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Phytochemical Profiling and Cytotoxic Evaluation of *Marasi* (*Curculigo latifolia*) Leaf Extract and Fractions Against MCF-7 Breast Cancer Cells

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Abstract

Breast cancer has the highest incidence among women in Indonesia. The exploration of natural products as sources of selective anticancer agents with minimal side effects continues to attract considerable attention. *Marasi* (*Curculigo latifolia*) is reported to contain secondary metabolites with potential antiproliferative properties. This study aimed to perform phytochemical profiling using thin-layer chromatography (TLC) and liquid chromatography–mass spectrometry (LC–MS), as well as to evaluate the cytotoxic activity of *C. latifolia* leaf extracts and fractions against MCF-7 breast cancer cells. TLC analysis indicated that the leaf extract of *C. latifolia* and its fractions comprise diverse secondary metabolites, such as phenolics, terpenoids, flavonoids, and tannins. Meanwhile, LC–MS profiling demonstrated that nitrogen-bearing heterocyclic compounds predominate, particularly triazine derivatives, tetrazoles, and benzothiazoles. Cytotoxicity assessment demonstrated that the extracts and fractions displayed low inhibitory activity against MCF-7 cells, with the strongest effect observed at an IC_{50} value of 234.8782 ppm, which remains above the accepted threshold for significant cytotoxic activity. Although the activity against MCF-7 cells was limited, further investigation involving other cancer models and isolation of active constituents is necessary to better understand and potentially enhance the biological activity of *C. latifolia* leaf.

Keywords: *Curculigo latifolia*; anticancer; MCF-7; cytotoxic; LC-MS; fractionation.

INTRODUCTION

Cancer is the third leading cause of death in Indonesia, with 242,988 deaths reported among 408,661 new cases in 2022, and its incidence continues to rise alongside population growth (Setiani, 2023). Breast cancer represents the most common malignancy among women, with a prevalence of 16.3% per 100,000 population. This disease results in significant physical complications, including chronic pain, insomnia, severe fatigue, and loss of appetite, as well as psychological effects such as depression and anxiety, which often necessitate strong family support (Setiani, 2023; Syamsuddin et al., 2025).

Breast cancer is associated with multiple risk factors, including genetic mutations such as BRCA1 and BRCA2, hormonal influences such as prolonged estrogen exposure, and environmental factors including alcohol consumption, obesity, and physical inactivity (Waks & Winer, 2019). Pathologically, the most prevalent subtype is invasive breast carcinoma, accounting for approximately 70–75% of cases. Diagnosis is commonly established through imaging techniques, histopathological biopsy, and immunofluorescence or immunohistochemical analyses (Xiong et al., 2025). The progression of breast cancer is strongly linked to dysfunction of the p53 tumor suppressor protein, which regulates cell cycle progression and apoptosis. Mutations in the p53 gene impair its regulatory function, allowing damaged cells to proliferate uncontrollably (Binayke et al., 2019).

Conventional breast cancer treatments include surgery (61.8%), radiation therapy (17.3%), chemotherapy (24.9%), and hormonal therapy; however, these approaches are often associated with significant side effects, therapeutic resistance, and high treatment costs (Shofa et al., 2022). Consequently, herbal-based traditional therapies have gained attention as alternative or complementary options due to their relatively lower side effects and potential to target specific molecular pathways (In et al., 2024). Several medicinal plants, including white turmeric, red betel, *Tinospora crispa*, *bandotan*, *dadap ayam*, *keladi tikus*, and *marasi* (*Curculigo latifolia*), have been reported to possess potential anticancer activity against breast cancer.

Curculigo latifolia, a member of the Hypoxidaceae family, is widely distributed in tropical Asia, including Indonesia. The leaves, fruits, and rhizomes have

traditionally been used to treat fever, stomachache, and wounds (Silalahi, 2023;Wahab & Rosnawati, 2021). Previous studies have reported that *C. latifolia* extracts contain flavonoids, alkaloids, terpenoids, phenolic compounds, and tannins, which exhibit antioxidants, anti-inflammatory, and antitumor activities (Zabidi et al., 2019; Kristiani & Susanti, 2021). Cytogenotoxicity assays have also indicated the presence of bioactive constituents capable of inhibiting cell division, suggesting its potential as an anticancer agent. Furthermore, species within the *Curculigo* genus have demonstrated activity against MCF-7, HeLa, and HepG2 cancer cell lines and have been reported to enhance the efficacy of chemotherapy while reducing its toxicity (Hejazi et al., 2018;Muthiam et al., 2025).

Studies evaluating the anticancer potential and cytotoxic activity of *Curculigo latifolia* leaf extracts and fractions remain limited, and comprehensive investigations are still lacking. *C. latifolia* is hypothesized to exhibit antiproliferative effects and influence p53 expression in MCF-7 breast cancer cells due to its metabolite, thereby potentially inhibiting mechanisms associated with breast cancer progression. Therefore, this study aimed to evaluate the cytotoxic activity and p53 expression in MCF-7 breast cancer cells treated with *C. latifolia* leaf extracts and fractions, as well as to perform phytochemical screening to identify the major bioactive constituents present. The findings are expected to contribute to the development of natural product-based strategies for breast cancer management.

