Cytotoxic Effects of Verbascoside on MCF-7 AND MDA-MB-231

Short title: Cytotoxic Effects of Verbascoside

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Abstract:
Verbascoside known as Acteoside/Kusaginin has attracted great attention due to its pharmacological features. In this study, we aimed to determine the cytotoxic effects of pure Verbascoside isolated from Phlomis nissolii L. plant in both MCF-7 and MDA-MB-231 cell lines in vitro. MCF-7 and MDA-MB 231 cells were treated with verbascoside (100, 48, 25, 10, 1, 0.5 and 0.1 μM) for 24, 48, and 72 hours. Cytotoxicity effect of verbascoside in MCF-7 and MDA-MB-231 cells was assessed by using TEBU-BIO cell counting kit 8. IC₅₀ values for 24, 48 and 72 hours verbascoside exposure of MCF-7 cells were determined as 0.127, 0.2174 and 0.2828 μM respectively. R² values were calculated as 0.9630, 0.8789 and 0.8752 respectively. Two-way ANOVA multiple comparison test results showed that 100 μM verbascoside has the highest cytotoxic effect in MCF-7 breast cancer cells after 72 hours of exposure. IC₅₀ values for 24, 48 and 72 hours verbascoside exposure of MDA-MB 231 cells were determined as 0.1597, 0.2584 and 0.2563 μM respectively. R² values were calculated as 0.8438, 0.5107 and 0.9203 respectively. Two-way ANOVA multiple comparisons test results showed that 100 μM verbascoside has the highest cytotoxic effect in MDA-MB 231 breast cancer cells after 24, 48 and 72 hours of exposure.

Key Words: Cytotoxicity, MCF-7, MDA-MB-231, Phlomis nissolii L., Verbascoside

Özet
Acteoside / Kusaginin olarak bilinen Verbascoside, farmakolojik özelliklerinden dolayı büyük ilgi görmüştür. Bu çalışmada, Phlomis nissolii L. bitkisinden izole edilen saf Verbascoside’ın MCF-7 ve MDA-MB-231 hücre hatlarında in vitro olarak sitotoksik etkilerini belirlemeyi amaçladık. MCF-7 ve MDA-MB 231 hücreleri, 24, 48 ve 72 saat süreyle 100, 48, 25, 10, 1,
0.5 and 0.1 μM verbascoside were administered to MCF-7 and MDA-MB-231 cell lines to evaluate cytotoxicity. Verbascoside's cytotoxic effects on MCF-7 and MDA-MB-231 cell lines were evaluated using the TEBU-BIO cell count kit. The IC50 values for MCF-7 cells were determined to be 0.127, 0.2174, and 0.2828 μM at 24, 48, and 72 hours, respectively. The R2 values were calculated as 0.9630, 0.8789, and 0.8752. The two-way ANOVA multiple comparison test results showed that 100 μM verbascoside had the highest cytotoxic effect on MCF-7 breast cancer cells at 72 hours. The IC50 values for MDA-MB 231 cells were 0.1597, 0.2584, and 0.2563 μM at 24, 48, and 72 hours, respectively. The R2 values were calculated as 0.8438, 0.5107, and 0.9203. The two-way ANOVA multiple comparison test results showed that 100 μM verbascoside had the highest cytotoxic effect on MDA-MB 231 breast cancer cells at 24, 48, and 72 hours.

**Key Words:** MCF-7, MDA-MB-231, Phlomis nissolii L., Cytotoxicity, Verbascoside

**Introduction**

Breast Cancer is the most frequent cancer type among women, impacting 2.1 million women each year (1). In 2018, the number of female deaths due to breast cancer was as high as 627,000. This value compromises 15% of all cancer deaths among women. Furthermore, breast cancer rates in women are higher in more developed regions than developing countries and threateningly, these rates are still increasing in every region globally (1). According to statistical data obtained by the Ministry of Health in North Cyprus, 1854 men and 1809 women were diagnosed with cancer between 2012-2016. Breast cancer has the highest incidence (62.2%) among women in North Cyprus, and this value is lower than incidence in Europe but unfortunately higher than breast cancer incidence in the world-wide (2). The current treatment strategies for breast cancer, including radiotherapy plus adjuvant chemotherapy, radiation therapy, hormone therapy, and surgery, have side effects (3). These may include rib fracture, second non-breast infield malignancies, tissue necrosis, and brachial plexopathy in radiation therapy, and reduced number of white and red blood cells, elevated risk of infection and anemia, diarrhea, fatigue, hair loss, sore throat, ulcers, nausea, constipation, loss of appetite, and change in color of the skin in chemotherapy (3). Due to these side effects, there has been a growing interest in alternative treatment modalities with reduced side effects (4). There are many studies that have identified anti-cancer properties of herbal medicines that are used in developing countries for medical treatment for many years (5).

Verbascoside (C29H36O15) known as acteoside/kusaginin is a phenyl ethanoid glycoside. Verbascoside has been isolated from many different plant species such as Verbascum sinuatum L. (6), Syringa vulgaris (7), Orobancherapum-genistae (8), Clerodendron trichotomum Thumb (9), Phlomis nissolii L. (Lamiaceae) (10), Buddleja brasiensis, Striga asiatica, Oteca europaea, Paulownia tomentosa var. tomentosa, Lippia javanica, Lantana camara, and Lippia citriodora (11). In addition, verbascoside is abundant in olive mill waste water (12, 13). There are total number of 34 species of the genus Phlomis species L. in Turkey and Aegean islands (14). The project performed on the 33 Phlomis species recorded in the Flora of Turkey has resulted in the isolation and characterization of 33 phenylethanoid glycosides of which verbascoside and forsythoside B were the common compounds for all of the Phlomis species (15). Recently, two compounds were isolated from the two endemic Phlomis species, P. brevibracteata and P. cypria growing in Cyprus (16). Verbascoside attracted great attention due to its pharmacological features (17) such as anti-inflammatory effect (18,19, 20, 21, 22, 23, 24); anti-oxidative effect (25, 26, 27, 28, 29, 30, 31, 32); neuroprotective effect (33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43) anti-microbial effect (44,45, 46); UV radiation protective...
effect (47, 48, 49, 50, 51); anti-metastatic effect (52) and cytotoxic effects on many types of cancer such as myelo and leukaemia (53, 54, 55, 56); human gastric carcinoma (57); colorectal cancer (58); human oral squamous cell carcinoma (59); glioblastoma (60) and inhibitory effect on tumour cell proliferation (61). In this study, we aimed to determine the cytotoxic effects of pure Verbascoside isolated from Phlomis nissolii L. plant in both MCF-7 and MDA-MB-231 cell lines in vitro.

