In vitro study and phytochemical profile of *Laportea decumana (Roxb.) Wedd* leaf extract

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ABSTRACT

Breast cancer ranks as the most commonly identified cancer in women on a global scale, with chemotherapy often limited by adverse effects and multidrug resistance. This has increased interest in medicinal plants as sources of bioactive compounds with improved safety profiles. Laportea decumana (Roxb.) Wedd, a traditional medicinal plant from Eastern Indonesia, has shown antioxidant and cytotoxic properties, yet its in vitro cellular effects and detailed phytochemical composition remain incompletely understood. GC–MS analysis was conducted on ethanol and n-hexane extracts of L. decumana leaves. In vitro cell viability and preliminary cellular response activity were evaluated on MCF-7 breast cancer cells using the MTT assay, and IC_{50} values were calculated. GC–MS analysis revealed that both extracts were dominated by cyclotrisiloxane, hexamethyl-, along with bioactive compounds such as phytol, neophytadiene, and n-hexadecanoic acid. Both extracts associated with moderate decreases in MCF-7 cell viability, with IC_{50} values of 175 µg/mL (ethanol extract) and 179.42 µg/mL (n-hexane extract), suggesting measurable in vitro cytotoxic activity within the tested concentration range. Laportea decumana leaf extracts exhibit a diverse phytochemical profile and demonstrate moderate in vitro growth-inhibitory effects against MCF-7 cells, supporting their potential as sources of bioactive compounds for preliminary in vitro evaluation rather than definitive therapeutic application.

Keywords: GC–MS, *in vitro* study, *Laportea decumana*, MCF-7 cells, phytochemical profile

INTRODUCTION

Breast cancer is the most commonly diagnosed malignancy among women globally and remains a critical challenge for global public health. According to estimates from the World Health Organization, approximately 2,088,849 breast cancer cases were reported, accounting for 24.2% of all malignancies affecting women, with an associated global mortality rate of 6.6%. In Indonesia, breast cancer imposes the greatest cancer burden, with 58,256 documented cases (30.9%) (WHO, 2019). Conventional breast cancer management relies on a combination of surgery, hormone therapy, radiotherapy, and chemotherapy (Ramli, 2017). From a nursing perspective, care is largely supportive and includes patient education, counseling, symptom management, and palliative interventions (Chan *et al*., 2020). Despite advances in treatment, chemotherapy is frequently associated with substantial adverse effects, such as nausea, vomiting, dysgeusia, weight loss, peripheral neuropathy, and alopecia (Ambarwati and Wardani, 2014). Moreover, the emergence of multidrug resistance (MDR) significantly reduces therapeutic effectiveness and limits long-term treatment outcomes (Bukowski *et al*., 2020). These challenges have stimulated growing

interest in safer, more tolerable therapeutic alternatives and adjunctive strategies.

Numerous medicinal plants have been evaluated for their anticancer potential, including *Sterculia quadrifida bark* (Rollando and Alfanaar, 2017), soursop leaves (*Annona muricata*) (Fatmawati *et al*., 2018), *Strobilanthes crispus* (Roring *et al*., 2017), and *Moringa oleifera* leaves (Susilowati and Anggraini, 2018). *Laportea decumana (Roxb.) Wedd,* locally known in Indonesia as daun gatal, is a member of the Urticaceae family that grows abundantly in Eastern Indonesia, particularly in Ambon, Maluku. In traditional practice, this plant has been used externally to reduce muscle pain and fatigue (Simaremare *et al.*, 2019). The plant is characterized by stinging trichomes containing formic acid and histamine, while its leaves are reported to contain various secondary metabolites, including glycosides, alkaloids, and steroidal/triterpenoid compounds (Simaremare, 2014; Thalib *et al.*, 2021).

