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Original Research

Anti-tumor activity of plant extracts against human breast cancer cells are different in monolayer and three-dimensional cell culture screening models: A comparison on 34 extracts

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ABSTRACT

Introduction: The monolayer cell culture model is a popular model for screening anti-tumor activity of plant extracts. However, almost the extracts selected for screening in this model have failed in subsequent animal models. Therefore, there is only about 5 % of candidates from the original thousands of drugs that are screened which ultimately reach clinical trial. This study aimed to compare the differences in anti-tumor activity of 34 plant extracts against breast cancer cells in 2 models of monolayer cell culture (2D) and in three-dimensional (3D) cell culture. **Methods**: Four breast cancer cell lines (MCF-7, $CD44^+CD24^-$ MCF-7, VN9, and $CD44^+CD24^-$VN9) were used to generate the 2D and 3D models (the 3D model was developed by culturing breast cancer cells in matrigel). The extracts were got from the plant extract library that prepared in the previous study. The anti-tumor activity was evaluated via half inhibitory concentrations(IC_{50} values). **Results**: Of the 34 extracts, E12, E7, E5 and E6 of them had an effect on MCF-7, $CD44^+CD24^-$MCF-7, VN9 and $CD44^+CD24^-$VN9 cells, respectively. The results indicated 10 potentially strong candidates for future drug development targeting hypoxic areas in breast cancer. **Conclusion**: The 3D culture model exhibited higher resistance to extracts than the 2D culture model. The $CD44^+CD24^-$cell population of both VN9 and MCF-7 cell lines showed higher drug resistance than the original cell lines (VN9 and MCF-7).

Key words: breast cancer, drug screening, natural extract, CD44+CD24-

INTRODUCTION

For the discovery of new drugs, screening of natural compounds that target the proliferation of cancer cells is important[1]. For libraries with hundreds to thousands of extracts, they need to be screened with high-performance screening methods. Such methods allow the screening of many compounds at different concentrations at the same time on each target cell or the combination of compounds- with uniformity and high accuracy[2,3].

Screening of extracts on cancer cell models in 2-dimensional (2D) monolayer culture is limited because the monolayer model lacks the tumor cell characteristics of physiological tumors in the body[4]. Meanwhile, screening done on a cancer model in 3-dimensional (3D) culture may be better for studying drug effects since the 3D culture model is more similar to the *in vivo* animal models (and possibly clinical trials); the 3D model more closely reflects characteristics of *in vivo* tumors, such as differentiation, tumor microenvironment, and distribution of hypoxia in certain populations[5–7]. Many methods have been developed to create 3D cells like tumors in the body; these methods include use of U-shaped bottom well , the hanging drop, and cell growth in bio-matrix[6,8]. The method of using a U-shaped bottom well is heavily used in 3D cell model studies. However, one downside is that not all cell types can develop into 3D cell mass by this method[9]. For hanging drop culture, the advantage is that gravity is used to precipitate the cells together and thereby stimulate the cells to stick together into 3D spheres[10]. This method has a disadvantage of using very gentle manipulations and is difficult to develop if screened at high throughput automation. Meanwhile, the method of using biological substrates (like Matrigel) offers great potential for the development of 3D cell model[11–13]. Matrigel is usually stored in frozen form, at a concentration of 10-15 mg/mL; it is thawed at 4 °C and gelated in a temperature range of 24-37 °C for 30 minutes. Matrigel promotes the differentiation of different cell lines (*e.g.* prostate, salivary gland, mammary epithelium, pancreas, Schwann cells, intestinal cells, and bone cells), of primary cell lines (*e.g.* sertoli cells, blood cells, cartilage cells, epithelial cells, endometrial cells, and fallopian epithelial cells), and even tissue explants (*e.g.*

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neural crest, immature follicles, and zygote) [14].

This study used matrigel to create a 3D cell model of breast cancer for the purpose of screening natural compounds that inhibit the growth of breast cancer cell . Modeling of 2D and 3D monolayer cancer cells was carried out in parallel (simultaneously) with the same evaluation agents, including Alarma Blue. The IC_{50} (half maximal inhibitory concentration) values were compared between 2D and 3D cancer cell models to evaluate and select the extract which showed different effects in these two models.