MATERIALS AND METHODS

Equipment and materials

The equipment used in this study included an oven, moisture balance, filter paper, rotary evaporator, analytical balance, test tubes, Sigma 3K12 centrifuge (B. Braun Biotech International), a 37 °C incubator with 5% CO_2 (Model 6200, Napco), Class II laminar airflow cabinet (Labconco), electric balance (Sartorius), ELISA reader spectrocolorimeter (SLT 240 ATC), Neubauer hemocytometer (Olympus CKX41), autoclave, sterile conical tubes (Nunclone), tissue culture flasks (Nunclone), 96- and 24-well microplates (Nunclone), micropipettes (20–200 µL and 200–1000 µL, Pipetman), vortex mixer, 15 mL conical tubes, magnetic stirrer, and inverted microscope (Axiovert-25) (Yanti et al., 2021) (Yanti et al., 2021).

The materials used included *C. latifolia* leaves, 96% ethanol, distilled water, n-hexane, ethyl acetate, silica gel F254 TLC plates, H_2SO_4, MCF-7 breast cancer cells, normal Vero cells, Dulbecco's Modified Eagle Medium (DMEM), M199 medium, cisplatin as a positive control, sodium bicarbonate, HEPES (Sigma), 1 M NaOH, 1 M HCl, fetal bovine serum (FBS) 10% v/v (Gibco), Fungizone (Amphotericin B) 0.5% v/v (Gibco), Penicillin–Streptomycin 2% v/v (Gibco), dimethyl sulfoxide (DMSO), 0.5% trypsin, MTT reagent, phosphate-buffered saline (PBS) pH 7.2, sodium dodecyl sulfate (SDS) 10% in 0.1 N HCl, methanol, blocking serum, hydrogen peroxide, prediluted blocking serum, primary antibodies against caspase-3 and p53, xylol, mounting medium, and the Novostatin Universal Detection Kit.

Sample Procurement

C. latifolia leaves were collected from community plantations in Sribunga Village, Buay Pemuka Bangsa Raja District, East Oku Regency, Indonesia. Preparation of the extracts and fractions was conducted at the Laboratory of the Faculty of Mathematics and Natural Sciences, Department of Biosciences, Universitas Sriwijaya. The anticancer activity was evaluated using the MTT assay method at the Laboratory of Universitas Gajah Mada, Yogyakarta.

Phytochemical Profiling

Preparation of *Curculigo latifolia* Leaf Ethanol Extract

Dried powdered leaves of *C. latifolia* were extracted using the maceration method with 96% ethanol as the solvent. A total of 500 g of leaf powder was immersed in 2 L of 96% ethanol. The mixture was stirred manually three times during the maceration process. After 24 h, the solution was filtered to separate the filtrate from the residue. The residue was air-dried to remove residual solvent and subjected to a second maceration for 48 h. A third extraction was subsequently performed for 72 h. The combined filtrates were concentrated using a rotary evaporator at 50 °C until the solvent was completely removed, yielding a thick ethanol extract of *C. latifolia* leaves (Ditjen POM, 1979, cited in Lumbangaol, 2020).

Fractionation of Ethanol Extract

Fractionation was performed using a liquid–liquid extraction method. The crude extract obtained from the previous extraction stage was diluted with distilled water in a 1:1 ratio (200 mL extract:200 mL distilled water). Subsequently, n-hexane was added gradually to the mixture to achieve a total volume of 1 L, added in portions of 200 mL (4 × 200 mL). After each addition, the mixture was transferred to a separatory funnel and shaken, and the n-hexane layer was collected until the organic phase appeared orange.

The aqueous layer was then further fractionated by the gradual addition of 1 L of ethyl acetate, also in portions of 200 mL (4 × 200 mL), followed by separation using a separatory funnel. This procedure yielded three fractions: n-hexane, ethyl acetate, and ethanol–water fractions. Each fraction was concentrated using a rotary evaporator followed by evaporation in a water bath to obtain viscous fractions for further testing. All fractions were evaluated for anticancer activity, and the fraction

exhibiting the highest activity was subsequently subjected to silica gel column chromatography for further separation.

Thin Layer Chromatography (TLC)

Phytochemical screening of the extract and fractions of *Curculigo latifolia* leaves was performed using the TLC method to identify major groups of secondary metabolites based on spot color and appearance. The extract and each fraction were dissolved in ethanol to obtain a 1% solution. An aliquot of each solution was spotted onto silica gel F_{254} TLC plates (1 cm × 6 cm). The plates were then placed in a developing chamber containing the appropriate mobile phase and allowed to develop for 15–20 min. After development, the plates were removed and air-dried. The chromatograms were subsequently sprayed with 1% H_2SO_4 reagent and heated on a hot plate to visualize the separated compounds. The resulting spot colors and patterns were observed and recorded for compound group identification.