Previous investigations have demonstrated that *Laportea decumana* leaf extracts possess strong antioxidant activity, pro-oxidant behavior under specific conditions, and cytotoxic effects against cancer cell lines (Cepeda *et al*., 2021; Simaremare *et al*., 2020; Thalib et al., 2022). Specific triterpenoids and steroidal constituents, such as stigmasterol, are known to regulate cancer-related signaling pathways through Akt/mTOR pathway inhibition, ROS induction, disruption of mitochondrial membrane potential, and reduced expression of anti-apoptotic proteins (Ameli *et al*., 2022; Bae *et al.*, 2020; Zhao *et al.*, 2021). Notably, *Laportea decumana* leaves have been confirmed to contain stigmasterol and hydroxylated C28 steroid compounds, further supporting their biological relevance (Basy *et al.*, 2022; Rollando *et al*., 2022). The MCF-7 breast cancer cell line is widely used in *in vitro* studies owing to its expression of estrogen receptors, epithelial characteristics, and relative resistance to certain chemotherapeutic agents, making it suitable for evaluating cellular responses and safety profiles (Shammout *et al*., 2021).

Based on the existing evidence, although *Laportea decumana* has shown promising antioxidant and cytotoxic potential, there remains a critical gap in studies that systematically correlate its GC–MS-based phytochemical profile with quantitative in vitro cellular responses. To our knowledge, no previous study has concurrently integrated detailed GC–MS compound identification of ethanol and n-hexane extracts with MTT-based cellular evaluation on MCF-7 cells to provide a compound-oriented interpretation of cellular effects. Therefore, the novelty of this study lies in the integrative approach that combines comprehensive GC–MS phytochemical profiling with in vitro cellular viability assessment, enabling a more mechanistic interpretation of how specific detected constituents may contribute to observed biological responses. This study seeks to evaluate the cellular safety of *Laportea decumana* leaf extract through in vitro assays while concurrently characterizing its phytochemical profile using GC–MS.

MATERIALS AND METHODS

The extraction of *Laportea decumana* (Roxb.) Wedd leaves was conducted at the Phytopharmaceutical Laboratory, Faculty of Pharmacy, Research Activity Center, Hasanuddin University, Makassar, Indonesia. The évaluation of its chemopreventive activity against MCF-7 breast cancer cells *in vitro* was performed at the HUM-RC Laboratory, 6th Floor, Hasanuddin University Teaching Hospital. The study was carried out from August 2021 to June 2022.

Preparation of cell culture medium

The complete culture medium was prepared by adding 10 mL of fetal bovine serum, 1 mL of penicillin–streptomycin, and 1 mL of amphotericin B to 100 mL of DMEM. All procedures were conducted aseptically inside a Class II Biological Safety Cabinet (BSC). The prepared medium was labeled with the medium name, preparation date, and preparer's name, then stored at 4°C. Prior to use, the medium was incubated until it reached room temperature.

Revival of frozen MCF-7 cells

Cryopreserved MCF-7 cells maintained at −80°C were allowed to thaw at ambient temperature. The recovered cells were introduced into 3 mL of culture medium in a sterile conical tube and gently washed. After centrifugation at 1,000 rpm for 5 min, the supernatant was discarded, and the cell pellet was re-suspended in 1 mL of fresh medium, transferred to a culture flask, and incubated at 37 °C with 5% CO_2 for 24 h. Culture medium renewal was performed every 24 hours until the cells reached approximately 80% confluence.

Cell harvesting

Cells were harvested once they reached maximum growth (approximately 80% confluency), indicated by the formation of a monolayer at the base of the culture flask. The harvesting process began by discarding the culture medium, followed by two washes with PBS to remove residual medium and debris. Adherent cells were subsequently detached using 500 µL of 0.25% trypsin–EDTA for 3 minutes. Trypsinization was stopped with 3 mL of culture medium, after which the cells were gently mixed, centrifuged at 1,000 rpm for 5 min, and resuspended in 900 µL of medium supplemented with 100 µL of DMSO. Cell density was determined using a hemocytometer, and the cells were either immediately processed for cytotoxicity assays or preserved for subsequent analyses.