This study used 34 natural plant extracts and two control drugs (Doxorubicin and Tiparazamine) on 4 cell lines (MCF-7, $CD44^+CD24^-$ MCF-7, VN9, and $CD44^+CD24^-$ VN9 cells).

METHODS

Cell lines

MCF-7 cell line was obtained from ATCC (Manassas, VA). VN9 cell line was obtained from the Stem Cell Institute, University of Science, VNU-HCM. MCF-7 and VN9 cells were cultured in DMEM/F12 (Sigma-Aldrich, St Louis, MO), 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO), 1% antibiotic-antimycotic (Sigma-Aldrich, St Louis, MO). The $CD44^+CD24^-$ cells were sorted from VN9 cells (and termed $CD44^+CD24^-$VN9) or from MCF-7 (and termed $CD44^+CD24^-$MCF-7) by magnetic-activated cell sorting (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany), and then expanded in M171 medium (Thermo Fisher Scientific, Waltham, MA) with MEGS Suplement (Thermo Fisher Scientific, Waltham, MA) for maintenance of stemness. The $CD44^+CD24^-$populations corresponded to the cancer stem cell (CSC) populations.

Chemicals

In the research study, the library of the 34 extract (**Table 1**), which were coded with '**E**' as the initial label (*i.e.* E1-E34), were obtained from the Division of Medicinal Chemistry, Faculty of Chemistry, University of Science, Vietnam National University Ho Chi Minh City, Vietnam. Doxorubicin hydrochloride and tirapazamine were purchased from Sigma-Aldrich.

Cell culture in monolayer (2D) and three-dimensional (3D) culture

For 2D models, single cells (MCF-7, $CD44^+CD24^-$MCF-7, VN9 or $CD44^+CD24^-$VN-9) were harvested and seeded in 96-well plates at a final density of 1000 cells per well, and grown for 5 days. Fresh medium was replenished every two days. Cancer cells were cultured in DMEM/F12, 10% FBS (Sigma-Aldirch), and 1% antibiotic-antimycotic (Sigma-Aldrich). $CD44^+CD24^-$cancer cells were cultured in M171 medium (Thermo Fisher Scientific) with MEGS supplement (Thermo Fisher Scientific). For the 3D model, 5 μL of 1000 single cells was mixed with 5 μL of matrigel (Sigma-Aldrich) on ice and placed on the edge of the well. The plate was incubated at 37 ^{o}C in 10 minutes for gel polymerization, and then 100 μL of pre-warmed medium was added on top of the gel. The pre-warmed medium was a requisite for manipulation of 3D culture to avoid melting the gel (**Figure 1**).

Cell viability assay and IC_{50} determination

After 5 days of culture, the cells and organoids were treated for 48 hours with the respective 34 extracts at the following concentrations: 31.25 μg/ml, 62.5 μg/ml, 125 μg/ml, 250 μg/ml, 500 μg/ml, or 1000 μg/ml. The concentrations of doxorubicin evaluated were: 62.5 nM, 125 nM, 250 nM, 500 nM, and 2000 nM; the concentrations of tirapazamine evaluated were: 15.625 μM, 31.25 μM, 62.5 μM, 125 μM, 250 μM, and 500 μM. Then, Alarma Blue (Sigma-Aldrich) was added to the wells at a final concentration of 10 μg/mL and incubated in the dark for 1 hour. The fluorescence intensity was read using an DTX880 system (Beckman Coulter, Brea, CA) at excitation wavelength of 535 nm, emission wavelength of 595 nm, and integration time of 500 μs. The data were normalized to control values (untreated wells) and IC_{50} values were calculated with GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA).

Statistical analysis

All experiments were performed in triplicate. Statistical significance was set at $P<0.05$. Data were analyzed by GraphPad Prism 7.

RESULTS

IC_{50} values of extracts are different on MCF-7 2D and 3D models

The IC_{50} results of doxorubicin and tirapazamine showed that both 2D and 3D models were successfully established for anti-tumor activity evaluation (**Table 2**). The IC_{50} results of the 34 plant extracts on MCF-7 breast cancer cells in 2D and 3D models are summarized in **Table 3**.

