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Cytotoxic Potential of Rare Plant *Salvia candidissima* subsp. *candidissima* on Breast Cancer Cells

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HIGHLIGHTS

- *Salvia* species have been suggested for their potential anti-cancer effects.
- *Salvia candidissima* Vahl. subsp. *candidissima* suppressed the survival of the breast cancer cells.
- Breast cancer cells nuclei become pyknotic and fragmented after treatment.
- The treatment group had greater levels of M30-antigen level in the MCF-7 cell line.

Abstract: Breast cancer is the leading cause of cancer-related deaths in women throughout the world. Research on natural anti-cancer products from plants has gained traction. *Salvia* L. species and their derivatives are rare in Turkey and have suggested for their potential anti-cancer effects. The aim of this study is to assess the potential cytotoxic/apoptotic activities of methanol extract of *Salvia candidissima* Vahl. subsp. *candidissima* (SCE) on MCF-7 and MDA-MB-231 breast cancer cells. A GCxGC-TOF/MS system and a dual stage commercial thermal desorption injector were used to determine the chemical components of SCE. MTT and ATP viability tests were used to investigate the anti-growth activity. The apoptosis-inducing effect was assessed using a fluorescence staining method. Caspase-cleaved keratin 18 (ccK18, M30-antigen) levels measured by M30-CytoDeath ELISA Kit. The results showed that SCE suppressed the survival of the MCF-7 and MDA-MB-231 breast cancer cells in a dose-dependent manner, based on the findings of both MTT and ATP cell viability tests and pyknotic cell nuclei were observed via fluorescent staining in both cell lines after 48 h of treatment. The treatment group had greater levels of caspase-cleaved keratin 18 in the MCF-7 cells than the untreated group. These results showed that SCE triggers apoptosis, causes cell death in MCF-7 and MDA-MB-231 cell lines. SCE may become promising therapeutic strategy in the treatment of breast cancer with further *in vitro* and *in vivo* studies.

Keywords: *Salvia candidissima* Vahl. subsp. *candidissima*; breast cancer; MCF-7; MDA-MB-231; cytotoxicity.

INTRODUCTION

Cancer is one of the world's leading causes of morbidity and mortality. According to the International Center for Cancer Research's (IARC) Global Cancer Statistics (GLOBOCAN) 2020 database, there were 19.3 million new cancer cases and 10.0 million cancer deaths in 2020 [1]. In 2020, the most frequently diagnosed cancer was lung in men (25.8%), followed by prostate (14.6%) and colorectal cancer (9%). Breast cancer is the main cause of death in women (23.9%), followed by thyroid (10.9%) and colorectal cancer (9.1%). Breast cancer ranks first among the cancer types seen in women and is the most common cause of death after lung cancer [2]. Despite the current treatment options used clinically, survival rates in breast cancer are not yet very satisfactory. In this context, new and effective therapeutic strategies are urgently required.

Medicinal herbs and their extracts are used all over the world to treat a range of diseases, including cancer. Therefore, new anticancer drugs derived from medicinal plants continue to play a critical role in improving the health of the global population [3].

Salvia is the broadest genus in the Lamiaceae plant family, with over 1000 species worldwide [4]. The genus *Salvia* is found in temperate climate zones around the world, including the Mediterranean, tropical Africa, Central Asia, the Pacific Islands, and America. Some regions such as Mexico, Turkey, Iran and Africa may contain more endemic species [5–8]. *Salvia* derives its name from the Latin word "salvare", which means "to heal", reflecting the legendary belief in its "magical" healing abilities for a variety of diseases throughout history [9]. For instance, *Salvia cavaleriei* H.Lév. is useful in carbuncle and traumatic injuries; *Salvia desoleana* Atzei & V.Picci is efficient for gynecological and gastrointestinal diseases [10].

In recent years, with the development of technology, the contents of *Salvia* plants have been determined and some substances such as terpenoids and phenolic acids have been shown in studies to have anti-cancer, antioxidant, antimicrobial and anti-inflammatory activities [11–13]. Tanshionone and salvicine, two more active compounds derived from *Salvia* species, have been studied as anti-cancer therapies [14, 15]. According to research, *Salvia* species limit malignant cell proliferation selectively by inducing apoptosis, necrosis, and cell cycle arrest [16].