Materials and methods
Cell culture conditions
The compound Verbascoside used in this study was provided from the studies performed on Phlomis species L. (Çalış et al., 2004). Human breast cancer cells MCF-7 and MDA-MB-231 (ATCC) were cultured in DMEM/F-12 media supplemented with 10% Fetal Bovine Serum, 4mg/ml insulin human, %1 penicillin streptomycin at 37˚C in a 5% CO₂ containing humidified chamber. The medium was refreshed every other day.

Cell viability/Cytotoxicity
MCF-7 and MDA-MB 231 breast cancer cells were plated in 96-well plates in triplicate with density of 5.000 cells/well. The cells were treated with verbascoside after 24 hours of culturing at different concentrations (100, 48, 25, 10, 1, 0.5 and 0.1 μM) for 24, 48, and 72 hours. CCK-8 (Tebu, France) analysis was performed according to manufacturer’s protocol. The absorbances were measured by using Versa max tunable microplate reader at 450 nm wavelength.

Statistical Analysis
GraphPad® Prism software version 8 was used to calculate IC₅₀ values by the application of non-linear regression curve fit analysis. Further statistical analysis was performed using Two-way ANOVA Multiple Comparisons Test to determine the significance of mean difference between control and varying concentrations of Verbascoside.

Results
Cytotoxic Effects of Verbascoside in MCF-7 Cells
To assess the cytotoxicity of verbascoside, MCF-7 breast cancer cells were treated with several concentrations of verbascoside (100, 48, 25, 10, 1, 0.5 and 0.1 μM) for 24, 48 and 72 hours. IC₅₀ values of verbascoside in MCF-7 cells are shown in table 1.

| Exposure Time to verbascoside | IC₅₀ (μM) | R²      |
|-------------------------------|----------|---------|
| 24 hour                       | 0.127    | 0.9630  |
| 48 hour                       | 0.2174   | 0.8789  |
| 72 hour                       | 0.2828   | 0.8752  |

Table 1: IC₅₀ and R² values for MCF-7 cell line

Significance of mean difference between control and other concentrations of verbascoside for MCF-7 cell line after 24h, 48h and 72h was determined by using Two-way ANOVA Multiple Comparisons Test and results are shown in figures 1, 2 and 3 respectively.
Two-way ANOVA multiple comparisons test results for MCF-7 cell line after 24h exposure to different concentrations of verbascoside showed that mean difference was not found as significant at 95% confidence level between the control and test group at 48, 25 and 10 μM verbascoside concentrations. However, significance was observed at 100, 1, 0.5 and 0.1 μM verbascoside concentrations and the control group after 24h exposure. When concentration of verbascoside was decreased from 100 to 10 μM, absorbancy increased so that number of alive
cells increased. When concentration of verbascoside was further decreased from 10 to 0.1 μM, absorbancy decreased so that number of alive cells decreased but number of dead cells increased. All absorbancy values were higher than the control group, indicating that 100, 48, 25, 10, 1, 0.5 and 0.1 μM concentrations of verbascoside were not effectively toxic to the MCF-7 breast cancer cells after 24h exposure (Figure 1).

Mean difference was not significant at 95% confidence level between control absorbancy value and absorbancy values obtained at 100, 48, 25, 10, 1, 0.5 and 0.1 μM verbascoside concentrations after 48h exposure of MCF-7 cell line. When concentration of verbascoside was decreased from 100 to 10 μM, absorbancy increased so that number of alive cells increased. When concentration of verbascoside was further decreased from 10 to 0.1 μM absorbancy decreased so that number of alive cells decreased but number of dead cells increased. All absorbancy values were higher than control so that 100, 48, 25, 10, 1, 0.5 and 0.1 μM concentrations of verbascoside were not effective on MCF-7 breast cancer cells after 48h exposure (Figure 2).

Mean difference calculated by Two-way ANOVA multiple comparisons test was not significant at 95% confidence level between control absorbancy value and absorbancy values obtained at 100, 48, 25, 10, 1, 0.5 and 0.1 μM verbascoside concentrations after 72h exposure of MCF-7 cell line. When concentration of verbascoside was decreased from 100 to 25 μM, absorbancy increased so that number of alive cells increased. When concentration of verbascoside was further decreased from 25 to 0.1 μM, absorbancy decreased so that number of alive cells decreased but number of dead cells increased. Absorbancy value at 100 μM verbascoside was the lowest among the other absorbancy values so that lowest number of alive cells but highest number of dead cells was at this concentration. 100 μM verbascoside had the highest cytotoxic effect on MCF-7 breast cancer cells after 72h exposure (Figure 3).

