection. Remarkably, with the increase in concentrations, the quantity of NO generation was significantly enhanced, as seen in both G361 and A549 cancer cells (Figures 5A–D). These effects were similar in both stem and leaf extracts when applied to G361 and A549 cancer cells.

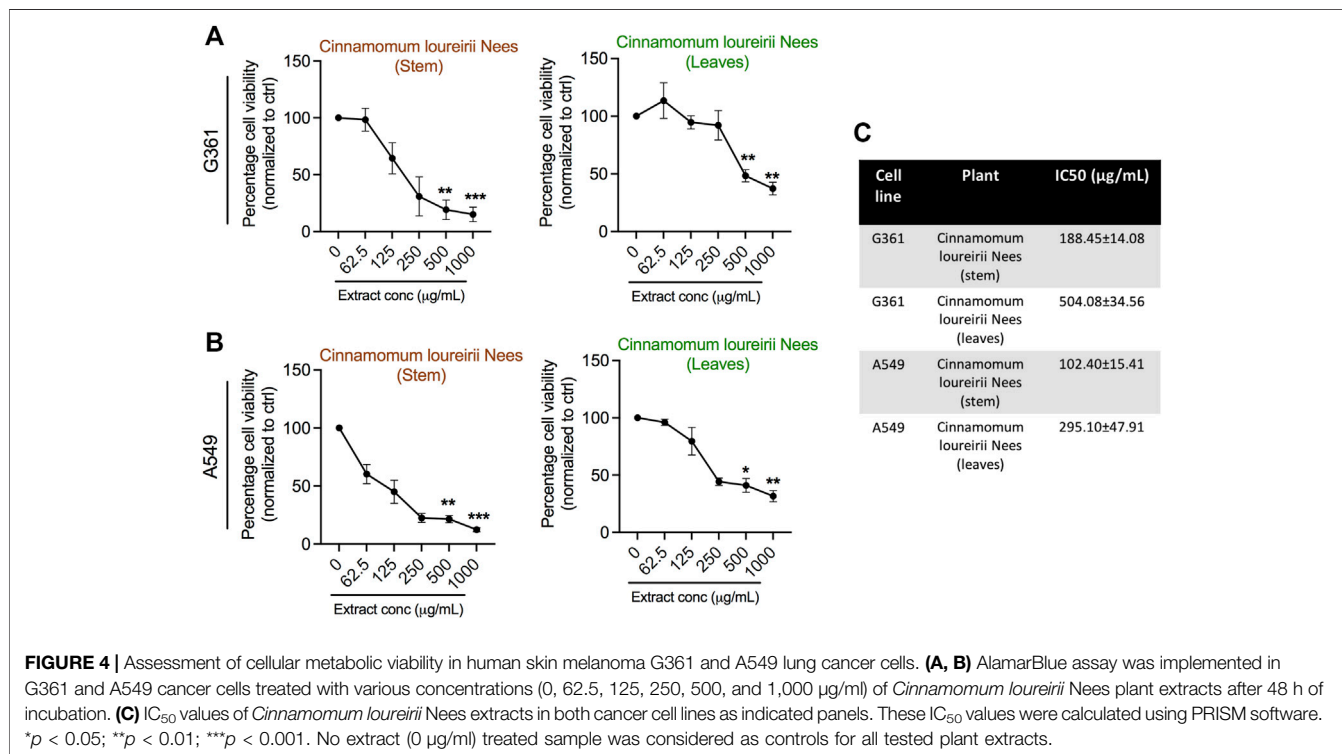
Apoptosis Cancer Cell Death Induction by Cell Cycle Arrest by *Cinnamomum loureirii* Nees Plant Extracts