Liquid Chromatography–Mass Spectrometry (LC–MS)

High-resolution mass spectrometry analysis was performed using a mass spectrometer coupled to an ultra-performance liquid chromatography (UPLC) system. Separation was achieved on a C18 column maintained at 50 °C, while the ambient temperature was set at 25 °C. The mobile phase consisted of acetonitrile containing 0.05% formic acid (solvent B) and water containing 5 mM ammonium formate (solvent A), delivered at a flow rate of 0.2 mL min^{-1} using a step-gradient program over 23 min. Prior to injection, samples were filtered through a 0.2 µm syringe filter, and 5 µL of the filtrate was injected into the system.

Mass spectrometric detection was conducted using electrospray ionization (ESI) in positive ion mode, with a mass scan range of 50–1200 m/z. The desolvation temperature was set at 350 °C, and the ion source temperature was maintained at 100 °C. The cone gas flow rate was 0 L h^{-1}, and the desolvation gas flow rate was 793 L h^{-1}. Collision energies ranged from 4 to 60 eV. Data acquisition, processing, and instrument control were performed using MassLynx software version 4.1 (Ismed et al., 2022).

Cytotoxic Evaluation

Media Preparation, Positive Controls and Samples

Complete Roswell Park Memorial Institute (RPMI) liquid culture medium was prepared by supplementing the medium with 10% fetal bovine serum (FBS) and 50 µL of antibiotics per 50 mL of medium. Cisplatin was prepared as a positive control. The test samples were prepared at various concentrations of 1000, 500, 250, 125, 62.50, 31.25, 15.63, and 7.81 µg mL^{-1}. Dimethyl sulfoxide (DMSO) was used as the solvent, with a final concentration below 5% to ensure non-toxicity to the cells. The working solution of the antiproliferation assay reagent, PrestoBlue™ Cell Viability Reagent, was prepared according to the manufacturer's instructions.

Cells Preparation

Cells with a minimum confluency of 70% were used for the experiment. The culture medium was removed, and the cells were washed twice with 1 mL of phosphate-buffered saline (PBS). Subsequently, 1 mL of Trypsin–EDTA solution was added and incubated for 5 min to detach the cells, as confirmed by observing floating cells under an inverted microscope. The detached cells were transferred into a tube containing complete medium to inactivate trypsin. The suspension was centrifuged at 3000 rpm for 5 min, the supernatant was discarded, and the cell pellet was resuspended in fresh medium.

Seeding of Cells into a 96-Well Plate

Cell number and viability were determined using the trypan blue exclusion method. The cell suspension was adjusted to a final density of 170,000 cells mL^{-1}, corresponding to 17,000 cells per well. For viability assessment, 10 µL of trypan blue solution was prepared in a sterile microtube, and 10 µL of the cell suspension was added and mixed gently. A hemocytometer and cover slip were cleaned with 70% ethanol and allowed to dry. Then, 10 µL of the cell–trypan blue mixture was carefully loaded into the hemocytometer chamber. Viable (unstained) cells were counted to determine the number of living cells per mL. The calculated cell suspension was seeded into 96-well plates and incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 24 h or until approximately 70% confluency was reached.

Cell Treatment with Samples, Positive Controls, and Negative Controls

Eight sterile 1.5 mL microtubes were prepared and labeled according to the required concentrations. Stock solutions of the samples were diluted into eight different concentrations using complete medium as the solvent. The 96-well plates containing adherent cells were removed from the incubator, and the medium in each well was carefully discarded. The plate layout was labeled to distinguish between sample-treated wells, positive control wells (cisplatin), and negative control wells. Using a micropipette, 100 µL of each prepared sample concentration and the cisplatin control were added to the designated wells. The plates were then incubated for an additional 48 h under standard culture conditions.

PrestoBlue™ Reagent Preparation and Absorbance Measurement

The cell viability assay employed the PrestoBlue™ reagent, which contains resazurin, a non-toxic and cell-permeable redox indicator. In its oxidized form, resazurin is blue and non-fluorescent; however, when reduced by intracellular reductase enzymes in metabolically active

viable cells, it is converted into resorufin, a pink and highly fluorescent compound. Yuliet et al. (2024) reported that this method is more sensitive and rapid than conventional assays because it does not require a cell lysis step and allows real-time monitoring of cell viability. Initial microscopic observations prior to reagent addition showed that the cells exhibited normal morphology, intact structure, and uniform distribution across all wells, indicating healthy growth conditions. After 24 h of incubation, the persistence of the blue coloration suggested that resazurin reduction had occurred, confirming that cellular metabolic activity remained active (Hidayat et al., 2023).

A working solution of PrestoBlue™ Cell Viability Reagent was prepared by mixing 1 mL of PrestoBlue reagent with 9 mL of complete medium (corresponding to 10 µL reagent per 90 µL medium). Subsequently, 100 µL of the prepared mixture was added to each well of the 96-well plate and incubated for 1–2 h at 37 °C until a visible color change occurred. In viable cells, the blue resazurin compound in the PrestoBlue reagent is reduced to pink, highly fluorescent resorufin. Absorbance was measured at 570 nm (resorufin) and 600 nm (resazurin) using a multimode microplate reader.