**Cytotoxic Effects of Verbascoside in MDA-MB 231 Cells**

MDA-MB 231 breast cancer cells were treated with several concentrations of verbascoside (100, 48, 25, 10, 1, 0.5 and 0.1 μM) for 24, 48 and 72 hours to assess the cytotoxicity of verbascoside by using TEBU-BIO cell counting kit 8 (CCK8). IC50 values of verbascoside in MDA-MB 231 cells are shown in table 2.

| Exposure Time to verbascoside | IC50 (μM) | R²        |
|-------------------------------|-----------|-----------|
| 24 hour                       | 0.1597    | 0.8438    |
| 48 hour                       | 0.2584    | 0.5107    |
| 72 hour                       | 0.2563    | 0.9203    |

Table 2: IC50 and R² values for MDA-MB 231 breast cancer cell line

Two-way Anova multiple comparisons test results for MDA-MB 231 breast cancer cell line after 24, 48 and 72 hours are shown in figures 4, 5 and 6.
Analysis of the results showed that mean difference between control absorbancy value and absorbancy values obtained at 100, 48, 25, 10, 1, 0.5 and 0.1 μM verbascoside concentrations was not significant at 95% confidence level. Absorbancy increased when concentration of verbascoside was decreased from 100 to 0.5 μM. This result showed that number of alive cells increased. Further decrease of concentration of verbascoside from 0.5 to
0.1 μM, caused decrease of absorbancy indicating decreased number of number of alive cells but number of dead cells increased. Absorbancy value at 100 μM verbascoside was the lowest among the other absorbancy values. This result indicated that lowest number of alive cells but the highest number of dead cells were at this concentration. 100 μM verbascoside had the highest cytotoxic effect on MDA-MB 231 breast cancer cells after 24h exposure (Figure 4).

Mean difference was not significant at 95% confidence level between control absorbancy value and absorbancy values obtained at 100, 48, 25, 10, 0.5, 1 and 0.1 μM verbascoside concentrations. When concentration of verbascoside was decreased from 100 to 25 μM, absorbancy increased so that number of alive cells increased. When concentration of verbascoside was further decreased from 25 to 0.1 μM, absorbancy decreased. This result indicated that number of alive cells decreased but number of dead cells increased. Absorbancy value at 100 μM verbascoside was the lowest among the other absorbancy values. This result showed that lowest number of alive cells but the highest number of dead cells was at this concentration. 100 μM verbascoside had the highest cytotoxic effect on MDA-MB 231 breast cancer cells after 48h exposure (Figure 5).

Although calculated mean difference between control absorbancy value and absorbancy values obtained at 48, 25, 10 and 1 μM verbascoside concentrations was not significant at 95% confidence level; mean difference was significant at 95% confidence level between control absorbancy value and absorbancy values obtained at 100, 0.5 and 0.1 μM verbascoside concentrations. Absorbancy increased when concentration of verbascoside was decreased from 100 to 0.5 μM indicating that number of alive cells increased. When concentration of verbascoside was further decreased from 0.5 to 0.1 μM, absorbancy decreased so that number of alive cells decreased but number of dead cells increased. Absorbancy value at 100 μM verbascoside was the lowest among the other absorbancy values so that lowest number of alive cells but the highest number of dead cells was at this concentration. 100 μM verbascoside had the highest cytotoxic effect on MDA-MB 231 breast cancer cells after 72h exposure (Figure 6).

Conclusion and Discussion
The prevalence of breast cancer have been rising rapidly in past decades, however diagnoses and treatment in the early stages is important (62). Despite advances in treatment in the early stage of breast cancer, many women experience recurrence and metastasis. Although the treatment strategies are limited, the main focus is on medical therapy. The importance of classical treatment methods in cancer therapy is indisputable (63). Increasing cancer cases and developing resistance to drugs has urged the need for new diagnostic and treatment approaches. Since the success of traditional treatments is limited, most cancer patients try complementary medical therapies. There has been a growing interest in alternative treatment modalities. Finding alternative therapies with less or no side effects are essential. In recent years, the alternative treatment modalities, such as natural products as anti-cancer drugs, have gained importance in breast cancer therapy. Thus, the main aim of this study was to evaluate the cytotoxic effect of Verbascoside isolated from *Phlomis nissolii* L. plant (Lamiaceae) in MCF-7 and MDA-MB 231 breast cancer cell lines in vitro.