Furthermore, based on the activity of *Cinnamomum* plant extracts in growth inhibition and/or cell death of cancer cells, we investigated the

ability of these ethanolic extracts to induce apoptosis in cancer cells. To check the apoptosis phenomenon, both the cells were treated with stem and leaf extracts at IC₅₀ concentrations, which were calculated from alamarBlue assay results (see Figure 4C). Flow cytometry results indicated that both the extracts were able to stimulate apoptotic cell death in G361 and A549 cancer cells. However, this effect was more prominent in G361 skin melanoma cells. It was noted that stem extracts promote a maximum range of 45.63 and 7.63% cell population in early and late apoptotic phases, respectively. Simultaneously, stem and leaves both can induce apoptosis in A549 cancer cells, but necrosis was also seen (Figures 6A,B). Next, to study whether the inhibitory effects of both stem and leaf extracts were associated with cell cycle alteration, we executed cell cycle analysis in G361 and A549 cancer cells using FACS analysis after 48 h after treatment at IC₅₀ concentrations of both extracts. Our G361 cell data showed that stem and leaf extract-induced cytotoxicity was related to the expansion of cell population in the G0/G1 phase and a significant decrease in S phases. In contrast, A549 cells treated with stem extracts displayed a noticeable accumulation of cells in the G2/M phase along with a reduction in the G0/G1 phase. However, this effect was not comparable in the case of A549 cells treated by leaf extract (Figures 6C, D). These results suggest that both *Cinnamomum* stem and leaf extracts affect differentially on cell tissue type regarding cell cycle arrest.

DISCUSSION

Earlier, herbal medicine has gained attraction for cancer treatment due to their phytochemical contents having