Calculation of absorbance value at a certain concentration using the following formula.

$$y = (Abs_{570} - Abs_{600}) - X_{media\ cell}$$

where y represents the corrected absorbance value used to construct the linear regression curve at a specific concentration. Abs_{570} and Abs_{600} correspond to the absorbance of the sample measured at wavelengths of 570 nm and 600 nm, respectively. $X_{media\ cell}$ represents the mean absorbance of the media control (blank). The corrected absorbance values were subsequently used to determine the percentage of cell viability and to calculate the IC_{50} value.

Calculation of IC_{50} value for anticancer activity

The IC_{50} value for anticancer activity was determined as the concentration required to reduce cell viability to 50% relative to untreated control cells, based on corrected absorbance measurements. A linear regression equation was generated from the relationship between sample concentration and corrected absorbance, expressed as follows.

$$y = ax + b$$

where y represents half of the mean corrected absorbance of the media plus solvent control, while x corresponds to the IC_{50} concentration value. The IC_{50} was obtained by substituting the calculated y value into the regression equation and solving for x.

Data analysis

The data obtained were analyzed quantitatively by calculating the percentage of expressed cells using ImageJ software (Java version 1.8.0_40, 64-bit). Observations were conducted in one field of view with three replications for each treatment. Statistical analysis of the IC_{50} values was performed using SPSS version 21.

RESULT AND DISCUSSION

Extract and Percentage Yield of *C. latifolia* Leaf

The preparation of *C. latifolia* leaf extract using the maceration method with ethanol as the solvent was carried out to obtain a concentrated ethanol extract for subsequent anticancer testing. The percentage yield of the extract is presented in Table 1. From 600 g of dried simplicia, 154.12 g of thick extract was obtained, corresponding to a yield of 25.69%.

Table 1. Weight of thick extract and percentage yield of ethanol extract of C. latifolia leaf.

Simplicia Weight (gram)	Thick Extract Weight (gram)	Yield percentage (%)
600	154.12	25.69%

The use of ethanol as a solvent is considered effective for extracting bioactive compounds. According to Mladenovic et al. (2018), a higher yield percentage generally correlates with a greater quantity of extracted bioactive constituents, indicating improved extract quality. The yield value of 25.69% is classified as high, as it exceeds the minimum standard of 10% established in the Indonesian Herbal Pharmacopoeia (2017). Rosawanti and Arfianto (2023) also reported that thick extract yields below 10% may indicate low bioactive content and reduced potential as a natural medicinal source. Furthermore, Fauziyah et al. (2022), stated that a high yield reflects a substantial concentration of bioactive compounds within the extract.

The extraction method employed was cold maceration, selected primarily to prevent degradation of heat-sensitive or thermolabile active compounds. Rosita et al. (2019) explained that cold maceration aims to produce a more natural extract while preserving the quality and structural integrity of the bioactive constituents present in herbal materials. The process was conducted at room temperature with a 24-hour soaking period and repeated three times. Asworo and Widwiastuti (2023) noted that cold maceration generally requires a longer extraction time compared to heat-assisted methods, depending on the characteristics of the raw material and the intended extraction objective. According to Bitwell et al. (2023), the principle of maceration involves the diffusion of active compounds from solid plant material into a liquid solvent during soaking, where the solvent dissolves the target compounds, which are

subsequently separated by filtration. The duration of maceration is adjusted according to the nature of the plant matrix and the physicochemical properties of the compounds being extracted.

The selection of ethanol as the extraction solvent was based on its amphiphilic nature, possessing both polar (hydroxyl, –OH) and nonpolar (methyl, –CH_3) functional groups, which enable the extraction of compounds across a broad polarity range. Simamora et al. (2021) reported that this dual characteristic allows ethanol to interact with both polar and moderately nonpolar constituents. During the extraction process, ethanol penetrates the plant cell wall, diffuses into the intracellular space, and dissolves bioactive compounds present in the cytoplasm and vacuoles. Adisti et al. (2023) highlighted that the effectiveness of ethanol is attributed to its high polarity, which facilitates rapid solubilization of target compounds. The concentration gradient between the intracellular and extracellular environments drives diffusion, resulting in the migration of dissolved compounds into the solvent until equilibrium is achieved. Furthermore, Rudiana et al. (2021) explained that ethanol effectively extracts bioactive constituents such as saponins, steroids, and flavonoids due to its distinctive solvent properties.

The efficiency of the extraction process is influenced by several factors, including the type of solvent, temperature, extraction time, particle size of the raw material, and the ratio of material to solvent Fajri & Daru (2022) reported that longer extraction times generally yield extracts with higher concentrations of dissolved compounds. In addition, particle size plays a crucial role, as smaller particles provide a larger surface area for solvent contact, thereby accelerating mass transfer and enhancing extraction efficiency. Collectively, these factors determine the overall effectiveness of the extraction process and the quality of the resulting extract.