IC50 values for MCF-7 breast cancer cell line after 24h, 48h and 72h exposure to different concentrations of verbascoside were found as 0.127, 0.2174 and 0.2828 μM respectively. R² values for 24h, 48h and 72h exposure to verbascoside were calculated as 0.9630, 0.8789 and 0.8752 respectively. 48, 25,10, 0.5 and 0.1 μM concentrations of verbascoside are not toxic on MCF-7 breast cancer cells after 24h, 48h and 72h exposure. 100 μM verbascoside has the highest cytotoxic effect on MCF-7 breast cancer cells only after 72h exposure. In a study, verbascoside was isolated from *Scrophularia subaphylla* L. and researchers examined the
effect of 1 to 1000 μg/mL verbascoside on MCF-7 cells and found IC₅₀ value as 0.39 (+/- 0.015) μg/mL after 48 hours of exposure (64). In another study, 5β,6β-dihydroxyantirrhide was isolated from *Pseuderanthemum carruthersii* (Seem.) Guill. var. atropurpureum (Bull.) Fosb. (Acanthaceae) leaves with thirteen different compounds including verbascoside and the cytotoxic activities of these chemicals and acetylcholinesterase inhibition against MCF-7 and HeLa cells at a concentration of 100 μg/mL were analyzed. Isoverbascoside and verbascoside showed fairly weak AChE inhibitory activity but showed cytotoxic activity against MCF-7 cells strongly (65). This result supports the results of our study. Because in our study, it was found that 100 μM verbascoside has the highest cytotoxic effect on MCF-7 breast cancer cells only after 72h exposure. This shows that 100 μM and higher concentrations of verbascoside have cytotoxic effect on MCF-7 cells. In another study, acteoside was isolated from the crude methanolic extract of *Leucas indica* flowers and a range of concentrations of acteoside (250.00, 125.00, 62.50, 31.25, 15.63, 7.81, 3.91, 1.95, 0.98 μg/ml) was tested on the MCF-7 cell line after 48h of incubation. Researchers evaluated in vitro cytotoxicity of acteoside on MCF-7 cell by using the MTT assay. This study tested a higher range of acteoside concentration on MCF-7 cell line than our present study that used a range of concentrations of 100, 48, 25, 10, 1, 0.5 and 0.1 μM verbascoside (acteoside) and obtained higher values of IC₅₀ and R² as 7.7 and 0.9968 than current study (66). Researchers also concluded that acteoside isolated from *Leucas indica* flowers extract showed significant cytotoxicity activity on MCF-7 cell line and results indicated that the antiproliferative effect strengthens with increase in the concentration of extract (p<2). This supports our present study which concluded that 100 μM verbascoside showed significant cytotoxicity on MCF-7 breast cancer cell line after 72h exposure). Results of another study with verbascoside isolated from the aerial parts of *Plantago lagopus* L. showed that verbascoside had strong cytotoxic activities against MCF-7 cell line and also, histological analysis proved the apoptotic cell death of MCF-7 cells after the treatment of 50–100 μg/mL verbascoside (67). In one report, effect of different concentrations of verbascoside isolated from *V. ovalifolium Donn ex Sims* (Scrophulariaceae) on cell viability of MCF-7 cells was measured using the MTT colorimetric assay after 48h of incubation. The IC₅₀ value for verbascoside was calculated as 58.3 μg/mL and it was observed that verbascoside decreased the viability by 69.6% in MCF-7 cells at 100 μg/mL but did not affect the viability of non-tumor MCF-10A cells (up to 100 μg/mL) (68). Acteoside may be effective to prevent MCF-7 breast cancer because of its antiestrogenic effect. Acteoside isolated from aerial parts of *Verbascum macrurum* exhibited an ER-mediated significant antiestrogenic activity at a low concentration range 10⁻⁷ - 10⁻⁹ M in both the ERα and ERβ assay systems, indicating that acteoside may act as antagonist by itself. Acteoside at low concentration (10⁻⁷ M) demonstrated a potent inhibitory effect against estradiol (10⁻⁹ M) mainly via ERα so that acteoside functions as antagonist for ERα-mediated transcription (69). In contrast, in another study, 12 chemical constituents from the *Callicarpa nudiflora* were isolated and their cytotoxicity was evaluated by the MTT assay. The cytotoxicity assay demonstrated that flavonoids luteoloside, lutedin-4’-O-β-D-glucoside, 6-hydroxyluteolin-7-O-β-glucoside, lutedin-7-O-neohesperidoside, rhoifolin, luteolin-7, and 4’-di-O-glucoside showed monolithic proliferation inhibitory activities against Hela, A549 and MCF-7 cell lines in various concentrations. Compounds 6-hydroxyluteolin-7-O-β-glucoside and rhoifolin and iridoid glycoside nudifloside exhibited higher cytotoxic activities. This study showed that main components of cytotoxic extract from *C. Nudiflora* are flavonoids while phenylethanoid glycosides are the predominant components but inactive to targeted cancer cell lines and the minor iridoid glycoside expressed weak cytotoxic activity (70). IC₅₀ values for MDA-MB 231 cell line after 24h, 48h and 72h of exposure to different concentrations of verbascoside were found as 0.1597, 0.2584 and 0.2563 μM respectively.
R² values for 24h, 48h and 72h exposure to verbascoside were calculated as 0.8438, 0.5107 and 0.9203 respectively. 48, 25,10,1, 0.5 and 0.1 μM concentrations of verbascoside are not toxic on MDA-MB 231 breast cancer cells after 24h and 48h and 72h exposure. 100 μM verbascoside has the highest cytotoxic effect on MDA-MB 231 breast cancer cells after 24h, 48h and 72 h exposure. There are few studies about the cytotoxic effects of verbascoside on MDA-MB 231 breast cancer cell line in literature. In a study, antiproliferative effect of *Strobilanthes crispus* containing verbascoside on MDA-MB 231 cells was evaluated by using MTT assay and IC₅₀ value of methanolic extract was found as 27.2 μg/mL (71). Another study examined the effect of dry olive mill residue water containing verbascoside and found that dry olive mill residue water inhibited MDA-MB 231 cell growth by EC value of 57.15 ± 1.04 c (72). Both of these studies supported the idea that plant extracts containing verbascoside have cytotoxic effects on MDA-MB 231 breast cancer cell line but researchers in these studies examined the cytotoxic effects of plant extracts containing verbascoside and any other chemicals on MDA-MB 231 cell line, not pure verbascoside like in our study.

**Suggestions**

This study proved that verbascoside isolated from *Phlomis species* L. has cytotoxic effects on MCF-7 and MDA-MB 231 breast cancer cells. Further studies can be performed to assess the underlying mechanisms for apoptotic induction of verbascoside extracted from *Phlomis species* L.. Also, detailed investigations can be performed to evaluate the synergic effects of verbascoside isolated from *Phlomis species* L. with other plant extracts used in breast cancer treatment.

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**Disclosure Statement**

The authors declare no conflict of interest.

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**Author Contributions**

H.Ş is the corresponding author. H.Ş and PT designed the study, provided data and conducted statistical analyses. MÇE contributed to research by supplying the materials used. AZ and İÇ contributed the research by supplying verbascoside that they isolated from *Phlomis species* L.. All authors contributed to the interpretation of data. All authors read and approved the final manuscript.