In the light of these, the goal of our research is to determine the chemical content of rare *Salvia candidissima* Vahl. subsp. *candidissima* collected from some regions in Turkey and to investigate their cytotoxic activities on human breast cancer cells, MCF-7 and MDA-MB-231.

MATERIAL AND METHODS

Collections and identification of plant materials

Salvia candidissima Vahl. subsp. *candidissima* was gathered in Turkey. B9 Van: Gürpınar district, Kurubaş passage, steppe and meadow, 2198 m a.s.l., 38°22′25″ N, 43°23′33″ E, coll. 13 June 2010, M. Fırat 27611 (VANF), and identified using flora of Turkey books by Mehmet Fırat [17]. Plant samples were dried in a sunless closed area with high air circulation at room temperature, for 10 days in June. The specimens were deposited in the Herbarium of Van Yüzüncü Yıl University’s Faculty of Science, Turkey.

Extraction of plant samples

The entire sample (leave and flower) was powdered, and 15 g of the plant material was extracted using 150 mL of methanol (Merck) in a Soxhlet device for 24 h. The crude extract was concentrated at 40 °C using a rotary evaporator, then lyophilized and stored at -20 °C until used in the other research. All procedures were performed as previously described [18].

Determination of chemical compounds of *Salvia candidissima* subsp. *candidissima*

The content of the leaves and flowers sections of *Salvia candidissima* Vahl. subsp. *candidissima* was assessed using the GCxGC-TOF/MS technique. A GCxGC-TOF/MS system was used in conjunction with a dual stage commercial thermal desorption injector. All chromatographic analysis was performed as previously described [18].

Determination of cytotoxicity

Chemicals and Cell culture

Lyophilized of SCE *(*The mixed of leaves and flowers) was prepared in DMSO (0.05 g/ 0.5 mL) for a stock solution and required concentrations for cell culture were prepared with the medium. In this investigation SCE were applied at dosages ranging from 0.05 to 100 µg/mL. MCF-7 and MDA-MB-231 cells were used in the study. Roswell Park Memorial Institute (RPMI) medium 1640 with L-glutamine (Gibco®; Thermo Fisher Scientific, New York, USA), penicillin G (100 U/mL) and streptomycin (100 µg/mL) (Life Technologies, Canada) were used to culturing the cells. 5% (MCF-7) and 10% (MDA-MB-231) fetal bovine serum (Invitrogen, Paisley, UK) were added in the cells and incubated in 37 °C and 5% CO_2 condition.

The MTT and ATP viability assays

The cells cultured in 96-well microplates ($5x10^3$/well) before being treated with SCE at various concentrations for 48 h. Only the medium and solvent (0.1 percent DMSO as final concentration) were given to the untreated cells. Two independent experiments, each one run in triplicates were performed. MTT viability assay was performed as previously described [19]. In addition, cell viability was determined by the ATP assay as previously described [20]. The ATP assay is more reliable and sensitive than the MTT assay, so it was used to corroborate the results of the MTT assay [19].

Fluorescence imaging for determination of cell death mode

Fluorescent dyes Hoechst 33342, Calcein-AM, and propidium iodide (PI) were used to determine the manner of cell death. Hoechst 33342 is a blue stain that can penetrate through the cells, bind DNA, and stain living or dead (apoptotic or necrotic) cells. When stained with Hoechst 33342 dye, apoptotic cells appear brighter and smaller compared to the healthy cells. Calcein-AM only stains living cells as green. Because PI only passes through damaged membranes, it describes late apoptotic or necrotic cells. MCF-7 and MDA-MB-231 cells were planted at a density of $5x10^3$ cells per well in 100 µL culture medium in a 96-well plate. The cells were then treated (use of IC_{90} doses according to the ATP assay results) for 48 h with SCE. Procedures for staining were carried out as previously reported [18]. Two independent staining's were performed.

Detection of caspase-cleaved cytokeratin 18 (M30-antigen)

M30 monoclonal antibody recognizes the CK18's fragment (M30 antigen) thereby proving the presence of apoptosis in the cells [21]. $5x10^3$ cells were seeded per well of a 96-well plate in 200 µl culture medium in duplicate for this assay. SCE were applied (IC_{90} doses obtained from ATP assay) to the cells for 48 h. A positive control for apoptosis, paclitaxel (3.21 M) was used. Two independent experiments, each one run in triplicates were performed. As previously described, the M30-CytoDeath ELISA (PEVIVA, Sweden) assay was used [22].