TLC analysis of the extract and fraction of *Curculigo latifolia* leaf

The fractionation process was carried out using the liquid–liquid extraction method, resulting in three fractions: n-hexane, ethyl acetate, and ethanol–water. These fractions were subsequently subjected to TLC analysis to identify the presence of secondary metabolite compounds in the *C. latifolia* leaf extract. Thin-layer chromatography (TLC) is a planar analytical technique that separates compounds based on differences in their affinity for the stationary phase (silica gel) and the mobile phase (organic solvent), primarily influenced by variations in compound polarity (Prasetyawan et al., 2024). The TLC profiles of the three fractions of *C. latifolia* leaves exhibited distinct spot patterns after application of the locating reagent, confirming the presence of phenolic compounds, terpenoids, flavonoids, and tannins (Table 2). These spot patterns not only indicate the presence of specific compound groups but also provide information regarding the degree of separation and relative purity of each fraction. This observation supports the validity of TLC as a rapid and effective preliminary screening method for identifying bioactive compounds (Putri et al., 2024).

Table 2. TLC Results of Compound Profiles in *C. latifolia* Leaf Extract and Fractions.

Fraction	Color	Compound	Rf value
Extract	Yellow	Phenol	0,84
	Purple	Terpenoid	0,76
	Purple	Terpenoid	0,66
	Yellow	Flavonoid	0,30
n-Hexane	Yellow	Phenol	0,94
	Purple	Terpenoid	0,8
	Purple	Terpenoid	0,58
	Brown	Tannin	0,44
	Yellow	Flavonoid	0,2
Ethyl Acetate	Purple	Terpenoid	0,86
	Purple	Terpenoid	0,72
	Yellow	Flavonoid	0,3
	Brown	Tannin	0,18
Ethanol-water	Brown	Tannin	0,12

Based on the fractionation results of *Curculigo latifolia* leaf extract using a series of solvents with increasing polarity, namely nonpolar n-hexane, semipolar ethyl acetate, and polar methanol–water, several groups of potential bioactive compounds were successfully identified through distinct Rf values observed in each fraction. Noviyanty et al. (2024) explained that fractionation is a method used to separate concentrated extracts according to polarity differences using two immiscible solvents. Sugiarti et al. (2020) further stated that multistage fractionation is typically conducted sequentially from nonpolar to polar solvents to achieve effective separation of compounds based on their polarity characteristics. The Rf values obtained from thin-layer chromatography are specific for each compound within a given eluent system and can serve as preliminary

identification markers. Similar Rf values suggest comparable physicochemical characteristics, whereas differences in Rf values indicate the presence of distinct compounds or variations in their properties (Pratiwi et al., 2023; Sunu, 2018).

Four groups of compounds were detected in the n-hexane fraction, namely phenols, terpenoids, tannins, and flavonoids, with the highest Rf value observed for phenolic compounds (Rf = 0.94). This relatively high Rf value suggests that the detected phenolic compounds are less polar, possibly consisting of phenolic derivatives with fewer hydroxyl groups or containing lipophilic substituents, resulting in greater affinity toward the nonpolar mobile phase. Indarto (2015) reported that although phenolic compounds are generally soluble in polar solvents, certain phenolic derivatives, such as those from the stilbenoid group, may also dissolve in semi-polar to nonpolar solvents. In the ethyl acetate fraction, the highest Rf value was observed for terpenoid compounds (Rf = 0.86), while the methanol–water fraction contained only tannins with an Rf value of 0.12.

The presence of flavonoids and terpenoids in both the n-hexane and ethyl acetate fractions indicates that these compound classes exhibit a broad polarity range and structural diversity. Apmarja et al. (2025) explained that flavonoids are commonly enriched in semi-polar fractions such as ethyl acetate due to their intermediate polarity, whereas terpenoids are generally more soluble in nonpolar solvents such as n-hexane, although their distribution may vary depending on plant species. In the ethyl acetate fraction, terpenoids appeared as two distinct spots with Rf values of 0.86 and 0.72, suggesting the presence of multiple terpenoid compounds with different molecular weights or polarity characteristics. Fitriani et al. (2022) confirmed that ethyl acetate fractions are often dominated by terpenoids because semi-polar solvents effectively extract secondary metabolites such as monoterpenoids and steroids.

The methanol–water fraction contained only tannins with a relatively low Rf value (0.12), indicating strong interaction with the polar silica gel stationary phase. This low Rf value is consistent with the highly polar nature of tannins, which are large polyphenolic compounds rich in hydroxyl groups and therefore exhibit limited mobility in TLC systems employing organic mobile phases. Noviyanty et al. (2024) stated that tannins are polar polyphenols that dissolve readily in polar solvents such as methanol and water. The use of a methanol–water mixture increases solvent polarity and enhances tannin extraction efficiency depending on the plant matrix. Overall, these fractionation results demonstrate the chemical complexity of secondary metabolites present in *C. latifolia* leaves, highlighting their potential as a source of bioactive compounds.