Preparation of test solutions

Ten milligrams of extract were dissolved in 500 µL DMSO and mixed with an equal volume of culture medium to prepare a 500 µg/mL stock solution, which was then serially diluted to final concentrations of 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/mL using the $M_1V_1 = M_2V_2$ equation. An identical procedure was applied to the n-hexane fraction, with serial dilutions prepared to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/mL. A concentrated 5-fluorouracil (5-FU) stock solution of 50,000 µg/mL was diluted to generate a 500 µg/mL working solution, which was further subjected to stepwise serial dilutions to achieve final test concentrations of 300, 150, 75, 37.5, 18.75, 9.75, and 3.6875 µg/mL. MCF-7 cells were seeded at ~1 × 10^4 cells/well in 96-well plates and allowed to attach for 24 h, followed by treatment with 0.5 mg/mL MTT for 4 h. Formazan crystals were dissolved in 10% SDS, absorbance was measured at 620 nm, and IC_{50} values were determined (Figure 1).

Active compound analysis using GC-MS

The extract (≈100 mg) was dissolved in 5 mL of chloroform in a 10 mL volumetric flask, sonicated for 5 minutes, diluted to volume with chloroform, and injected (1 µL) into the GC–MS instrument. *Laportea decumana* extracts were analyzed by GC–MS on an Agilent 7890A with a DB-5MS column, using an oven program of 50–325 °C at 5 °C/min (held 5 min), and compounds were identified via the NIST library.

Statistical design

The experimental results were presented descriptively without formal statistical analysis, focusing on observed cellular responses and assay outcomes.

RESULTS AND DISCUSSION

Phytochemical profile of *Laportea decumana* (Roxb.) Wedd leaf extracts

GC–MS analysis of the ethanol extract of *Laportea decumana* (EE-LDrW) identified a total of nine compounds (Table 1). Of these, one compound remained unidentified, while two compounds were detected at trace levels (<1% relative abundance). Therefore, only six major compounds were selected for further discussion, namely cyclotrisiloxane, hexamethyl-; 1,2-bis(trimethylsilyl)benzene; neophytadiene; phytol; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; and n-hexadecanoic acid, arranged in decreasing order of abundance (Table 1). Notably, the unidentified fraction (peak no. 9) comprised 28.76% of the total chromatographic area (Table 1). This fraction corresponds to the cumulative area of several minor constituents that were not individually identified, either due to their low relative abundance or the absence of reliable matches in the mass spectral library, suggesting the

presence of minor constituents below detection or library-matching thresholds. Several identified constituents, including phytol, neophytadiene, and *n*-hexadecanoic acid, have been reported to possess antioxidant, antimicrobial, and cytotoxic activities, supporting their potential contribution to the biological effects observed in this study (Ghante and Jamkhande, 2019; Ozyurt *et al.*, 2011)**.**

GC–MS profiling of the n-hexane extract (ENH-LDrW) revealed seven major compounds: cyclotrisiloxane, hexamethyl-; 1,4-bis(trimethylsilyl)benzene; 1-heptatriacotanol; 9,19-cyclolanostan-3-ol, acetate (3β); and 1-[3,3-dimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclopentyl]-2-hydroxy-ethanone (Table 2). Only 8.24% of the total composition remained unidentified, indicating a relatively well-resolved phytochemical profile compared with the ethanol extract. The detection of cyclolanostane-derived compounds supports the presence of triterpenoid-related structures, which are widely reported to modulate membrane integrity, oxidative balance, and cell proliferation pathways (Bishayee *et al*., 2011; Petronellia *et al.*, 2009).

The qualitative phytochemical assessment confirmed alkaloids, flavonoids, tannins, triterpenoids, and saponins as constituents of *Laportea decumana* leaf extracts (Tables 1 and Table 2). This profile partially differs from the findings of Simaremare (2014), who reported alkaloids, glycosides, and triterpenoid/steroid compounds but did not detect flavonoids, tannins, or saponins. These differences may be attributed to variations in analytical techniques—thin-layer chromatography in the present study versus test-tube assays in previous research—as well as differences in geographical origin of plant material (Ambon–Maluku versus Papua), which are known to influence secondary metabolite biosynthesis (Astuti *et al.*, 2014).