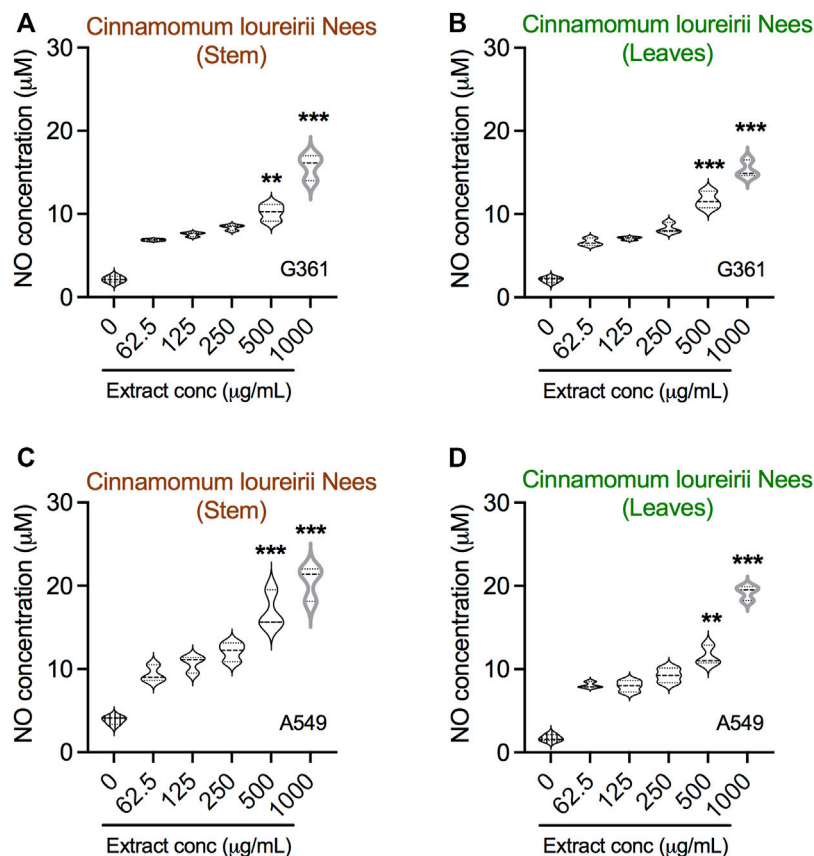
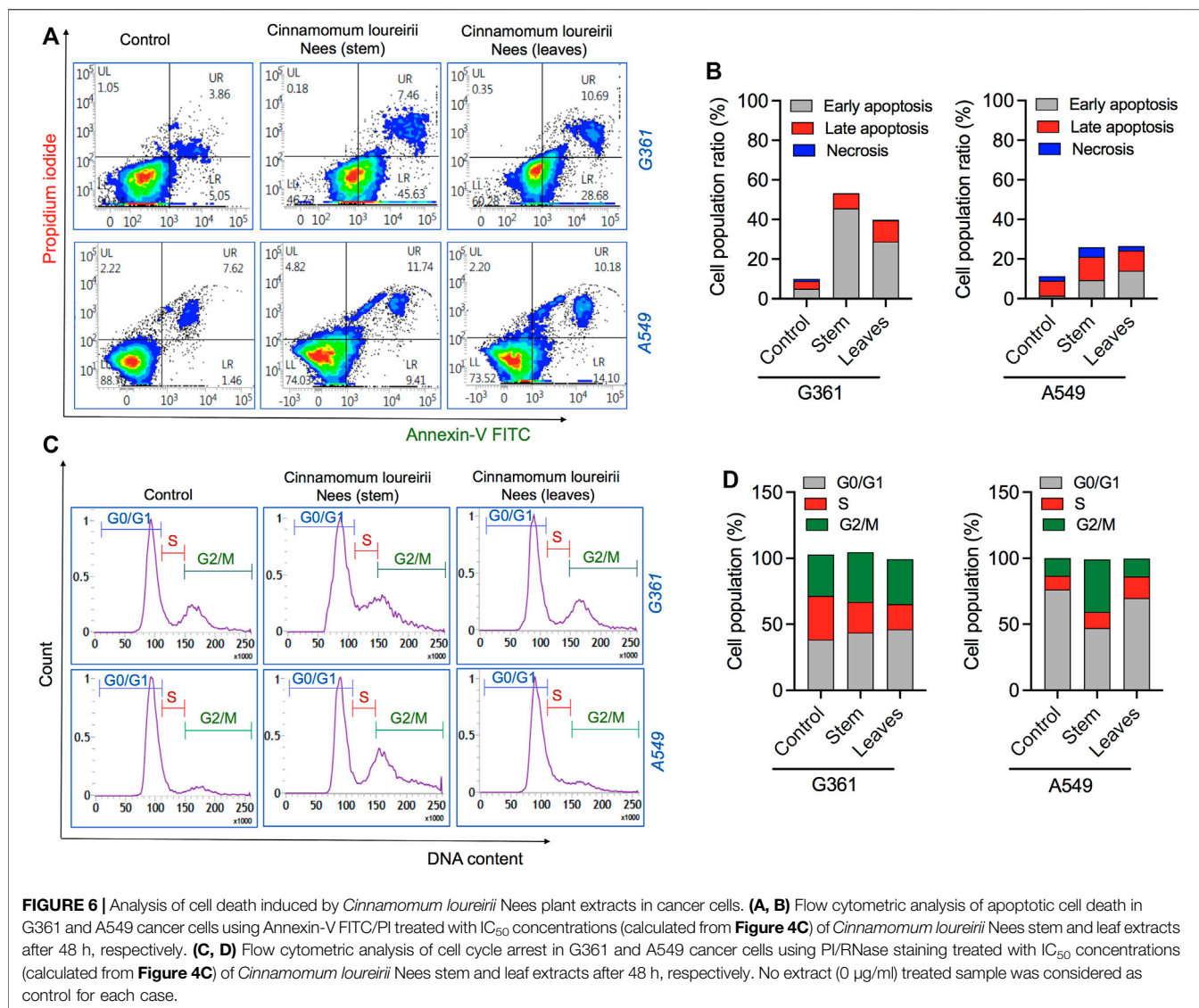


FIGURE 5 | Detection of nitric oxide in *Cinnamomum loureirii* Nees plant extracts treated cancer cells. **(A–D)** Assessment of NO in G361 and A549 cancer cells treated with various concentrations (0, 62.5, 125, 250, 500, and 1,000 µg/ml) of *Cinnamomum loureirii* Nees stem and leaf extracts after 48 h, respectively. ** $p < 0.01$; *** $p < 0.001$. No extract (0 µg/ml) treated sample was considered as controls for each case.