The variation in Rf values among the fractions reflects the effectiveness of multistage fractionation in separating compounds based on polarity differences. The n-hexane fraction predominantly contained nonpolar to semipolar compounds, the ethyl acetate fraction was enriched with semipolar compounds, and the methanol–water fraction contained polar constituents. These findings indicate that sequential solvent fractionation is an effective preliminary strategy for separating and concentrating target compounds prior to further purification. The diversity of detected bioactive compounds, particularly flavonoids, phenols, and terpenoids, supports the potential of *C. latifolia* leaves as a source of pharmacologically active substances with antioxidant, anti-inflammatory, antimicrobial, and cytotoxic properties. Gusungi et al. (2020) reported that flavonoids, alkaloids, and triterpenoids exhibit anticancer activity by inhibiting cell proliferation and activating apoptotic pathways.

LC-MS analysis of the extract and fraction of Curculigo latifolia *leaf*

Analysis by Liquid Chromatography-Mass Spectrometry (LCMS) of *marasi* leaf extract successfully detected 15 compound peaks with varying molecular masses (m/z), indicating the chemical complexity of the secondary metabolites contained therein (Figure 1). Not all peaks in the chromatogram could be identified. This occurred because some peaks showed a fit conf value below 60%. Additionally, there were several peaks that could not be identified as natural compounds based on the database used. Therefore, the compounds selected for further analysis were those with a fit conf value above 80%. The selected compounds also align with previous studies, as they have been reported previously. The results of the LC-MS chromatogram interpretation of the *marasi* leaf ethanol extract are shown in Table 3.

The peak at a retention time of 1.41 minutes shows a measured mass (120.0609 m/z), appearing at a retention time of 1.41 with the molecular formula C_8H_8O and a fit conf of 97.19%. This compound has the highest fit conf value, indicating a very high level of agreement with the database. Based on the ChemSpider database, the molecular formula C_8H_8O corresponds to the compound acetophenone. This compound belongs to the phenolic group and is commonly found in various plants as a secondary metabolite. Acetophenone and its derivatives have been reported in several plant genera, such as *Paeonia, Apocynum*, and *Acorus*. Acetophenone also exhibits antioxidant, anticancer, analgesic, antidiabetic, cardioprotective, and neuroprotective activities (Ahmadpourmir et al., 2024). Therefore, this compound is recommended as a tentative compound present in the ethanol extract of marasi leaves.

The second tentative compound was detected at a retention time of 5.87 minutes with an area percentage of 3.35% and a measured mass (196.1108 m/z). This compound is predicted to be a liliolide with the molecular formula $C_{11}H_{16}O_3$ and a fit conf value of 80.24%. This value exhibits a high degree of similarity to

liliolide compounds in the HMDB database. This compound has been reported to be present in n-hexane and ethyl acetate extracts of *C. latifolia* leaves as a terpenoid or lactone derivative (Nur et al., 2023). Liliolide has been reported to exhibit cytotoxic activity against HepG2 cells, a human liver cancer cell line, thereby demonstrating potential as a natural anticancer agent. Additionally, this compound has been reported to possess neuroprotective and anti-inflammatory activities (Silva et al., 2021).

The peak at a retention time of 9.06 minutes with a measured mass (180.1146 m/z) and a molecular formula of $C_{11}H_{16}O_2$ is predicted to be the compound dihydroactinidiolide. This compound has a fit conf value of 47.24%, indicating that its match with the HMDB database is still relatively low. However, it is still recommended as a tentative compound due to its similarity in chemical characteristics and biosynthetic relationship to the liliolide compound that appeared at a retention time of 5.87 minutes with a high fit conf value exceeding 80%. The dihydroactinidiolide compound possesses various biological activities, particularly as a neuroprotective agent, an antibacterial agent, and a flavoring agent in black tea and tobacco (Das et al., 2018).