Although earlier studies reported the presence of stigmasterol and hydroxylated C28 steroid compounds in *L. decumana* (Basy *et al.*, 2022; Rollando *et al*., 2022), these compounds were not detected in the present GC–MS analysis. Instead, cyclotrisiloxane, hexamethyl- emerged as the dominant compound in both extracts. Variability in phytochemical composition may reflect differences in plant age, environmental exposure, soil composition, and climatic conditions (Astuti *et al*., 2014). Cyclotrisiloxane, hexamethyl- has previously been reported to exhibit antimicrobial, antioxidant, and antiplasmodial activities (Kosasih *et al.*, 2020; Krishna *et al.*, 2015). However, its biological activity is context-dependent and influenced by exposure duration and concentration.

In vitro cellular safety and cytotoxic activity on MCF-7 cells

Once phytochemical profiling was conducted, the term “cellular safety” in this study refers specifically to a preliminary *in vitro* assessment of cell viability following short-term exposure to the extracts, and does not imply clinical, *in vivo*, or therapeutic safety. Cellular safety in MCF-7 breast cancer cells was evaluated using the MTT assay. The MTT assay measures mitochondrial metabolic activity through the reduction of tetrazolium salt to formazan by viable cells, with absorbance values directly proportional to cell viability (Mosmann, 1983).

Both ethanol (EE-LDrW) and n-hexane (ENH-LDrW) extracts demonstrated measurable cytotoxic effects against MCF-7 cells (Table 3). The ethanol extract showed the highest activity, with an IC_{50} value of 175 µg/mL (R^2 = 0.951), indicating a strong and consistent concentration–response relationship. The n-hexane extract exhibited a comparable IC_{50} of 179.42 µg/mL (R^2 = 0.844). In contrast, the reference chemotherapeutic agent 5-fluorouracil (5-FU) displayed a higher IC_{50} value of 361.20 µg/mL (R^2 = 0.218), reflecting lower potency under the same experimental conditions.

The morphological changes of MCF-7 cells following treatment are presented in Figure 1. The control group (EE-LDrW) exhibited relatively intact cell morphology with dense cell populations and prominent formazan crystal formation (indicated by red

arrows), reflecting active mitochondrial metabolism. In contrast, cells treated with ENH-LDrW showed noticeable morphological alterations, including reduced cell density and signs of cell shrinkage. More pronounced cytotoxic effects were observed in the 5-FU-treated group, where extensive cell damage and decreased cell populations were evident. The reduced presence of formazan crystals in treated groups further supports the decline in cell viability, consistent with the MTT assay results (Table 3). These observations confirm that morphological alterations correlate with the cytotoxic effects measured quantitatively.

According to Paul and Ramasubbu (2017), crude plant extracts exhibiting IC_{50} values below 1000 µg/mL are considered biologically active, indicating that both extracts fall within a relevant cytotoxic range. Previous studies reported that n-hexane and ethyl acetate fractions of *L. decumana* exhibited higher toxicity, whereas the ethanol fraction showed limited activity (Simaremare *et al.*, 2020). In contrast, this study demonstrates that slightly higher cytotoxicity in the ethanol extract. This variation could result from differences in extraction methods, solvent polarity, compound interactions, and the plant's growth environment. For instance, coastal and sun-exposed areas like Ambon can enhance phenolic and terpenoid accumulation (Astuti *et al.*, 2014).

Methodological differences may also contribute to divergent findings. Earlier studies employed the Brine Shrimp Lethality Test (BSLT) to determine LC_{50} values, whereas the present study directly assessed IC_{50} values using the MTT assay on human cancer cells. These assays differ fundamentally in biological relevance and sensitivity (Masud *et al*., 2022)

Interestingly, a dose-dependent effect was observed, as indicated by the changes in cell viability across different concentrations (Table 3) indicated a non-monotonic pattern, in which lower concentrations of *L. decumana* extracts induced greater reductions in cell viability, whereas higher concentrations resulted in diminished cytotoxic effects. This behavior contrasts with the classical dose-dependent cytotoxicity observed for 5-FU and may reflect the complex chemical composition of crude plant extracts. At higher concentrations, certain constituents may exert cytoprotective or antagonistic effects, masking pro-apoptotic activity. Similar non-linear dose–response patterns have been reported in phytochemical and toxicological studies (Masud *et al*., 2022).