numerous biological activities (Mann, 2002). Currently, natural products or their structural derivatives contain approximately 45–55% of the drugs used for cancer chemotherapies. Using the human genome project, researchers could identify selective gene targets for innovative anticancer drugs and pharmaceutical business challenges to attain these drug formulations *via* the use of chemistry and high-performance screening methods. Nonetheless, the large libraries of compounds fail to provide the essential formation needed for the formulation of new anticancer drugs. To overcome these problems, the much more elusive use of natural-product patterns shared with chemistry to fabricate selective analogs could have a great rate of success. Several pieces of literature provide information on the crucial role of natural products in the development of the novel anticancer candidates, and their significance in the optimization of unique molecular leads from natural products (Cragg and Newman, 2009; (Demain and Vaishnav, 2011; Basmadjian et al., 2014). The phytogetic nanoparticles are widely considered as antimicrobial agents, wound healing processes in cancer therapy, drug delivery approaches, and bioenergy or biosensors applications (Augustine and Hasan, 2020; Verma and Rani, 2021). A recent report suggests that zinc oxide nanoparticles synthesized using *Deverra tortuosa* plant extracts have the

potential to induce a cytotoxic effect in different tissue-specific cancer cells (Selim et al., 2020). Similarly, the formation of silver nanoparticles using *Sesbania grandiflora* leaf extracts has been shown to be cytotoxic against breast carcinoma cells (Das et al., 2013). Magnetic iron oxide nanoparticles synthesized from *Phyllanthus niruri* were also found to be biocompatible and have high antibacterial efficacy against both Gram-positive and Gram-negative bacterial strains (Sheel et al., 2020). A similar group of researchers also highlights the importance of green synthesized nanoparticles prepared by leaf extract of *Andrographis peniculata* against zebrafish embryos as shown by *in vivo* analysis (Kumari et al., 2019) and the use of hybrid silver nanoparticles synthesized using leaf extracts of *Calotropis gigantea* in cancer cells death (Verma et al., 2016).

Cinnamon is a form of spice that has been largely utilized from ancient times in most countries (Dutta and Chakraborty, 2018). The function of this spice has been broadly studied in its benefits for human health. Generally, plant products contain polyphenols (Chang, 2002); accordingly, we have seen that our *C. loureirii* Nees plant stem and leaf extracts have a mixture of numerous phytochemicals as determined by chemical analysis techniques, including FTIR and UV-Vis spectroscopy. Moreover, FTIR analysis shows the potential functional groups of the major



compounds existing in plant extracts (**Figures 3A–D**). **Table 2** provides more clarity on the functional groups present in the stem and leaf ethanolic extracts of *C. loureirii* Nees plant. It has been stated that *Cinnamomum* has anticancer properties via many molecular signaling mechanisms (Kwon et al., 2019). The main ability of any potential anticancer drug is its efficiency in inhibiting the growth of cancer cells. Schoene et al. (2005) showed that *Cinnamomum* extracts could promote antiproliferative effects in different blood cancer cell types. Besides, the essential oil of *Cinnamomum* has been revealed to decrease receptor tyrosine kinase activities in squamous cell carcinoma, reducing the tumor burden in those cells (Yang et al., 2015). In our study, we observed that our *C. loureirii* Nees plant stem and leaf extracts were able to suppress cancer cell death in skin melanoma and lung carcinoma cells (**Figure 4**). It is worth mentioning that it is the first study on cancer cell inhibition using *C. loureirii* Nees plant ethanolic extracts to the best of my knowledge. In another study, *C. loureirii* extracts are beneficial

as a neuroprotective agent by preventing Alzheimer's disease. They identified that potent acetylcholine inhibitors were present in their *C. loureirii* ethanolic extracts (Kim C. R. et al., 2016). Earlier, it has been reported that phenolic components often exist in wild plants (Rana and Blazquez, 2007). Using liquid chromatography–mass spectrometry analysis, we observed that our *C. loureirii* Nees ethanolic stem extracts have a major amount of compounds (**Figure 7**) such as vanilloloside [as a wound-healing (Harikarnpakdee and Chowjarean, 2018), neuroprotector (Jung et al., 2010)], epicatechin [as an anticancer agent (Takanashi et al., 2017), antioxidant (Caro et al., 2019), antidiabetic (Kim et al., 2004)], diisobutyl phthalate [as a water-based adhesive, estrogenic (Harris et al., 1997) DNA damage inducer (Sicińska et al., 2021)], and many more described in **Table 3**.