Statistical analyses

For the statistical analyses GraphPad Prism 8.0 (Demo Version, GraphPad. San Diego, CA) was used. One-Way ANOVA test was performed to calculate the significance. $p<0.05$, $p<0.01$, $p<0.001$ values were considered statistically significant. Cell viability results were expressed as mean ± SD (standard deviation). IC_{50} and IC_{90} doses are defined as the dose inhibiting 50% and 90% of viability of the cells respectively. It is calculated based on the MTT and ATP assay dose response results calculated with GraphPad program.

RESULTS

Chemical analyses of *Salvia candidissima* subsp. *candidissima* extract (SCE)

The chemical composition of SCE was analyzed using GCxGC-TOF/MS system and the qualitative and quantitative compositions were shown in Table 1. One hundred twenty compounds were detected in SCE. The major components of leaves were caryophyllene oxide (9.61%), eucalyptol (7.98%), sclareol (6.39%), 1-docosene (4.84%), α-terpineol (4.75%) and α-pinene (4.25%). Eucalyptol (6.11%), camphene (5.62%), 2-propan-1-ol (5.38%), verbenone (4.89%) and E-3-caren-2-ol were found to be the major compounds in the flowers. The overall compositions in SCE leaves and flowers are quite different.

Table 1. Total compounds of SCE

Compound[a]	RI[b]	% Area[c]	
		Flower	Leave
Caryophyllene oxide	1582	0,88	9,61
Eucalyptol	1042	6,11	7,68
Sclareol	2346	[d]	6,39
1-Docosene	2195	[d]	4,84
α-Terpineol	1189	3,99	4,75
α-Pinene	939	1,93	4,25
o-Cymene	1020	3,32	3,33
2-Propen-1-ol	1448	5,38	3,26
Hexanoic acid	967	0,91	2,93
Camphene	953	5,62	2,90
(-)-Spathulenol	1577	0,64	1,97
Verbenone	1204	4,89	1,90
beta-Bisabolene epoxide	1509	[d]	1,82
β-Pinene	981	3,87	1,78
Acetic acid	600	[d]	1,68
p-Cymen-8-ol	1179	3,40	1,63
Carveol	1197	1,37	1,49
4-Carene	1069	1,36	1,48
Borneol	1165	2,57	1,46
cis-Pinocarveol	1182	1,86	1,42
2-Caren-10-al	1289	3,33	1,27
Nonanal	1100	0,27	1,25
Octanoic Acid	1167	0,67	1,17
Heptanal	901	0,3	1,15
β-Ionone	1485	[d]	1,15
Nonanoic acid	1267	0,52	1,06
α-Thujenal	1193	[d]	1,03
Limonene	1024	3,21	1,01
γ-Terpinene	1054	0,99	0,93
Eicosane	2000	0,46	0,88
(E)-3-Caren-2-ol	1134	4,69	0,87
Myrtenol	1194	2,03	0,81
Nonadecane, 2-methyl-	1966	[d]	0,81
Heptacosane	2700	[d]	0,78
Heptanoic acid	1083	0,33	0,73
Terpinolen	1086	0,86	0,62
(1R)-(+)-Norinone	1137	1,27	0,56
α-Campholenal	1125	1,01	0,55
Octanal	998	0,3	0,52
trans-Carveol	1217	0,78	0,45
β-Phellandrene	1053	2,95	0,44
β-Eudesmol	1649	0,7	0,43