**References**

1. World Health Organization. Breast Cancer (2019). Retrieved from https://www.who.int/cancer/prevention/diagnosis-screening/breast-cancer/en/
2. TRNC Ministry of Health, Kuzey Kıbrıs Türk Cumhuriyeti’nde Kanser Kayıtçılık Projesi (2019). Retrieved from https://saglik.gov.ct.tr/Portals/107/KK-Kidem%202012-2016%20BesYllkKanserIstatistikleri%20%282%29.pdf.
3. Agrawal S. Late effects of cancer treatment in breast cancer survivors. *South Asian J Cancer*. 2014;3(2):112-5.
4. Mitra S, Dash R. Natural Products for the Management and Prevention of Breast Cancer. *Evid Based Complement Alternat Med*. 2018 Feb 26; 2018:8324696. doi: 10.1155/2018/8324696.
5. Greenwell M, Rahman PK. Medicinal Plants: Their Use in Anticancer Treatment. *Int J Pharm Sci Res*. 2015 Oct 1;6(10):4103-4112. doi: 10.13040/IJPSR.0975-8232.6(10).4103-12.
6. Scarpati ML, Monache D. Isolation from *Verbascum sinuatum* of two new glucosides, verbascoside and isoverbascoside. *Ann Chim*, 1963: 53(4): 356-367.
7. Birkofer L, Kaiser C, Thomas U. Acteosid und neoacteosid: Zukerester aus Syringa vulgaris. *Z Naturforsch B*, 1968: 23:1051-8.
8. Andary C., Wylde R., Laffite C., Privat G., & Winternitz F. Structures of verbascoside and orobanchoside, caffeic acid sugar esters from Orobanche rapum-genistae. *Phytochemistry*, 1982; 21, 1123-1127.
9. Sakurai A, Kato T. A new glycoside, kusaginin isolated from Clerodendron trichotomum. *Bull Chem Soc Jpn*, 1983: 56(5):1573-1574.
10. Kırmızibekmez H, Piacente S, Pizza C, Dönmez AA and Çaliş İ. Iridoid and Phenylethanoid Glycosides from Phlomis nissolii and P. capitata. *Z. Naturforsch*, 2004:59b: 609 – 613.
11. Alipieva K, Korkina L, Orhan IE, Georgiev MI. Verbascoside--a review of its occurrence, (bio)synthesis and pharmacological significance. *Biotechnol Adv*. 2014 Nov 1;32(6):1065-76. doi: 10.1016/j.biotechadv.2014.07.001.
12. De Marco E, Savarese M, Paduano A, Sächer R. Characterization and fractionation of phenolic compounds extracted from olive oil mill waste waters. *Food Chem*, 2006: 104,2:858–867.
13. Dell'Aquila ME, Bogliolo L, Russo R, Martino NA, Filioli Uranio M, Ariu F, Amati F, Sardanelli AM, Linsalata V, Ferruzzi MG, Cardinali A, Minervini F. Prooxidant effects of verbascoside, a bioactive compound from olive oil mill wastewater, on in vitro developmental potential of ovine prepubertal oocytes and bioenergetic/oxidative stress parameters of fresh and vitrified oocytes. *Biomed Res Int*. 2014;2014:878062. doi: 10.1155/2014/878062.
14. Huber-Morath A. *Phlomis*, in *Flora of Turkey and the East Aegean Islands*, Vol. 7, ed. P.H. Davis, 1982: pp 102-126, Edinburg University Press, Edinburgh.
15. Çaliş İ, Saracoğlu İ, Ersöz T, Kırmızibekmez H, Yağcı MN, Harput Ş. Chemotaxonomy of Turkish *Phlomis* L. (Lamiaceae) Genus . The Scientific and Technological Research Council of Turkey, 2004:Project Number: SBAG-2304.
16. Hanoğlu A. Phytochemical Studies on the Endemic *Phlomis* species growing in Northern Cyprus (K.K.T.C.* de Yetişen Endemik Phlomis Türleri Üzerine Fitokimyasal Araştımlar)*. Near East University, Graduate School of Health Sciences, 2019:PhD Thesis, T.R.N.C.
17. Schönbließer SA, Bittner LK, Pallua JD, Popp M, Abel G, Bonn GK, Huck CW. Simultaneous quantification of verbenalin and verbascoside in Verbena officinalis by ATR-IR and NIR spectroscopy. *J Pharm Biomed Anal*. 2013 Oct;84:97-102. doi: 10.1016/j.jpba.2013.04.038.
18. Lee JH, Lee JY, Kang HS, et al. The effect of acteoside on histamine release and arachidonic acid release in RBL-2H3 mast cells. *Archives of Pharmacal Research*.2006 Jun; 29(6):508-513. DOI: 10.1007/bf02969425.
19. Li Y, Gan L, Li GQ, Deng L, Zhang X, Deng Y. Pharmacokinetics of plantamajoside and acteoside from Plantago asiatica in rats by liquid chromatography-mass spectrometry. *J Pharm Biomed Anal*. 2014 Feb;89:251-6. doi: 10.1016/j.jpba.2013.11.014.
20. Mazzon E, Esposito E, Di Paola R, Riccardi L, Caminiti R, Dal Tos R, Pressi G, Cuzzocrea S. Effects of verbascoside biotechnologically produced by Syringa vulgaris plant cell cultures in a rodent model of colitis. *Naunyn Schmiedebergs Arch Pharmacol*. 2009 Jul;380(1):79-94. doi: 10.1007/s00210-009-0400-5.

21. Rao YK, Fang SH, Hsieh SC, Yeh TH, Tseng YM. The constituents of Anisomeles indica and their anti-inflammatory activities. *J Ethnopharmacol*. 2009 Jan 21;121(2):292-6. doi: 10.1016/j.jep.2008.10.032.