Recent literature indicates that NO has a potential role in cell growth inhibition and apoptosis at higher concentrations (Reveneau et al., 1999). Their study showed the

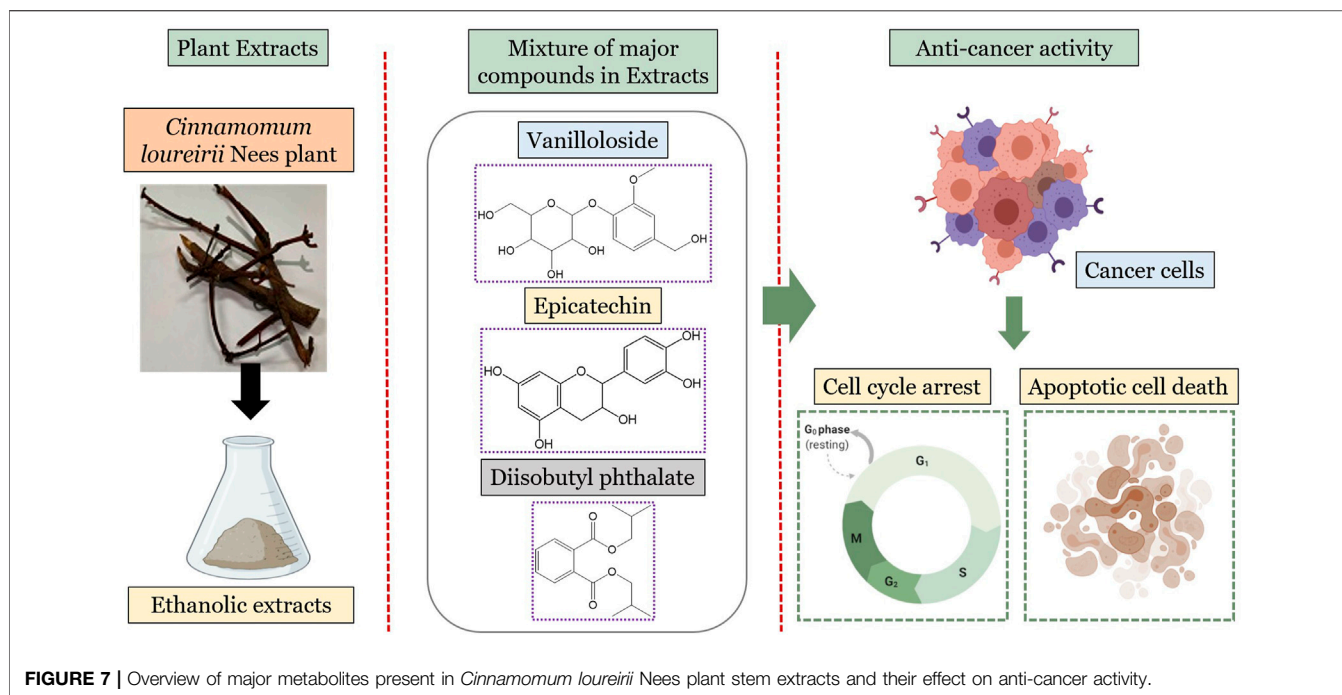
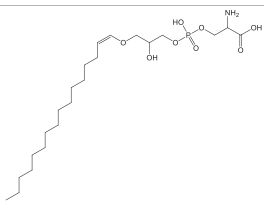
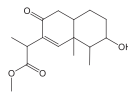



TABLE 3 | Phytochemicals identified in *Cinnamomum loureirii* Nees stem by LC-QTOF-MS analysis.