Cont. Table 1

Hexanal	801	0,25	0,42
4-Methyl-2-propyl-furan	828	0,63	0,41
Pentanoic acid	911	0,08	0,37
2-Octanone	988	0,18	0,37
Camphor	1143	1,72	0,34
Heptadecane	1700	0,21	0,33
Furan, 2-pentyl-	992	0,18	0,32
Cuminal	1239	1,33	0,29
3-Heptanone, 5-methyl-	936	[d]	0,28
trans-Verbenol	1140	2,03	0,26
Sabina ketone	1156	1,01	0,26
3-Pinanone	1173	1,43	0,2
Hexahydrofarnesyl acetone	1921	[d]	0,19
2-Propanone, 1-hydroxy-	672	0,38	0,18
2-Decanone	1190	[d]	0,18
Furfural	828	0,13	0,17
Melilotal	1181	0,64	0,16
Thymol	1289	[d]	0,16
(+)-Carvotanacetone	1246	0,62	0,15
2-Nonanone	1087	0,11	0,13
Hexadecane	1600	0,05	0,13
2-Furanmethanol	866	[d]	0,12
2-Furancarboxaldehyde, 5-methyl-	942	0,84	0,12
Benzaldehyde	952	0,22	0,12
Hexanoic acid, 2-ethyl-	1027	[d]	0,12
2(3H)-Furanone, dihydro-5-propyl-	1130	[d]	0,12
n-Decanoic acid	1364	0,08	0,12
1-Octene	792	[d]	0,11
Phenol	980	[d]	0,11
2(5H)-Furanone	871	0,08	0,1
(-)-cis-Sabinol	1140	0,34	0,1
cis-Z-α-Bisabolene	1506	0,15	0,1
Butyrolactone	1299	0,08	0,09
Butanoic acid	763	[d]	0,08
1-Octen-3-one	973	[d]	0,08
Butyl 3-methylbutanoate	1047	0,05	0,08
2(3H)-Furanone, ethyl-4-hydroxymethyl-	1139	0,15	0,08
Decanal	1204	0,05	0,08
8-Hydroxycarvotanacetone	1309	[d]	0,08
Dibutyl phthalate	1922	0,16	0,08
2-Heptanone	889	[d]	0,07
2,5-Furandione, 3-methyl-	896	0,07	0,07
Isobornyl formate	1235	0,4	0,07

Cont. Table 1

2-Nonenal	1157	[d]	0,06
Pulegone	1233	0,37	0,06
2-Allyl-4-methylphenol	1376	0,09	0,06
α-Farnesene	1500	0,17	0,06
Octadecane, 2-methyl-	1867	[d]	0,06
α-Copaene	1374	0,14	0,05
Propanoic acid	668	0,04	0,04
2(5H)-Furanone, 3-methyl-	983	0,04	0,04
Phenol, 2-methyl-	1053	0,1	0,04
Nonadecane	1900	[d]	0,03
Methyl pyrazine	819	0,06	0,02
Pyrazine, 2,5-dimethyl-	908	[d]	0,02
Diethyl Phthalate	1590	0,02	0,02
Benzeneacetaldehyde	1036	0,08	0,01
Myrtanal	1180	1,29	[d]
2-Carene	1001	0,84	[d]
(-)-Thujone	1102	0,83	[d]
beta-Caryophyllene	1418	0,69	[d]
Camphenilone	1083	0,45	[d]
2,3-Epoxycarane	1113	0,42	[d]
Tricyclene	919	0,25	[d]
Ledol	1602	0,23	[d]
Dihydrocarveol acetate	1306	0,22	[d]
(-)-trans-Pinocarvyl acetate	1298	0,19	[d]
Nerol oxide	1154	0,17	[d]
Ledene oxide-(II)	1890	0,16	[d]
(-)-Myrtenyl acetate	1235	0,14	[d]
Camphenol, 6-	1111	0,1	[d]
2,4-Hexadien-1-ol	912	0,09	[d]
Ethanone, 1-(2-furanyl)-	910	0,07	[d]
5-Hepten-2-one, 6-methyl-	985	0,07	[d]
2-Acetylpyridine	1035	0,06	[d]
Tetradecane	1400	0,06	[d]
2-Methoxy-4-vinylphenol	1313	0,04	[d]
2,3-Butanediol	785	0,03	[d]
Unknown		6,05	6,72

(a) According to the GCxGC-TOF/MS software; designations based on NIST mass spectral library and comparison of their Kovats retention indices. (b) For column Rxi 5ms, Kovats retention indices of each component were gathered from the literature. (c) The percentage of each component is calculated as peak area of the analyte divided by peak area of the total ion chromatogram times 100. (d) Not detected.

Anti-growth activity of *Salvia candidissima* subsp. *candidissima* extract (SCE)

The MTT test was used to determine the anti-growth activity of SCE (0.05-100 µg/mL, 48 h) on breast cancer cell lines (MCF-7 and MDA-MB-231), which was subsequently confirmed by a more sensitive ATP viability assay (Figure 1). In both types of cells, the ATP level was significantly reduced after SCE treatments in a dose-dependent manner ($p<0.05$) (Figure 1b). MTT and ATP assay findings were used to obtain the IC_{50} and IC_{90} values of SCE (Table 2). According to the ATP assay results, IC_{50} values for SCE were calculated 6.86 µg/mL and 5.20 µg/mL; IC_{90} values for 37.74 µg/mL and 47.41 µg/mL for MCF-7 and MDA-MB-231 cell lines, respectively.