There were 12/34 extracts which showed effects on both 2D and 3D culture models. These 12 extracts were: E4, E10, E11, E12, E17, E18, E20, E21, E22, E27,

Table 1: List of 34 natural extracts used in this study

Code of extract	Plant (solvent)	Code of extract	Plant (solvent)
E4	*Buchanania Latifolia* – (CH_3OH)	E26	*Anisoptera costata* – (CH_3OH)
E7	*M. Camptosperma* – (CH_3OH)	E27	*Anisoptera costata* – (CH_3OH)
E8	*D. Dyeri* – (CH_3OH)	E28	*Willughbeia cochinchinensis* – (CH_3OH)
E9	*H. recopei* – (CH_3OH)	E30	*Streblus ilicifolius* – (CH_3OH)
E10	*H. recopei* – (CH_3OH)	E31	*B. pandurate* – (CH_3OH)
E11	*S. thorelii* – (CH_3OH)	E32	*Paramignya trimera* – (CH_3OH)
E12	*S. thorelii* – (CH_3OH)	E35	*Mangifera mekongiensis* – (CH_3OH)
E13	*D. turbinatus* – (CH_3OH)	E36	*Embelia ribes* – (CH_3OH)
E14	*D. turbinatus* – (CH_3OH)	E37	*Willughbeia cochinchinensis* – ($C_4H_8O_2$)
E15	*D. costatus* – (CH_3OH)	E38	*Artocarpus heterophyllus* – ($C_4H_8O_2$)
E16	*D. costatus* –(CH_3OH)	E39	*Mangifera mekongiensis* – ($C_4H_8O_2$)
E17	*Hopea odorata* – (CH_3OH)	E40	*Taxus wallichiana* – (CH_2Cl_2)
E19	*Vatica odorata* – (CH_3OH)	E41	*Caesalpinia sappan* – (CH_2Cl_2)
E20	*Vatica odorata* – (CH_3OH)	E42	*Trigona minor* – (Hexan)
E21	*Dipterocarpus alatus* – (CH_3OH)	E43	*B. pandurate* - (Chloroform)
E22	*Shorea roxburghii* – (CH_3OH)	E45	*Swintonia floribunda* – (CH_3OH)
E25	*K. laurifolia* – (CH_3OH)	E46	*Mangifera reba Pierre 1897* – (CH_3OH)

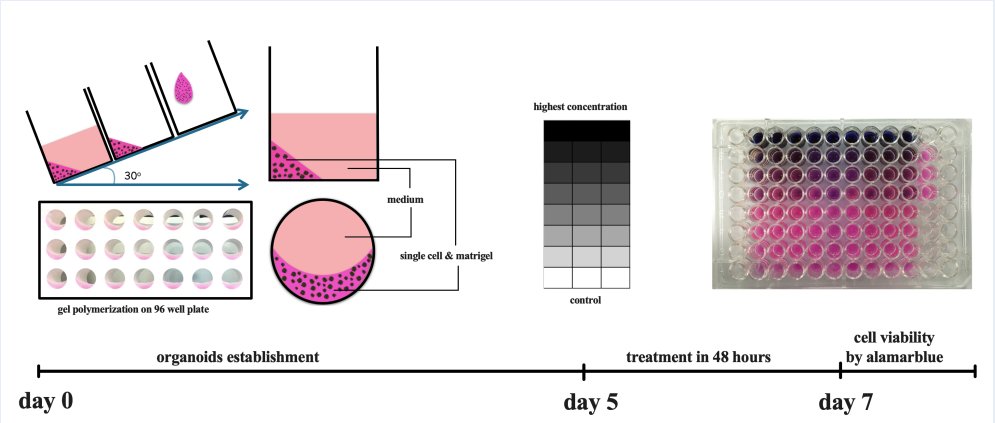

Figure 1: The 3D cell culture method using matrigel. The matrigel and the cells wered seed with density of 1000 cells/well. The matrigel was established on the edge of the well after 30 mins in 37 ^oC which has crescent shape. After 5 days in progress, the drug testing was process in 48 hours.