RT (min)	Compound	Formula	Chemical structure	Species	m/z	Error (ppm)
0.831	D-1-[(3-Carboxypropyl)amino]-1-deoxyfructose	C ₁₀ H ₁₉ NO ₇		(M + H) ⁺	226.12	1.9
1.109	(1x,2x)-Guaiacyl glyceryl 3-glucoside	C ₁₆ H ₂₄ O ₁₀		(M + Na) ⁺	339.13	0.06
1.529	Vanilloside	C ₁₄ H ₂₀ O ₈		(M + Na) ⁺	339.1	1.11
4.223	Epicatechin	C ₁₅ H ₁₄ O ₆		(M + H) ⁺	291.08	1.55
5.903	(7R,8R,E)-8-Methyl-6-(2-methylpropylidene)octahydroindolizine-7,8-diol	C ₁₃ H ₂₃ NO ₂		(M + H) ⁺	226.18	1.92
6.369	(1R,2S,4R,8R)-p-Menthane-1,2,8,9-tetrol	C ₁₀ H ₂₀ O ₄		(M + Na) ⁺	227.12	3.83
7.284	xi-5-Hydroxydodecanoic acid	C ₁₂ H ₂₄ O ₃		(M + H) ⁺	217.18	2.5
7.417	5-Methylheptan-3-one	C ₈ H ₁₆ O		(M + H) ⁺	129.12	3.27

(Continued on following page)

TABLE 3 | (Continued) Phytochemicals identified in *Cinnamomum loureirii* Nees stem by LC-QTOF-MS analysis.

RT (min)	Compound	Formula	Chemical structure	Species	m/z	Error (ppm)
8.232	PS(P-16:0/0:0)	C ₂₂ H ₄₄ NO ₈ P		(M + NH ₄) ⁺	499.31	0.3
8.315	Methyl (3b,11x)-3-Hydroxy-8-oxo-6-eremophilin-12-oate	C ₁₆ H ₂₄ O ₄		(M + H) ⁺	281.17	1.83
8.398	Diisobutyl phthalate	C ₁₆ H ₂₂ O ₄		(M + Na) ⁺ (M + H) ⁺	301.14	1.96

antiproliferative effect in breast cancer cells could be achieved with high NO generation induced by plant extracts in a concentration-dependent manner. Similarly, our *C. loureirii* Nees plant extracts were able to increase the production of NO in both cancer cells in a dose-dependent fashion (Figure 5). This might be largely possible due to the increasing effect of the inducible nitric oxide synthase gene, which may result in the enhanced NO in cells (Alalami and Martin, 1998), thereby responsible for cell death and apoptosis through cellular damage, including DNA, protein, and other cellular components. This was consistent with our result of observed apoptotic cell death in G361 and A549 cancer cells after treatment with *C. loureirii* Nees stem and leaf ethanolic extracts. Cell cycle results showed that the effect of stem and leaf extracts was different regarding cancer cell types on cell growth arrest, suggesting them more specific for each cell cycle that has been used for treatment (Figure 6). Furthermore, it would be motivating to know the basic mechanisms involved in such cell cycle effects induced by *C. loureirii* Nees stem and leaf extracts using different cancer cell tissue types.

CONCLUSION

In conclusion, the presented study showed that *C. loureirii* Nees ethanolic stem and leaf ethanolic extracts have an anticancer effect, which could be capable of inducing cell death phenomena via apoptosis. Moreover, our phytoconstituent characterization analysis showed the presence of polyphenols and flavonoids present in the *Cinnamomum* Nees extracts. Prominently, some vital compounds such as vanillogoside, epicatechin, and many more that exist in *Cinnamomum* extracts make them a potential candidate for health benefits. Further works are necessary to isolate particular active phytoconstituents from the extracts to be used as an efficient anticancer agent to elucidate their significance in future chemotherapies. In this regard, the application of phytochemicals in the form of nanoemulsion or nanoparticles

amplifies the therapeutic effect and provides a new way to solve the difficulty in the treatment of dreadful diseases such as cancer. Therefore, the utilization of phytochemicals in nanotechnology will be a promising approach.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

Conceptualization, NK and JK; methodology, NK and NKK; formal analysis, NK, HO, and YL; chemical characterization, LN; investigation, NK and NKK; resources, NK, NKK, EC, and JK; writing—original draft preparation, NK; writing—review and editing, NK, NKK, EC, and JK; funding acquisition, NK and JK; supervision, NK and JK. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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