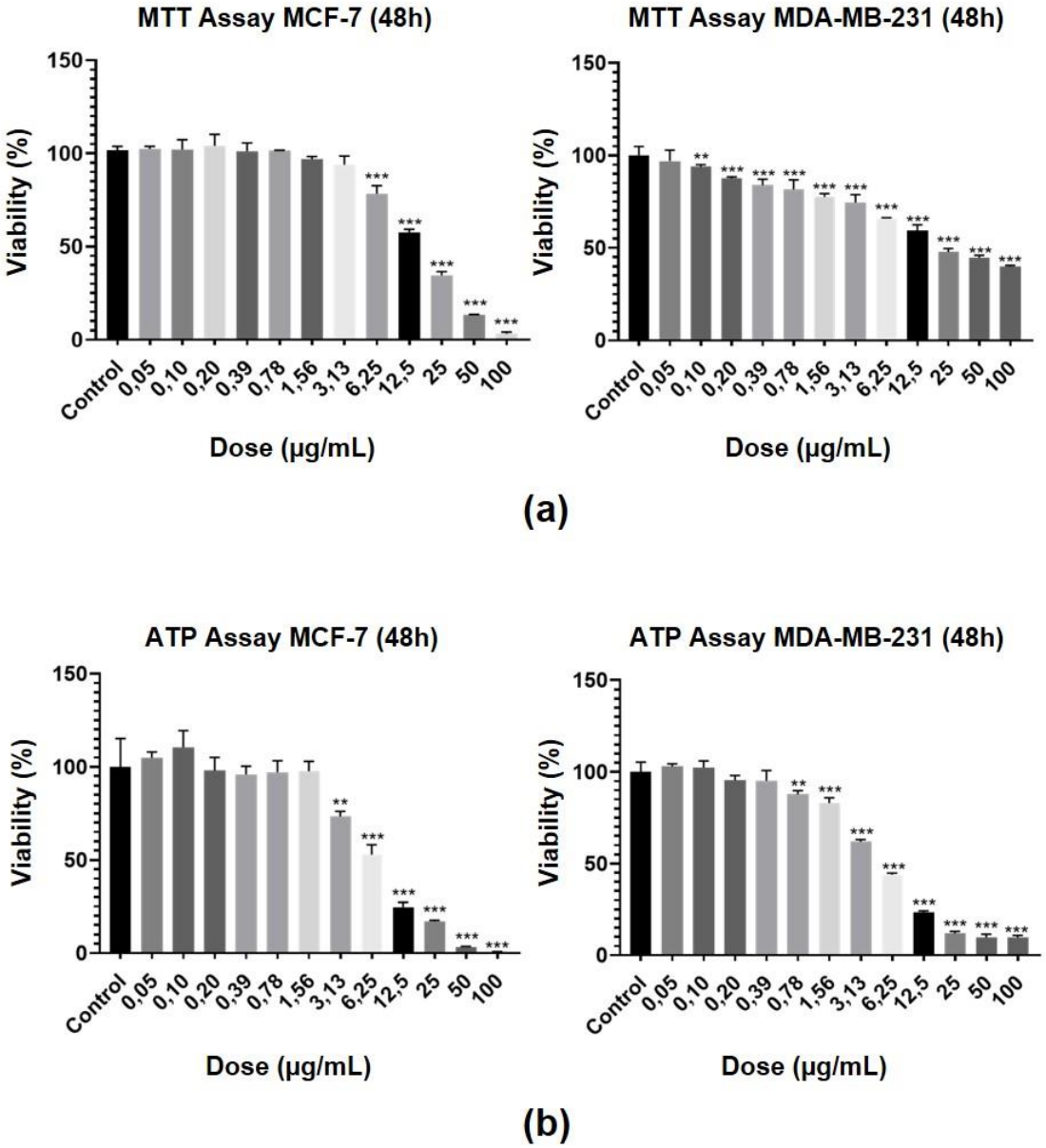

Figure 1. MTT (a) and ATP (b) viability tests were used to assess the viability of MCF-7 and MDA-MB-231 cancer cell lines following 48 h of treatment with varied concentrations (0.05-100 µg/mL) of SCE. Data represented mean ± Standard deviation (n=2). *Denotes statically significant differences in comparison with control (** p<0.01; *** p<0.001).