22. Lenoir L, Rossary A, Joubert-Zakeyj H, Vergnaud-Gauduchon J, Farges MC, Fraisse D, Texier O, Lamaison JL, Vasson MP, Felgines C. Lemon verbena infusion consumption attenuates oxidative stress in dextran sulfate sodium-induced colitis in the rat. *Dig Dis Sci*. 2011 Dec;56(12):3534-45. doi: 10.1007/s10620-011-1784-x.

23. Kostyuk VA, Potapovich AI, Suhan TO, de Luca C, Korkina LG. Antioxidant and signal modulation properties of plant polyphenols in controlling vascular inflammation. *Eur J Pharmacol*. 2011 May 11;658(2-3):248-56. doi: 10.1016/j.ejphar.2011.02.022.

24. Pesce M, Franceschelli S, Ferrone A, De Lutiis MA, Patruno A, Grilli A, Felaco M, Speranza L. Verbascoside down-regulates some pro-inflammatory signal transduction pathways by increasing the activity of tyrosine phosphatase SHP-1 in the U937 cell line. *J Cell Mol Med*. 2015 Jul;19(7):1548-56. doi: 10.1111/jcmm.12524.

25. Vertuani S, Beghelli E, Scalambra E, Malisardi G, Cocetti S, Dal Tos R, Baldisserotto A, Manfredini S. Activity and stability studies of verbascoside, a novel antioxidant, in dermo-cosmetic and pharmaceutical topical formulations. *Molecules*. 2011 Aug 18;16(8):7068-80. doi: 10.3390/molecules16087068.

26. Caturla N, Funes L, Pérez-Fons L, Micol V. A randomized, double-blinded, placebo-controlled study of the effect of a combination of lemon verbena extract and fish oil omega-3 fatty acid on joint management. *J Altern Complement Med*. 2011 Nov;17(11):1051-63. doi: 10.1089/acm.2010.0410.

27. Mestre-Alfaro A, Ferrer MD, Sureda A, Tautier P, Martinez E, Bibiloni MM, Micol V, Tur JA, Pons A. Phytoestrogens enhance antioxidant enzymes after swimming exercise and modulate sex hormone plasma levels in female swimmers. *Eur J Appl Physiol*. 2011 Sep;111(9):2281-94. doi: 10.1007/s00421-011-1862-y.

28. Carrera-Quintanar L, Funes L, Vilades E, Tur J, Micol V, Roche E, Pons A. Antioxidant effect of lemon verbena extracts in lymphocytes of university students performing aerobic training program. *Scand J Med Sci Sports*. 2012 Aug;22(4):454-61. doi: 10.1111/j.1600-0838.2010.01244.x.

29. Cardinali A, Peri S, Minervini F, D’Antuono I, Linsalata V, Lattanzio V. Verbascoside, isoverbascoside, and their derivatives recovered from olive mill wastewater as possible food antioxidants. *J Agric Food Chem*. 2012 Feb 22;60(7):1822-9. doi: 10.1021/jf204001p.

30. Sgarbossa A, Dal Bosco M, Pressi G, Cuzzocrea S, Dal Tos R, Menegazzi M. Phenylpropanoid glycosides from plant cell cultures induce heme oxygenase 1 gene expression in a human keratinocyte cell line by affecting the balance of NRF2 and BACH1 transcription factors. *Chem Biol Interact*. 2012 Aug 30;199(2):87-95. doi: 10.1016/j.cbi.2012.06.006.

31. Alipieva K, Korkina L, Orhan IE, Georgiev MI. Verbascoside--a review of its occurrence, (bio)synthesis and pharmacological significance. *Biotechnol Adv*. 2014 Nov 1;32(6):1065-76. doi: 10.1016/j.biotechadv.2014.07.001.

32. Di Giancamillo A, Rossi R, Vitari F, Carollo V, Deponti D, Corino C, Domeneghini C. Changes in nitrosative stress biomarkers in swine intestine following dietary intervention with verbascoside. *Histol Histopathol*. 2013 Jun;28(6):715-23. doi: 10.14670/HH-28.715.
33. Sheng GQ, Zhang JR, Pu XP, Ma J, Li CL. Protective effect of verbascoside on 1-methyl-4-phenylpyridinium ion-induced neurotoxicity in PC12 cells. Eur J Pharmacol. 2002 Sep 13;451(2):119-24. doi: 10.1016/s0014-2999(02)02240-9.

34. Pu X, Song Z, Li Y, Tu P, Li H. Acteoside from Cistanche salsa inhibits apoptosis by 1-methyl-4-phenylpyridinium ion in cerebellar granule neurons. Planta Med. 2003 Jan;69(1):65-6. doi: 10.1055/s-2003-37029.

35. Backhouse N, Delport C, Abalbaza C, Farias M, Goity L, Arrau S, Negrete R, Castro C, Miranda H. Antinociceptive activity of Buddleja globosa (matico) in several models of pain. J Ethnopharmacol. 2008 Sep 2;119(1):160-5. doi: 10.1016/j.jep.2008.06.022.

36. Deng M, Ju X, Fan D, Tu P, Zhang J, Shen Y. Verbascoside rescues the SH-SY5Y neuronal cells from MPP+ induced apoptosis. Chin Pharmacol Bull, 2008:24(10):1297–1302.

37. Wang H, Xu Y, Yan J, Zhao X, Sun X, Zhang Y, Guo J, Zhu C. Acteoside protects human neuroblastoma SH-SY5Y cells against beta-amyloid-induced cell injury. Brain Res. 2009 Aug 4;1283:139-47. doi: 10.1016/j.brainres.2009.05.101.