Table 2: The IC_{50} of doxorubicin and tirapazamin on cell lines

Cell lines	Models	IC_{50} DOX (ng/mL)	IC_{50} TPZ (μg/mL)
VN9	2D	1476	292
	3D	1868	128
$CD44^+CD24^-$ VN9	2D	98.52	315.2
	3D	1711	105.4
MCF-7	2D	1674	159.4
	3D	2354	68.14
$CD44^+CD24^-$ MCF-7	2D	278.3	174.9
	3D	3131	147

Abbreviations: **DOX**: doxorubicin, **TPZ**: tirapazamin, IC_{50}: half inhibitory concentration

Table 3: The IC_{50} values of 34 extracts on MCF-7 breast cancer cell line

Extracts	IC_{50} values (μg/mL)		Extracts	IC_{50} values (μg/mL)	
	2D model	3D model		2D model	3D model
E4	187.5	383.7	E25	597.4	870.8
E7	248.2	332.8	E27	165.3	242.5
E8	478.7	533.1	E28	299.7	673.3
E9	701.4	653.5	E30	1476	794
E10	310	154.7	E31	257.3	308.5
E11	342.9	198.3	E32	235.2	225.1
E12	303.5	160.4	E35	4450	615.9
E13	1779	1061	E36	2187	575.5
E14	348.6	593	E37	326.1	308.8
E15	1106	639.8	E38	368.1	692.2
E16	316.8	361.9	E39	345.6	270.6
E17	159.4	232.4	E40	**70**	1419
E18	112.4	230	E41	526.2	2063
E19	489.5	621.8	E42	499.7	359.6
E20	**86.42**	168.4	E43	306	620.4
E21	**57.67**	71.97	E45	155	361.9
E22	**83.58**	87.92	E46	135.4	387.6

Table 4: The IC_{50} values of 34 extracts on $CD44^+CD24^-$ MCF-7 breast cancer cell line

Extracts	IC_{50} values (μg/mL)		Extracts	IC_{50} values (μg/mL)	
	2D model	3D model		2D model	3D model
E4	**66.2**	360.3	E25	173.6	587.8
E7	**69.42**	307.7	E27	**80.45**	214.1
E8	103.2	937.8	E28	134.9	481.6
E9	153	887.8	E30	508.4	935.9
E10	**50.48**	162.2	EE31	**85.04**	253.5
E11	**58.14**	217.5	E32	**56.65**	223
E12	**61.95**	162	E35	**73.95**	624.6
E13	262.4	1243	E36	507	1215
E14	**74.52**	375.2	E37	103.5	322.7
E15	258.3	699.7	E38	229.8	980.4
E16	**70.91**	146.4	E39	**62.65**	386.6
E17	**20.31**	**56.97**	E40	274.5	1648
E18	145	**39.89**	E41	303.3	1257
E19	**31.88**	227.4	E42	102.3	293.9
E20	**35.6**	89.56	E43	**60.97**	449.6
E21	**26.71**	252.9	E45	**32.16**	295.5
E22	809.5	110.4	E46	**71.98**	459.6

Abbreviation: IC_{50}: half inhibitory concentration

E45, and E46. However, most of the extracts predominantly had effects on the 2D model. In fact, 27 extracts on the 3D models were correlated with increased resistance by the cancer cells as compared to the resistance on the 2D models. Specifically, there were 7 extracts that had an IC_{50} values in the 3D model which were lower than in the 2D culture model. The 7 extracts were: E10, E12, E15, E30, E35, E36, and E42 (**Figure 2**). Thus, they are potential candidates for further use in the 3D culture model of MCF-7 breast cancer.

The results of hit extracts on $CD44^+CD24^-$ MCF-7 in 2D and 3D models

There were 7/34 extracts that had effects on both 2D and 3D culture models. These 7 extracts were: E7, E10, E12, E17, E18, E19, E21, and E45. However, the majority of the extracts predominantly showed effects on the 2D model (**Table 5**). As seen in **Table 5**, cells grown in the 3D model showed more resistance to the effects of the 32 extracts than the cells grown in the 2D model. In particular, there were 2 extracts which had IC_{50} values in the 3D model that were lower than the values in the 2D model; those 2 extracts were E26 and E22 (**Figure 3**). Therefore, they are potential candidates for further research in the 3D culture model of the MCF-7 breast cancer stem cell (CSC). Comparing with the results on the MCF-7 cell line, it was observed that the $CD44^+CD24^-$ sub-population of MCF-7 cells has a much higher resistance to the same extracts tested.