Table 2. Anti-growth parameters (IC_{50} and IC_{90}) of SCE measurement by the MTT and ATP assay after the treatment for 48 h.

Cell Line	MTT Assay		ATP Assay	
	*IC_{50} (µg/mL)	**IC_{90} (µg/mL)	*IC_{50} (µg/mL)	**IC_{90} (µg/mL)
MCF-7	16,59	66,50	6,86	37,74
MDA-MB-231	22,61	<100	5,20	47,41

*IC_{50} is defined as the dose inhibiting 50% of viability **IC_{90} is defined as the dose inhibiting 90% of viability.

Fluorescence imaging for determination of cell death mode

The cell death mode generated by SCE (IC_{90}=37.74 µg/mL for MCF-7 and IC_{90}=47.41 µg/mL for MDA-MB-231 cells) were investigated for 48 h using fluorescence imaging and nuclear morphology (Figure 2). After comparison with the control group, the cell shrinks, the cell nuclei become pyknotic and fragmented, all of which are well-known apoptotic hallmarks. It was found that the cells were in the late stages of apoptosis or killed by secondary necrosis since they showed positive results in the presence of PI.

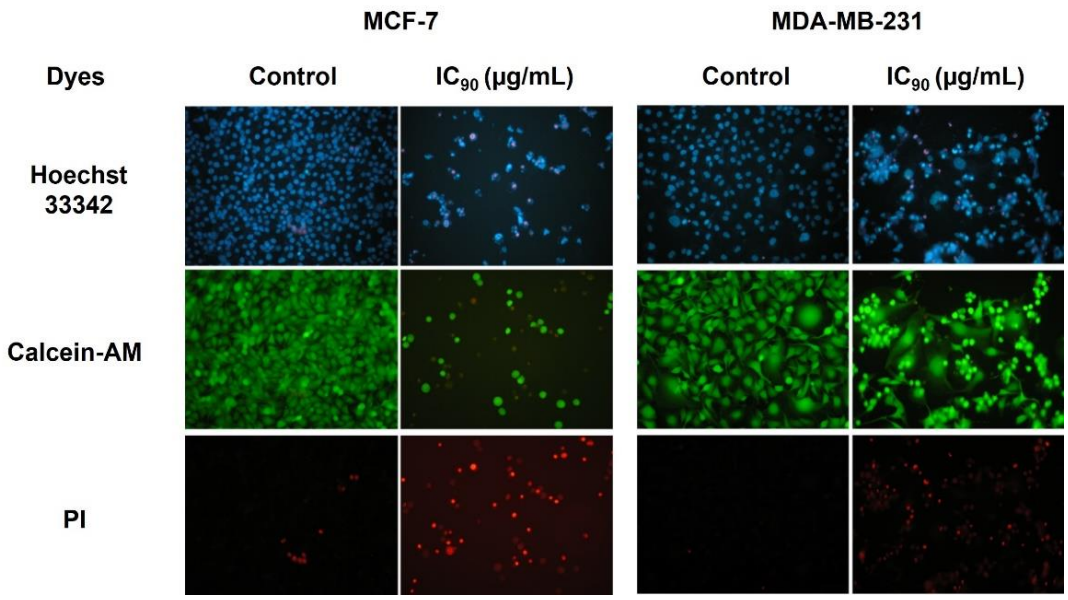

Figure 2. Fluorescence imaging of MCF-7 and MDA-MB-231 cells to determine cell death mode. The cells were treated with SCE for 48 h then stained and visualized using fluorescence microscope. Hoechst 33342 staining is shown in the upper panel, while Calcein-AM and Propidium Iodide (PI) staining are shown in the middle and bottom panels, respectively. Magnification 10X.

M30-Antigen levels

We measured the quantitites of caspase-cleaved cytokeratin 18 (M30-antigen) as a marker of apoptosis after SCE treatment since it caused pyknosis and fragmentation of nucleus. A positive control for apoptosis induction Paclitaxel was used (3.12 µM). In MCF-7 cell line there was an increase in M30-antigen levels after SCE treatment compared to the control group (***p <0,001) (Figure 3). Besides, there was no significant difference between treatment and control group in the MDA-MB-231 cell line. The fact that no increase in M30-antigen levels was observed is due to the very low cytokeratin 18 level in MDA-MB-231 cells [23,24]. These results demonstrated that SCE showed an apoptotic effect on MCF-7 cell line.