38. Esposito E, Mazzon E, Paterniti I, DalToso R, Pressi G, Caminiti R, Cuzzocrea. PPAR-α contributes to the anti-inflammatory activity of verbascoside in a model of inflammatory bowel disease in mice. PPAR Res, 2010:917312.http://dx.doi.org/10.1155/2010/917312.

39. Kahraman C, Tatlil II, Orhan IE, Akdemir ZS. Cholinesterase inhibitory and antioxidant properties of Verbascum mucronatum Lam. and its secondary metabolites. Z Naturforsch C J Biosci. 2010 Nov-Dec;65(11-12):667-74. doi: 10.1515/znc-2010-11-1206.

40. Filho AG, Morel AF, Adolpho L, Ilha V, Giralt E, Tarragó T, Dalcol II. Inhibitory effect of verbascoside isolated from Buddleja brasiliensis Jacq. ex Spreng on prolyl oligopeptidase activity. Phytother Res. 2012 Oct;26(10):1472-5. doi: 10.1002/ptr.4597.

41. Lin J, Gao L, Huo SX, Peng XM, Wu PP, Cai LM, Yan M. [Effect of acteoside on learning and memory impairment induced by scopolamine in mice]. Zhongguo Zhong Yao Za Zhi. 2012 Oct;37(19):2956-9. Chinese.

42. Wang HQ, Xu YX, Zhu CQ. Upregulation of heme oxygenase-1 by acteoside through ERK and PI3 K/Akt pathway confer neuroprotection against beta-amyloid-induced neurotoxicity. Neurotox Res. 2012 May;21(4):368-78. doi: 10.1007/s12640-011-9292-5.

43. Kurisu M, Miyamae Y, Murakami K, Han J, Isoda H, Irie K, Shigemori H. Inhibition of amyloid β aggregation by acteoside, a phenylethanoid glycoside. Biosci Biotechnol Biochem. 2013;77(6):129-32. doi: 10.1271/bbb.130101.

44. Azimi H, Fallah-Tafti M, Khakshur AA, Abdollahi M. A review of phytotherapy of acne vulgaris: perspective of new pharmacological treatments. Fitoterapia. 2012 Dec;83(8):1306-17. doi: 10.1016/j.fitote.2012.03.026.

45. Funari CS, Gullo FP, Napolitano A, Carneiro RL, Mendes-Giannini MJ, Fusco-Almeida AM, Piccente S, Pizza C, Silva DH. Chemical and antifungal investigations of six Lippia species (Verbenaceae) from Brazil. Food Chem. 2012 Dec 1;135(3):2086-94. doi: 10.1016/j.foodchem.2012.06.077.

46. Maquiaveli CDC, Rochetti AL, Fukumasu H, Vieira PC, da Silva ER. Antileishmanial activity of verbascoside: Selective arginine inhibition of intracellular amastigotes of Leishmania (Leishmania) amazonensis with resistance induced by LPS plus IFN-γ. Biochem Pharmacol. 2017 Mar 1;127:28-33. doi: 10.1016/j.bcp.2016.12.018.

47. Korkina L, Pastore S. The role of redox regulation in the normal physiology and inflammatory diseases of skin. Front Biosci (Elite Ed). 2009 Jun 1;1:123-41.

48. Pastore S, Lulli D, Fidanza P, Potapovich AI, Kostyuk VA, De Luca C, Mikhal'chik E, Korkina LG. Plant polyphenols regulate chemokine expression and tissue repair in human keratinocytes through interaction with cytoplasmic and nuclear components of epidermal
growth factor receptor system. Antioxid Redox Signal. 2012 Feb 15;16(4):314-28. doi: 10.1089/ars.2011.4053.

49. Kostyuk VA, Potapovich AI, Lulli D, Stancato A, De Luca C, Pastore S, Korkina L. Modulation of human keratinocyte responses to solar UV by plant polyphenols as a basis for chemoprevention of non-melanoma skin cancers. Curr Med Chem. 2013;20(7):869-79.

50. Muñoz E, Avila JG, Alarcón J, Kubo I, Werner E, Céspedes CL. Tyrosinase inhibitors from Calceolaria integrifolia s.l.: Calceolaria talcana aerial parts. J Agric Food Chem. 2013 May 8;61(18):4336-43. doi: 10.1021/jf400531h.

51. Potapovich AI, Kostyuk VA, Kostyuk TV, de Luca C, Korkina LG. Effects of pre- and post-treatment with plant polyphenols on human keratinocyte responses to solar UV. Inflamm Res. 2013 Aug;62(8):773-80. doi: 10.1007/s00011-013-0634-z.

52. Korkina LG. Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. Cell Mol Biol (Noisy-le-grand). 2007 Apr 15;53(1):15-25.

53. Wartenberg M, Buddé P, De Marcéès M, Grünheck F, Tsang SY, Huang Y, Chen ZY, Hescheler J, Sauer H. Inhibition of tumor-induced angiogenesis and matrix-metalloproteinase expression in confrontation cultures of embryoid bodies and tumor spheroids by plant ingredients used in traditional chinese medicine. Lab Invest. 2003 Jan;83(1):87-98. doi: 10.1097/01.lab.0000049348.51663.2f.

54. Zhang F, Jia Z, Deng Z, Wei Y, Zheng R, Yu L. In vitro modulation of telomerase activity, telomere length and cell cycle in MKN45 cells by verbascoside. Planta Medica, 2002:68(29):115-8.

55. Lee KW, Kim HJ, Lee YS, Park HJ, Choi JW, Ho J, Lee KT. Acteoside inhibits human promyelocytic HL-60 leukemia cell proliferation via inducing cell cycle arrest at G0/G1 phase and differentiation into monocyte. Carcinogenesis. 2007 Sep;28(9):1928-36. doi: 10.1093/carcin/bgm126.