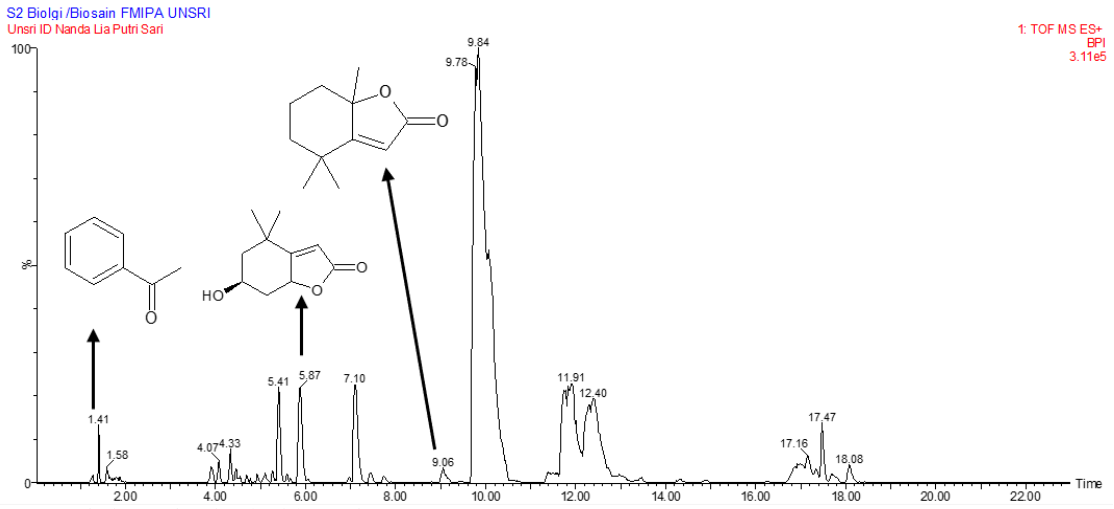

Figure 1. LC–MS Analysis Results of *C. latifolia* Leaf Extract.

Table 3. LC–MS Analysis Results of Compounds Identified in *C. latifolia* Leaf.

No.	Retention time	% Area	Measured Mass (m/z)	Molecular Formula	Tentative Identification	Fit Conf (%)	Reported Biological Activity
1.	1.41	0.75	120.0609	C_8H_8O	Asetofenon	97.19	Antioxidant, anticancer, analgesic, antidiabetic, cardioprotective, and neuroprotective activities
2	5.87	3.35	196.1108	$C_{11}H_{16}O_3$	Liliolide	80.24	Anti-inflammatory, anticancer, and neuroprotective activities
3	9.06	0.56	180.1146	$C_{11}H_{16}O_2$	Dihydroactinidiolide	47.24	Neuroprotective, antibacterial

Anticancer Activity Test

Cancer Cell Count Results

The anticancer activity of *C. latifolia* leaf ethanol extract and its fractions were evaluated using the MTT assay method. IC_{50} values for each treatment were calculated, and statistical analysis was performed using SPSS version 21. Based on the MTT assay results, the number of viable cancer cells after treatment is presented in Table 4. The cytotoxic activity of the extract and fractions against MCF-7 cells is also summarized in Table 4. The results demonstrated varying cytotoxic response patterns among the different fractions of C. latifolia leaf against MCF-7 cells.

In the crude extract fraction, the percentage of viable cells showed an inconsistent pattern. At the highest concentration (250 ppm), cell viability dropped to 39.82%, indicating an inhibitory effect. However, at intermediate concentrations (175–62.5 ppm), it exceeded 100%, reaching 109.95% at 31.25 ppm. This indicates that the crude extract likely contains a mixture of compounds with different or opposing biological activities. At certain concentrations, they exhibited proliferative or metabolic stimulation effects on MCF-7 cells, while toxic effects only emerged at higher concentrations.

The ethanol–water fraction exhibited very weak cytotoxic activity, with cell viability remaining relatively stable between 74.99% and 103.88%. This fraction likely contains polar compounds, such as glycosides, which may be less effective in inhibiting breast cancer cell proliferation or may contribute to maintaining cell viability. The absence of a clear dose–response relationship indicates that the active compounds may not be efficiently extracted into polar solvents or are present at concentrations insufficient to produce significant cytotoxic effects. Similarly, the ethyl acetate fraction showed cell viability values ranging from 91.18% to 100.24%, without marked cytotoxic activity. Although this fraction contains semipolar compounds such as flavonoid aglycones or terpenoids, which are commonly associated with anticancer properties, the results suggest that these constituents may not be sufficiently potent against MCF-7 cells or are present in suboptimal proportions to induce apoptosis.

The most notable findings were observed in the n-hexane fraction. At higher concentrations (250–125 ppm), cell viability increased markedly to 158.35%, exceeding the control. This response indicates that the n-hexane fraction may contain lipophilic compounds that act as mitogenic or proliferative agents in MCF-7 cells. Nonpolar constituents such as steroids, lipids, or essential oil components may interact with cellular membranes or lipophilic receptors, thereby promoting cell proliferation rather than inducing cell death. At lower concentrations (62.5–7.8125 ppm), cell viability remained close to or slightly above the control but generally above 90%, suggesting that the stimulatory effect is more pronounced at higher concentrations.

Table 4. Results of Cancer Cell Viability After Treatment with C. latifolia Leaf Extract and Fractions.

Extract and fraction	Concentration								
	3.9062	**7.8125**	**15.625**	**31.25**	**62.5**	**125**	**175**	**200**	**250**
Extract	98.09	87.72	99.49	109.95	105.74	101.86	101.38	90.48	39.82
n-Hexane	130.52	133.82	135.46	131.92	114.66	102.69	87.03	75.61	44.94
Ethyl Acetate	100.24	94.06	92.67	97.56	99.86	91.18	100.19	96.98	96.98
Ethanol-water	103.88	91.66	97.46	95.88	88.21	74.99	84.14	81.65	85.05

MCF-7 breast cancer cells were selected as the test model in this study based on several important considerations. MCF-7 cells represent one of the most widely used estrogen receptor-positive (ER+) breast cancer cell lines, originally derived from human breast adenocarcinoma tissue isolated in 1970 from a 69-year-old patient (COMŞA et al., 2015). These cells are estrogen-dependent and express estrogen receptor alpha (ERα) and progesterone receptors (PR), making them a standard model in hormone therapy studies. The cell line was established through in vitro culture and immortalization of primary tumor cells, resulting in a stable and reproducible model widely utilized in laboratory research. Cultivation of MCF-7 cells is typically performed in medium supplemented with fetal bovine serum (FBS) and, when necessary, estrogen to maintain cell proliferation and viability (Yuliet et al., 2024).