Cyclotrisiloxane, hexamethyl- was the most abundant compound detected in both extracts. While it has been reported to possess antimicrobial and antioxidant activities (Kosasih *et al.*, 2020; Krishna *et al*., 2015), prolonged exposure has also been associated with interference in DNA repair pathways involving BRCA1 and BRCA2 (Farasani and Darbre, 2017). In the present study, short-term exposure (≤72 h) at IC_{50} concentrations resulted in moderate inhibition of MCF-7 cell viability (15.39%), suggesting limited cytotoxic effects under controlled in vitro conditions.

The high levels of phytol, neophytadiene, and n-hexadecanoic acid in the ethanol extract likely underlie EE-LDrW's stronger antioxidant, antimicrobial, and cytotoxic activities relative to ENH-LDrW (Ghante and Jamkhande, 2019; Salvador *et al.,* 2017). Collectively, *Laportea decumana* leaf extracts show a rich phytochemical profile and display moderate in vitro growth-suppressing activity against MCF-7 breast cancer cells. Importantly, the IC_{50} values reported here should be interpreted only as an initial indicator of *in vitro* cellular response and preliminary cytotoxic selectivity, rather than as evidence of confirmed safety or anticancer efficacy *in vivo*.

CONCLUSION

This study demonstrates that *Laportea decumana* (Roxb.) Wedd leaf extracts possess a diverse phytochemical composition and exhibit measurable biological effects under *in vitro* conditions. GC–MS analysis revealed that both ethanol and n-hexane extracts are dominated by cyclotrisiloxane, hexamethyl-, along with several bioactive compounds such as phytol, neophytadiene, and n-hexadecanoic acid, which are known to contribute to

antioxidant and cellular growth-modulating effects. *In vitro* assessment using the MTT assay revealed that both extracts produced quantifiable decreases in MCF-7 breast cancer cell viability, yielding IC_{50} values that fall within the biologically relevant range for crude plant extracts. Further studies involving compound isolation, mechanistic investigations, and evaluation on non-cancerous cell lines are warranted to better define safety margins and clarify their potential biological relevance beyond preliminary laboratory observations.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

List of abbreviations

EE-LDrW: Ethanol Extract- Laportea decumana (Robx) wedd; **ENH-LDrW**: Extract N-hexane-Laportea decumana (Roxb.) Wedd; **5FU**: 5-Fluorouracil; **DMEM**: Dulbecco's Modified Eagle Medium; **BSC**: Biological Safety Cabinet; **MCF-7** : Michigan Cancer Foundation-7; **EDTA**: Ethylene diaminetetraacetic Acid; **DMSO**: Dimethyl Sulfoxide; **GC–MS**: Gas Chromatography–Mass Spectrometry; **MTT**: 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; **BRCA1**: Breast Cancer Gene 1; **BRCA2**: Breast Cancer Gene 2.

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Table 1: Chemical composition of *Laportea decumana* (Robx) wedd ethanol extract (EE-LDrW) by qualitative GC-MS method

S/N	RT (min)	Phytochemical Profile	T.P. (%)
1	29.44	trans-Arbusculone	0.27
2	30.70	Neophytadiene	9.32
3	31.58	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.6
4	33.41	n-Hexadecanoic acid	2.03
5	35.74	9-Octadecyne	0.26
6	36.05	Phytol	4.96
7	47.26	1,2-Bis(trimethylsilyl)benzene	25.46
8	50.61	Cyclotrisiloxane, hexamethyl	26.34
9	-	u.i	28.76
Total			100

RT = Retention time, T.P = Total persentage berdasarkan % sum area, u.i = unidentified compound

Table 2: Chemical composition of Laportea decumana (Robx) wedd extract n-hexan extract (ENH-LDrW) by qualitative GC-MS method