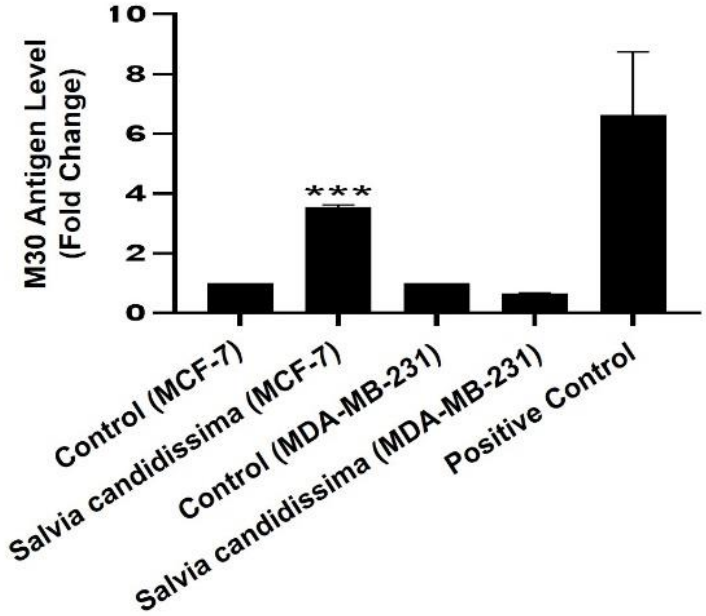

Figure 3. Caspase-cleaved cytokeratin 18 (M30-antigen) levels significantly increase in MCF-7 cell line after 48 h SCE. A positive control for apoptosis induction, Paclitaxel was (3.12 µM) used. Data represented mean ± Standard deviation (n=2). *Denotes statically significant differences in comparison with control (*** p<0.001).

DISCUSSION

Herbal medications have been widely used to cure cancer since ancient times. Breast cancer is the most frequent cancer in women around the world, with more than one million new cases diagnosed each year. Novel compounds should be discovered in order to cure breast cancer. Numerous studies have shown that Salvia species have antioxidant and anticancer properties [11].

Using the GCxGC-TOF/MS system, we analyzed the chemical content of this rare plant, *Salvia candidissima* Vahl. subsp. *candidissima*. We extract *Salvia candidissima* Vahl. subsp. *candidissima* with methanol solvent, but there are many kinds of solvents such as ethanol and CH_2Cl_2 that are used in various studies. These solvents can affect the cytotoxicity of the extract in cancer cells, so it can be used in further studies.

In our study, the most abundant compounds in SCE leaves were caryophyllene oxide, eucalyptol, and sclareol. Hao and coauthors classify the major chemical compounds of different *Salvia* species, and they divide these compounds into seven major categories: sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, steroids, polyphenols, and others [10]. Caryophyllene oxide, which we found the highest content in our study, is in the sesquiterpenoids group, and the other content Sclareol is in the diterpene group.

Caryophyllene and Caryophyllene oxide are two key active components of plants that have been shown to have anti-inflammatory [25], anticarcinogenic [26,27], antimicrobial [28], antioxidative [29], and analgesic activities [30]. Studies found that Caryophyllene oxide possess significant anti-cancer activities in numerous cancer cell lines including HeLa (human cervical adenocarcinoma cells), A-2780 (human ovarian cancer cells), AGS (human gastric adenocarcinoma cells), SNU-1 (human gastric cancer cells), SNU-16 (human stomach cancer cells), HCT-116 and HT-29 (colon cancer cells), PANC-1 (pancreatic cancer cells) and HepG2 (human hepatocellular carcinoma cells) [31-33]. Because of its epoxide exocyclic and methylene functional groups, Caryophyllene oxide appears to have stronger anticancer effects. These groups bind covalently to DNA bases and proteins by sulfhydryl and amino groups and cause suppression of cellular proliferation by decreasing cyclin D1 and induction of apoptosis by decreasing IAP-1, IAP-2 (inhibitor of apoptosis 1 and 2), bcl-2 (B-cell lymphoma 2), bcl-xL (B-cell lymphoma extra-large) proteins [34]. Furthermore, research has shown that these chemicals suppress the PI3K/AKT/mTOR/S6K1 signaling pathways, which are crucial for cell proliferation [35]. The other compound, sclareol, has been shown in numerous studies to have anti-cancer properties. For instance, Wang and coauthors demonstrated that sclareol suppresses osteosarcoma cancer cell proliferation by inducing apoptosis, which is followed by cell cycle arrest in the G1-phase [36]. In another study, by targeting caveolin-1 (Cav1) and copper-zinc superoxide dismutase (SOD1) proteins, sclareol dramatically sensitized many cancer cells to the anticancer effect of bortezomib [37].