The results of hit extracts on VN9 cultured in 2D and 3D models

There were 5/34 extracts which showed effects on both 2D and 3D culture models. The 5 extracts were: E4, E7, E20, E21, and E45. However, most of the extracts had predominant effects on the 2D models (**Table 6**). As **Table 6** demonstrates, 29 extracts on the 3D models were correlated with increased resistance by the cancer cells, as compared to their resistance on the 2D models. In particular, 5 extracts had IC_{50} values in the 3D model that were lower than the values in the 2D model. The 5 extracts were: E15, E18, E22, E25 and E30 (**Figure 4**). Therefore, these are potential

Table 5: Summary of hit extracts on each cell types and models

Cells	2D model	3D model	The extracts more sensitive on 3D than 2D
MCF-7	E20, E21, E22, E40	-	E10, E12, E15, E30, E35, E36, E42
$CD44^+CD24^-$ MCF-7	E4, E7, E10, E11, E12, E14, E16, E17, E19, E20, E21, E27, E31, E32, E35, E39, E43, E45, E46	E17, E18, E20	E26, E22
VN9	-	-	E15, E18, E22, E30
$CD44^+CD24^-$ VN9	E4, E7, E10, E11, E12, E14, E16, E17, E19, E20, E21, E31, E32, E35, E39, E45	E7, E21	E18, E22

Table 6: The IC_{50} values of 34 extracts on VN9 breast cancer cell line

Extracts	IC_{50} values (μg/mL)		Extracts	IC_{50} values (μg/mL)	
	2D model	3D model		2D model	3D model
E4	238.9	518.2	E25	4681	722.9
E7	345.6	297	E27	497.8	345.1
E8	1287	588.2	E28	1806	613.5
E9	916.8	535.7	E30	4976	1568
E10	712.6	270.9	E31	293.5	463.9
E11	559.2	686.4	E32	403.6	347
E12	635.9	496.2	E35	5799	3004
E13	7756	2706	E36	7437	3605
E14	531.4	638.3	E37	559.3	977.5
E15	5744	1088	E38	3158	2260
E16	377.7	987.1	E39	697.3	847.6
E17	211	431	E40	267.2	1212
E18	2055	430.6	E41	1083	871.1
E19	357.5	654	E42	2136	963.8
E20	103.8	122.6	E43	247.5	504.1
E21	304.2	146.1	E45	196.1	262
E22	2964	324.1	E46	1080	417.3

Abbreviation: IC_{50}: half inhibitory concentration

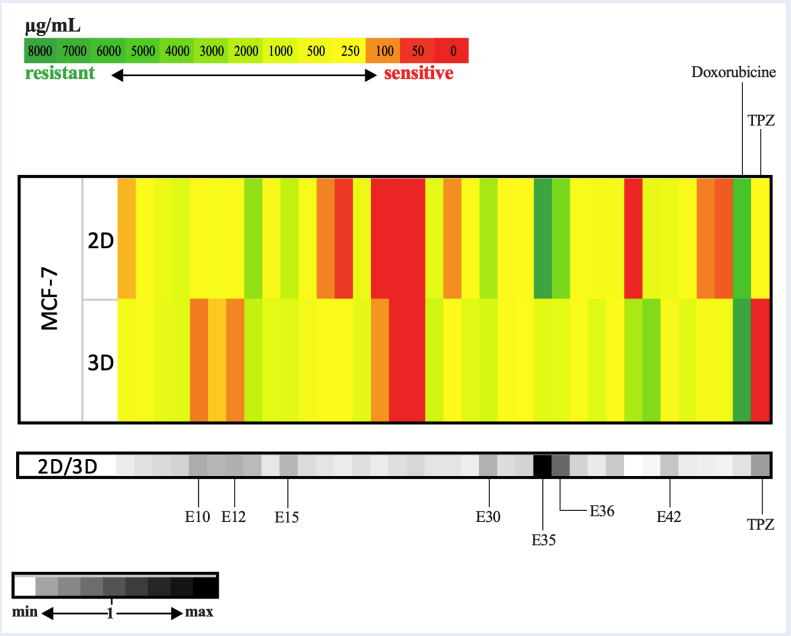

Figure 2: **Comparing the IC_{50} values of 34 extracts, doxorubicin and tirapazamine on MCF-7 breast cancer cell line**. **Scale 1**: Red corresponds to sensitivity, green corresponds to high resistance. **Scale 2**: Black corresponds to, ratio of 2D/3D concentration is greater than 1. Gray white corresponds to, ratio of 2D/3D concentration is less than 1. **Abbreviations**: **TPZ**: tirapazamine, **2D**: mononuclear cell culture, **3D**: three-dimensional cell culture model, **IC_{50}**: half inhibitory concentration