S/N	RT (min)	Phytochemical Profile	T.P. (%)
1	4.147	(S)-(+)-1-Cyclohexylethylamine	0.31
2	8.66	1-Butanamine, N-butyl	0.06
3	30.70	Neophytadiene	0.47
4	30.83	2-Piperidinone, N-[4-bromo-n-butyl]	0.05
5	31.21	trans-Z-.alpha.-Bisabolene epoxide	0.13
6	31.58	9-Eicosyne	0.18
7	32.53	Hexadecanoic acid, methyl ester	0.12
8	33.37	7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin	0.28
9	33.87	Hexadecanoic acid, ethyl ester	0.26
10	35.74	trans-Z-.alpha.-Bisabolene epoxide	0.15
11	35.86	1-[3,3-Dimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclopentyl]-2-hydroxy-ethanone	2.39
12	36.05	cis-Z-.alpha.-Bisabolene epoxide	0.31
13	36.96	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol	0.78
14	37.09	1-Heptatriacotanol	8
15	43.24	4-(2,2-Dimethyl-6-methylenecyclohexylidene)-3-methylbutan-2-one	0.5
16	44.08	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)	4.63
17	47.07	2H3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.	0.21
18	47.28	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	1.23
19	47.82	1,4-Bis(trimethylsilyl)benzene	25.06
20	49.36	1,2-Bis(trimethylsilyl)benzene	1.74
21	52.70	Cyclotrisiloxane, hexamethyl-	44.9
22	-	u.i	8.24
Total			100

RT = Retention time, T.P = Total persentage berdasarkan % sum area, u.i = unidentified compound

Table 3: Cytotoxic assay extract of Laportea decumana (Robx) wedd (EE-LDrW) in breast cancer cells MCF-7 in vitro

Sample	Concentration (µg/mL)	Average Absorb	% Viability	IC_{50} (µg/mL)
Ethanol Extract Laportea decumana (Robx) wedd (EE-LDrW)	7.81	0.3927	62.26	175*
	15.6	0.3647	57.06	
	31.25	0.3755	59.06	
	62.5	0.3907	61.88	
	125	0.4018	63.94	
	250	0.4254	68.31	
	500	0.5061	83.26	
Ekstrak n-heksan Laportea decumana (Robx) wedd (ENH-LDrW)	7.81	0.4431	71.6	179.42*
	15.6	0.3979	63.21	
	31.25	0.3851	60.84	
	62.5	0.3899	61.74	
	125	0.4021	63.99	
	250	0.4783	78.11	
	500	0.5721	95.51	
5FU	4.875	0.3343	75.56	361.20
	9.75	0.3343	79.01	
	18.75	0.3639	73.69	
	37.5	0.3716	67.17	
	75	0.3584	64.92	
	150	0.3457	61.84	
	300	0.325	57.01	

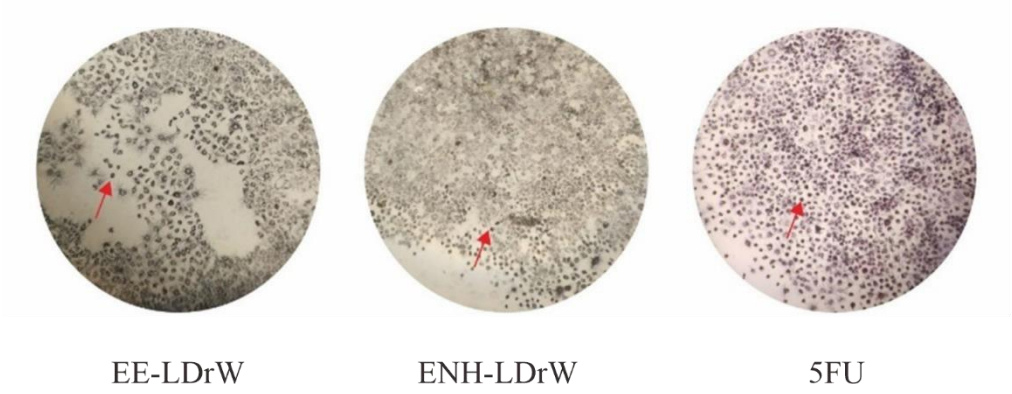

Figure 1: Morphology of MCF-7 cells after MTT treatment (Red mark indicates the presence of crystal formations)

EE-LDrW (Ethanol Extract- Laportea decumana (Robx) wedd), ENH-LDrW (Extract N-hexane-Laportea decumana (Roxb.) Wedd.), 5FU (5-Fluorouracil)