The potential cytotoxic effects of SCE (0.05-100 µg/mL, 48 h) on the MCF-7 and MDA-MB-231 breast cancer cell lines were investigated by the MTT and ATP assays. After the treatments, the cell viability was significantly decreased in both MCF-7 and MDA-MB-231 breast cancer cells in a dose-dependent manner. According to an ATP assay's IC_{50} dosages, we found that SCE showed stronger cytotoxic action in MDA-MB-231 cell lines than in MCF-7 cell lines. The breast cancer cell nuclei become pyknotic, fragmented after treatment according to fluorescent images. In MCF-7 cell line, there was an increase in M30-antigen levels after SCE treatment compared to the control group. These results showed that cell death occurred by apoptotic pathway.

There are many cytotoxicity studies of *Salvia* species in breast cancer cells. The antiproliferative effect of *Salvia triloba* L. and *Salvia dominica* Sw. on MCF-7 and T47D breast cancer cell lines were investigated using the sulforhodamine B (SRB) assay in one study. The ethanol extracts were biologically active with IC_{50} values of 29.89 ±0.92 and 38.91 ±2.44 µg/mL for *Salvia triloba* against MCF-7 and T47D cells, respectively, and 5.83 ±0.51 and 12.83 ±0.64 µg/mL for *Salvia dominica* against MCF7 and T47D cells, respectively [38]. In the same study, annexin-V and propidium iodide (PI) fluorescent stains demonstrated that the ethanolic extracts of *Salvia triloba* and *Salvia dominica* promoted apoptosis and to a lesser degree, necrosis-driven cell death. Zhang and coauthors revealed that isocryptotanshinone (ICTS) compound that is isolated from the *Salvia miltiorrhiza* Bunge chemical effectively suppressed the proliferation of MCF-7 and MDA-MB-231 human breast cancer cells, A549 human lung cancer cells and HepG2 human hepatocellular carcinoma cells *in vitro*. MCF-7 cells were the most sensitive to ICTS of the cell lines studied. ICTS promoted apoptosis in MCF-7 cells, according to Western blot analysis for apoptotic proteins and Hoechst 33342 staining [39]. Different solvent fractions derived from *Salvia chloroleuca* Rech.f. & Aellen were identified as a potential apoptotic agent in MCF-7 cell lines in another study. The IC_{50} values

calculated as 60.25 µg/mL in the methanol fraction of extract [40]. The results obtained in all these studies support our results.

Salvia species have been shown to have anticancer effects on various tumor cell lines. The methanolic extract of *Salvia officinalis* L. was found to decrease cell proliferation in human lymphoma cell line U937 and leukemic cell line KG-1A in a dose dependent manner while having no cytotoxic activity on normal cell line, HUVEC [41]. There are also many drugs combination studies in the literature. In the one study investigate sclareol and cyclophosphamide combination in the MCF-7 breast cancer cells and researcher discovered that sclareol reduced cell viability and promoted cell death and its co-administration with cyclophosphamide increased its anti-cancer activity [42]. Ambrož and coauthors have indicated that Caryophyllene oxide enhances the anticancer effects of doxorubicin in CaCo-2 colorectal adenocarcinoma cells [43].

Although numerous studies have been conducted *in vitro*, there is minimal evidence to support the anti-tumor activity of these drugs in animal models. In future studies, extracts of the different *Salvia* species and their various compounds can be tested in animal models.

CONCLUSION

Salvia species, which are rare in Turkey, have been suggested for their potential anti-cancer effects. Our research demonstrated that SCE exhibits cytotoxic effects on breast cancer cells in cell viability assays and apoptotic effects on both fluorescent images and M30-antigen level results. These results give us hope for the development of new anticancer drugs from SCE, but it would be worthwhile to further investigate the cytotoxic activity in different cell lines, together with *in-vivo* studies.

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