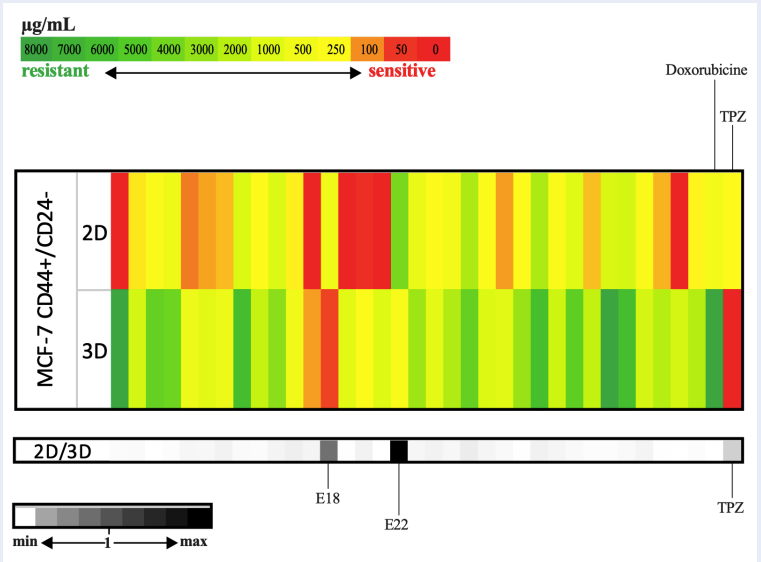

Figure 3: **Comparing the IC_{50} values of 34 extracts, Dox and TPZ on $CD44^{+}CD24^{-}$ MCF-7 breast cancer cell line**. **Scale 1**: Red corresponds to sensitivity, green corresponds to high resistance. **Scale 2**: Black corresponds to ratio of 2D/3D concentration is greater than 1. Gray white corresponds to ratio of 2D/3D concentration is less than 1. **Abbreviations**: **Dox**: doxorubicin, **TPZ**: tirapazamine, **2D**: mononuclear cell culture, **3D**: three-dimensional cell culture model

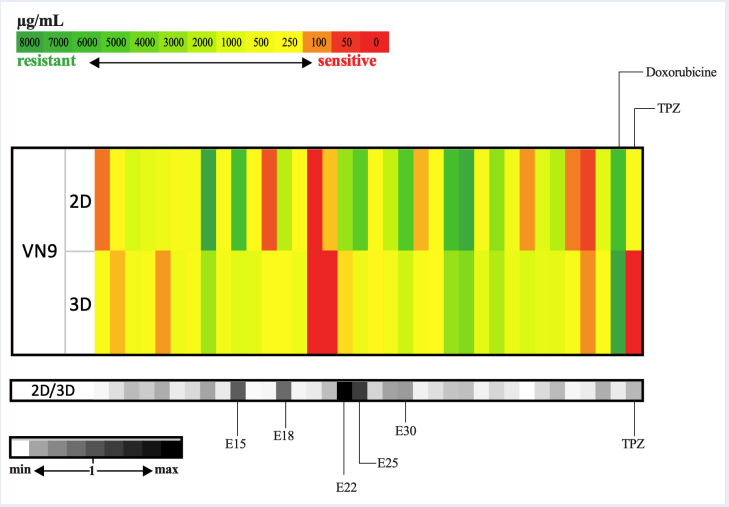

Figure 4: **Comparing the IC_{50} values of 34 extracts, Dox and TPZ on VN9 breast cancer cell line**. **Scale 1**: Red corresponds to sensitivity, green corresponds to high resistance. **Scale 2**: Black corresponds to ratio of 2D/3D concentration is greater than 1. Gray white corresponds to ratio of 2D/3D concentration is less than 1. **Abbreviations**: **Dox**: doxorubicin, **TPZ**: tirapazamine, **2D**: mononuclear cell culture, **3D**: three-dimensional cell culture model