56. Mingyue Chen, Yaqin Zhang Bin Huang, Xueming Yang, Yunong Wu, Bin Liu, Yi Yuan, Gen Zhang. “Evaluation of the Antitumor Activity by Ni Nanoparticles with Verbascoside”. Hindawi Publishing Corporation, Journal of Nanomaterials, Volume, 2013: Article ID 62349, 6 pages,2013.http://doi.org/10.1155/2013/623497

57. Zhang Y, Yuan Y, Wu H, Xie Z, Wu Y, Song X, Wang J, Shu W, Xu J, Liu B, Wan L, Yan Y, Ding X, Shi X, Pan Y, Li X, Yang J, Zhao X, Wang L. Effect of verbascoside on apoptosis and metastasis in human oral squamous cell carcinoma. Int J Cancer. 2018 Aug 15;143(4):980-991. doi: 10.1002/ijc.31378.

58. Zhou L, Feng Y, Lin Y, Liu X, Sui H, Chai N, Chen X, Liu N, Ji Q, Wang Y, Li Q. Verbascoside promotes apoptosis by regulating HIPK2-p53 signaling in human colorectal cancer. BMC Cancer. 2014 Oct 5;14:747. doi: 10.1186/1471-2407-14-747.

59. Zhang Y, Yuan Y, Wu H, Xie Z, Wu Y, Song X, Wang J, Shu W, Xu J, Liu B, Wan L, Yan Y, Ding X, Shi X, Pan Y, Li X, Yang J, Zhao X, Wang L. Effect of verbascoside on apoptosis and metastasis in human oral squamous cell carcinoma. Int J Cancer. 2018 Aug 15;143(4):980-991. doi: 10.1002/ijc.31378.

60. Jia WQ, Wang ZT, Zou MM, Lin JH, Li YH, Zhang L, Xu RX. Verbascoside Inhibits Glioblastoma Cell Proliferation, Migration and Invasion While Promoting Apoptosis Through Upregulation of Protein Tyrosine Phosphatase SHP-1 and Inhibition of STAT3 Phosphorylation. Cell Physiol Biochem. 2018;47(5):1871-1882. doi: 10.1159/000491067.

61. Wartenberg M, Buddé P, De Marcéès M, Grünheck F, Tsang SY, Huang Y, Chen ZY, Hescheler J, Sauer H. Inhibition of tumor-induced angiogenesis and matrix-metalloproteinase expression in confrontation cultures of embryoid bodies and tumor spheroids by plant ingredients used in traditional chinese medicine. Lab Invest. 2003 Jan;83(1):87-98. doi: 10.1097/01.lab.0000049348.51663.2f.
62. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005 Mar-Apr; 55(2):74-108. doi: 10.3322/canjclin.55.2.74.

63. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol. 2006 May;71(10):1397-421. doi: 10.1016/j.bcp.2006.02.009.

64. Delazar A, Asnaashari S, Nikkhah E, Asgharian P. Phytochemical analysis and antiproliferative activity of the aerial parts of Scrophularia subaphylla. Res Pharm Sci. 2019 Jun;14(3):263-272. doi: 10.4103/1735-5362.258495.

65. Nga V T Constituents of the leaves of Pseudantherum carruthersii (Seem.) Guill var. atropurpureum (Bull.) Fosb. 2017, ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

66. Vinayagam A, Sudha PN. In Vitro Cytotoxicity Activity of Acteoside From Leucas Indica Flowers. Indian Journal of Applied Research, 2014:4 (2):1-3.

67. Harput US, Genc Y, Saracoglu I. Cytotoxic and antioxidative activities of Plantago lagopus L. and characterization of its bioactive compounds. Food Chem Toxicol. 2012 May;50(5):1554-9. doi: 10.1016/j.fct.2012.01.019.

68. Vasincu A, Neophytou CM, Luca SV, Skalicka-Woźniak K, Miron A, Constantinou Al. 6-O-(3″, 4″-di-O-trans-cinnamoyl)-α-l-rhamnopyranosylcatalpol and verbascoside: Cytotoxicity, cell cycle kinetics, apoptosis, and ROS production evaluation in tumor cells. J Biochem Mol Toxicol. 2020 Mar;34(3):e22443. doi: 10.1002/jbt.22443.

69. Zoi Papoutsi, Eva Kassi, Sofia Mitakou, Nektarios Aligiannis, Anna Tsiapara, George P. Chrousos, Paraskevi Moutsatsou. Acteoside and martynoside exhibit estrogenic/antiestrogenic properties. The Journal of Steroid Biochemistry and Molecular Biology, Volume 98, Issue 1,2006: 63-71

70. Ma YC, Zhang M, Xu WT, Feng SX, Lei M, Yi B. [Chemical constituents from Callicarpa nudiflora and their cytotoxic activities]. Zhongguo Zhong Yao Za Zhi. 2014 Aug;39(16):3094-101. Chinese.

71. Asmah Rahmat, Susi Edrini, Abdah Md. Akim, Patimah Ismail, Taufiq Yap Yun Hin and Mohd Fadzelly Abu Bakar. Anticarcinogenic Properties of Strobilanthes crispus Extracts and its Compounds in vitro. International Journal of Cancer Research, 2006: 2(1): 47-49.

72. Patricia Ramos, Sónia A.O. Santos, Ângela R. Guerra, Olinda Guerreiro, Laura Felicio, Eliana Jerónimo, Armando J.D. Silvestre, Carlos Pascoal Neto, Maria Duarte. Valorization of olive mill residues: Antioxidant and breast cancer antiproliferative activities of hydroxytyrosol-rich extracts derived from olive oil by-products, Industrial Crops and Products, Volume 46, 2013, Pages 359-368, https://doi.org/10.1016/j.indcrop.2013.02.020.