Misgiati et al. (2024) reported that the MCF-7 cell line has been extensively characterized and widely utilized in oncology research due to its positive estrogen receptor expression, allowing investigation of hormone-related mechanisms in breast cancer. In addition, MCF-7 cells exhibit stable and reproducible proliferative characteristics, making them suitable for cytotoxicity evaluation. Complete DMEM medium was used as the negative control because it provides all essential nutrients required for optimal cell growth, including amino acids, vitamins, inorganic salts, glucose, and serum-derived growth factors. Rahim et al. (2023) stated that under such optimal culture conditions, any observed decrease in cell viability in treated groups can be attributed specifically to the cytotoxic effects of the tested fractions rather than to nutrient deficiency or suboptimal growth conditions. Previous studies evaluating other plant extracts against MCF-7 cells have demonstrated notable cytotoxic activity. For example, extracts of soursop (*Annona muricata*) exhibited significant antiproliferative effects, with the ethyl acetate fraction showing the highest potency, reflected by an LC_{50} value of 2.86 µg mL^{-1}. The observed antiproliferative activity was associated with apoptosis induction, characterized by decreased expression of the anti-apoptotic protein Bcl-2 and increased activation of caspase-9 and caspase-3 (Hadisaputri et al., 2021).

Determination of IC_{50}

Following a series of anticancer assays of *C. latifolia* leaf extract on MCF-7 breast cancer cells, the IC_{50} value was determined. The IC_{50} represents the concentration of a compound or extract required to inhibit 50% of target cell growth, calculated from the dose–response curve. A lower IC_{50} value indicates greater potency, as a smaller concentration is needed to achieve half-maximal

inhibition. In preliminary anticancer screening, IC_{50} serves as a standard parameter to compare compound effectiveness, determine appropriate dose ranges for further testing, and evaluate selectivity toward cancer cells relative to normal cells. The IC_{50} values obtained for *C. latifolia* extract and fractions are presented in Table 5.

The IC_{50} value of 234.8782 ppm obtained for *C. latifolia* leaf extract in this study is classified as weak, as compounds with IC_{50} values above 100 ppm are generally considered to have low anticancer activity. In comparison, established chemotherapeutic agents such as doxorubicin exhibit IC_{50} values in the nanomolar to micromolar range against MCF-7 cells. The relatively high IC_{50} value observed indicates that the extract was not effective in inhibiting MCF-7 cell proliferation within the tested concentration range, suggesting limited potential for development as a single-agent therapy for estrogenic receptor-positive breast cancer.

However, these findings do not entirely exclude other possible therapeutic benefits of *C. latifolia* leaf extract. The extract may demonstrate synergistic effects when combined with other agents, exhibit activity against different cancer cell types, or possess non-cytotoxic properties such as anti-inflammatory or immunomodulatory effects. Further investigations are warranted, including optimization of extraction methods, advanced fractionation, structural modification of active constituents, and detailed molecular mechanism studies to better elucidate its interaction with MCF-7 cells.

Table 5. IC_{50} value of extract and fraction of *C. latifolia* Leaf against MCF-7 breast cancer cell.

Extract and fraction	IC_{50}	Cytotoxic Activity
Extract	234.8782	Weak
n-Hexane	237,7029	Weak
Ethyl Acetate	284,3578	Weak
Ethanol-water	864,6694	Very weak

CONCLUSION

C. latifolia leaf extract and its fractions contain various secondary metabolites, including phenolic compounds, terpenoids, flavonoids, and tannins as identified by TLC analysis, while LC–MS revealed the predominance of nitrogen-containing heterocyclic compounds, including triazine derivatives, tetrazoles, and benzothiazoles. Cytotoxic evaluation using the PrestoBlue assay demonstrated no significant reduction in MCF-7 cell viability within 24 h, and observations at 48 h showed a consistently high percentage of viable cells, in some cases exceeding the control, particularly in the n-hexane fraction, which exhibited a proliferative tendency at higher concentrations. The IC_{50} value of 234.8782 ppm indicates weak cytotoxic potency, exceeding the commonly accepted activity threshold of 100 ppm, suggesting that the extract is not sufficiently effective as a single anticancer agent against estrogen receptor-positive MCF-7 breast cancer cells. Nevertheless, further research involving other cancer models and isolation of active constituents is required to better understand and potentially enhance the biological activity of C. latifolia leaf.

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