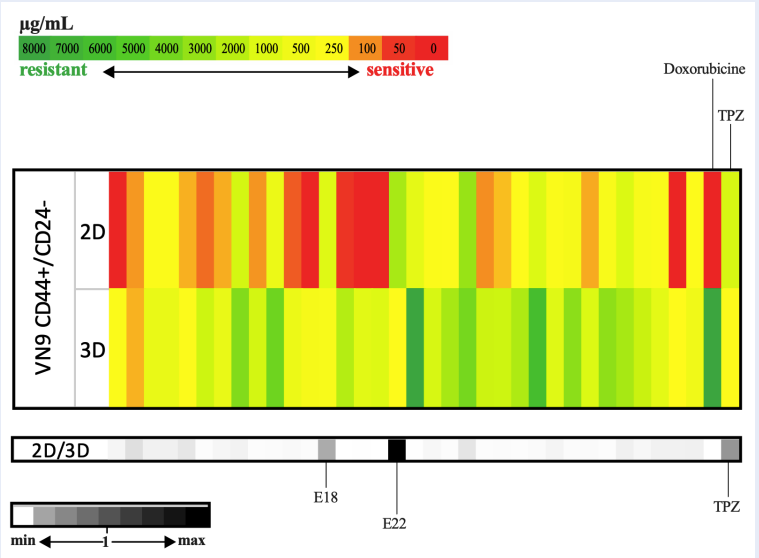

Figure 5: **Comparing the IC_{50} values of 34 extracts, Dox and TPZ on $CD44^{+}CD24^{-}$ VN9 breast cancer cell line**. **Scale 1**: Red corresponds to sensitivity, green corresponds to high resistance. **Scale 2**: Black corresponds to ratio of 2D/3D concentration is greater than 1. Gray white corresponds to ratio of 2D/3D concentration is less than 1. **Abbreviations**: **Dox**: doxorubicin, **TPZ**: tirapazamine, **2D**: mononuclear cell culture, **3D**: three-dimensional cell culture model

candidates for further studies in the 3D culture model of VN9 breast cancer.

Results of hit extracts on CD44$^+$CD24$^-$ VN9 cultured in 2D and 3D

There were 6/34 extracts affected both the 2D and 3D culture models: E4, E7, E10, E12, E18, and E45. Most of the extracts, however, mainly affected the 2D models (**Table 7**). As shown in **Table 7**, 32 extracts on the 3D models were correlated with increased resistance by the cancer cells, as compared to their resistance on the 2D models. In particular, there were 2 extracts which had IC_{50} values in the 3D model that were lower those in the 2D culture model; these extracts were E18 and E22 (**Figure 5**). Thus, they are potential candidates for further studies in the 3D VN9 breast CSC model. Comparison of screening results of VN9 with CD44$^+$CD24$^-$ phenotype versus the original VN9 demonstrated that the CSC cell line (CD44$^+$CD24$^-$ VN9) was more resistant to the extracts in the 3D culture model. Therefore, in this study, the number of extracts tested that showed an effect on this cell line was 2, indicating that VN9 CSC can carry more resistant characteristics than normal cells.

DISCUSSION

The use of bio-matrix substrates (such as matrigel) to create 3D culture models is very convenient for drug screening. Use of a gel forming method- that contains the cells on the side of the culture well in a 96-well plate- facilitates easy manipulation without disrupting the gel structure or limiting cell growth in the form of single layer in the center of the well. This method also allows the creation of a 3D cell mass with a size of 100 μm within 5 days of culture. The drug test is conducted in 48 hours such that the entire drug testing procedure can be summarized in 7 days. In order to minimize errors when comparing 2D and 3D models, we conducted all experiments with both models in parallel. For both 2D and 3D models, the threshold of extracting effect was lower than 200 μg/mL.

A number of published studies have show that 3D breast cell culture better reflect the histological, biological, and molecular features of primary tumors than the same cells cultured using traditional 2D techniques [15]. In a study by Imamura *et al.*, on a 3D breast cancer model, the breast cell mass was found to have the presence of a hypoxic cell population [7]; it is for this reason that the cell mass becomes sensitive to tiparazamine. In our study, we show that 10 extracts have the same effect as tiparazamine on breast cancer cells, and that they might be suitable candidates for hypoxia-targeted drug development (**Table 5**). Furthermore, in their study, Imamura and colleagues also showed that expression of Ki-67 was less in 3D breast cancer cell mass than in 2D, suggesting that the greater G0-dormant subpopulation was responsible for drug resistance in 3D culture.

Many studies have show that the breast cancer cell population with phenotype CD44$^+$CD24$^-$ possesses higher tolerability to chemotherapy, hormone therapy, and radiotherapy [16–21]. Thus, for the drug screening in our study, these 4 breast cancer cell lines were suitable for our evaluations: MCF-7, CD44$^+$CD24$^-$MCF-7, VN9, CD44$^+$CD24$^-$ VN9. Morever, a promising outcome from out study is the identification of 10 extracts which have a more sensitive effect on the 3D culture model than the 2D culture model. These 10 extracts include: E10, E12, E15, E18, E22, E26, E30, E35, E36, and E42. These could be suitable candidates for the next steps towards developing drugs that target the hypoxic region in breast cancer. Therapies targeting cancer cells in areas of hypoxia and studies to discern mechanisms have garnered increase interest for cancer treatment. Hypoxia-related mechanisms such as overexpression of hypoxia-inducible factor (HIF) are also important avenues of research. Inhibiting HIF activity and changing the molecules involved in HIF offer hope for identifying molecular target to inhibit tumor growth or even completely halt growth [22]. HIF-1 also induces an increase in adenosine 2B receptor expression, thereby promoting the enrichment of breast cancer stem cells by activating protein kinase C-δ [23].

Therefore, in the study herein, it was shown that the use of a 3D model of breast cancer cell culture for drug screening reflects a huge difference in drug resistance and drug sensitivity when compared to the 2D culture model. The matrigel 3D culture model is significant for screening compounds related to hypoxia-based therapy for breast cancer.

CONCLUSION

Medium-throughput screening on breast cancer cell models MCF-7, CD44$^+$CD24$^-$ MCF-7, VN9, and CD44$^+$CD24$^-$ VN9, in 2D and 3D culture, with 34 extracts showed that resistance to these extracts occurred when cancer cells were cultured in 3D. Resistance to extracts also manifested in the CD44$^+$CD24$^-$ cell populations (*i.e.* CSC populations). There were 12/34 and 7/34 extracts which affected MCF-7 and CD44 $^+$CD24$^-$ MCF-7 cells, respectively. For the Vietnamese breast cancer cell line

Table 7: The IC_{50} values of 34 extracts on $CD44^+CD24^-$ VN9 breast cancer cell line

Extracts	IC_{50} values (μg/mL)		Extracts	IC_{50} values (μg/mL)	
	2D model	3D model		2D model	3D model
E4	**38.15**	116.8	E25	292.7	2456
E7	69.24	**74.54**	E27	114.4	402.6
E8	112.8	255.3	E28	108.6	793.3
E9	118	249.6	E30	915.5	1218
E10	**74.13**	104	E31	**67.14**	480.1
E11	**59.78**	475	E32	**76.9**	479.4
E12	**73.57**	252.2	E35	**99.37**	738.9
E13	426.4	1107	E36	364.7	1855
E14	**68.61**	442.8	E37	119.1	333.4
E15	236	1296	E38	160.5	1022
E16	**56.12**	229.6	E39	**73.18**	362.4
E17	**27.7**	162.5	E40	137.5	992.6
E18	340.2	144.8	E41	372.8	790.6
E19	**47.05**	685.8	E42	146.6	515
E20	**38.34**	315.4	E43	126.1	301.5
E21	**30.58**	342.5	E45	**39.07**	**90.3**
E22	777.9	**92.74**	E46	**90.46**	193

Abbreviation: IC_{50}: half inhibitory concentration

(VN9), there were 5/34 and 6/34 extracts which affected the VN9 and $CD44^+CD24^-$ VN9 cells, respectively. Overall, our study results indicated 10 potential candidates for future drug development targeting hypoxia in breast cancer.

ABBREVIATIONS

Dox: Doxorubicin
HIF: Hypoxia-Inducible Factor
TPZ: Tirapazamine
VN9: Vietnamse breast cancer cell line #9

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

AUTHOR CONTRIBUTIONS

Nhan Phan designed the project and carried out the experiments. Khuong Pham contributed to feasibility experiments. Mai Nguyen provided the extract. Nhan Phan analyzed the data and wrote the paper with contributions from all authors. Phuc Pham, Kiet Truong and Ngoc Phan suggested the idea, corrected the scientific matters, english wording and review all paper.

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