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The Effect of *Corchorus olitorius* L. Extract on Viability of Breast Cancer Cells: A friend or a foe?

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Abstract

This *Corchorus olitorius* L. is known to be a medicinal plant widely consumed in the Eastern Mediterranean region and has anti-bacterial, anti-inflammatory and anti-cancer properties. Breast cancer has one of the highest mortality rates among all cancers. Therefore, the main aim of the study is to evaluate the effectiveness of *C. olitorius* plant on viability of estrogen receptor (ER) positive breast cancer cell line, MCF-7. *C. olitorius* leaves first extracted with ethanol, then LC-MS/MS analysis was done for identification of phytochemicals. MTT assay was used for assessment of cell viability of MCF-7 cells. Cells were treated with *C. olitorius* extracts at five concentrations (5, 10, 20, 50, 100 µg/ml) and four different incubation periods (24, 48, 72, 96 h). LC-MS/MS analysis identified seven phytochemicals in the extract, mainly quercetin and caffeoylquinic acid derivatives. MTT results showed that the extract was only slightly effective in terms of reduction of cell viability at 50 and 100 µg/ml doses which were incubated for 24 and 48 h. Lower concentration doses did not show any effect in cell viability of MCF-7 cells. Longer incubation periods tend to increase cell viability of breast cancer cells. Quercetin identified within the extract might interfere with ER and promote MCF-7 cell proliferation. Therefore, in ER positive breast adenocarcinoma, quercetin intake and doses should carefully be monitored. More studies regarding the relationship between *C. olitorius*, quercetin and breast cancer should be done for further clarification of the topic.

Key Words: *Corchorus olitorius* L., MCF-7, Breast Cancer, Cell Viability

1. Introduction

Corchorus olitorius L. or molohiya in its traditional name belongs to Tiliaceae family in botany and is known to be a medicinal plant widely consumed in the Eastern Mediterranean area and the Middle East (İşeri et al. 2013). According to earlier studies *C. olitorius* was known with its diuretic, sedative and laxative properties (Duke, 1986). With enlightenment of recent literature, it has been shown that *C. olitorius* indeed has anti-bacterial, anti-cancer and anti-inflammatory effects (Pal et al. 2006;

Adegoke et al. 2009; Li et al. 2012; Handoussa et al. 2013; Soykut et al. 2018). *C. olitorius* contains phytochemicals such as quercetin, chlorogenic acid as well as antioxidant vitamins and minerals, which are thought to aid the exertion of its medicinal properties (Azuma et al. 1999; Handoussa et al. 2013).

Breast cancer is known to be the most common cancer in women and has a high mortality rate especially in the developing world (WHO, 2019). There are several factors which contribute to the development of breast cancer, including sex, genetics, breastfeeding and lifestyle. Hormones actively play part in breast cancer progression and so; cancer cells can be classified according to the presence of estrogen receptor (ER) (ER positive and ER negative cells) (WCRF, 2019). Use of medicinal plants with potential anti-cancer properties to either directly slow progression of cancer or to increase effectiveness of chemotherapeutic drugs/agents with dual action is regarded as a new approach for cancer treatment (Kasiri et al. 2019). Therefore the main aim of the study is to evaluate the effectiveness of phytochemical rich *C. olitorius* on the viability of breast cancer cell line, MCF-7.

2. Material and Method

2.1. Extraction

C. olitorius leaves were collected during the summer months and dried. The plant sample was registered with Near East Herbarium at Near East University under the Herbarium number 6904. The dried leaves of *C. olitorius* (100 g) were powdered (Waring Commercial Blender, United States of America, USA) and extracted with 80% ethanol while incubated overnight at room temperature with frequent stirring.

The extract was vacuum filtered and concentrated by rotary evaporator (BUCHI Rotavapor R-210). The extract was evaporated and lyophilized (Christ Alpha 1-4 LD Plus, Germany) and the final yield of crude extract was found to be 14.8 grams. Extraction composition, LC-MS/MS analysis was done by Becer et al. (2020).

2.2. Cell line and cell culture

MCF-7 cell line which is breast adenocarcinoma was purchased from ATCC: HTB-22. MCF-7 cells are also known for being estrogen and progesterone receptor positive, and HER2 negative. Cells were maintained in their usual medium; RPMI-1640 (Biochrom, FG 1215). In total, 10% heat inactivated fetal bovine serum (Capricorn Scientific, FBS-11B), 1% penicillin-streptomycin (Biochrom, A2213) and 1% glutamine amino acid (EMD Millipore, K0282) were also added to the culture medium for sustaining optimal medium. Cells were cultured in a humidified atmosphere at 37°C in 5% CO_2 until 80% confluency.

2.3. Cell viability – MTT assay

The extract was first dissolved with dimethylsulfoxide DMSO, (Sigma-Aldrich). The final concentration of DMSO in cell lines was less than 0.05% to prevent any possible effect on the cytotoxicity levels. Then extract was further diluted in culture medium with five concentrations; 5 µg/mL, 10 µg/mL, 20 µg/mL, 50 µg/mL and 100 µg/mL.

MCF-7 cells were seeded in 96-well culture dishes at a density of 5×10^4 cells in each well. The cells were treated with each extract dilutions and were incubated for 24, 48, 72 and 96 h. The cell viability was estimated by MTT assay. MTT solution (Biotium, #30006) was heated to 37°C, after the addition of 10 µl solution to each seeded well. The cells were then incubated for 4 h at 37°C in 5% CO_2. After this, 200 µl DMSO was added to each well to prevent crystallinization formazan salts. The absorbance was measured at 570 nm by a spectrophotometer (Versa Max, Molecular Device, Sunnyvale, USA).

3. Results and Discussion

3.1. Identification of Corchorus olitorius L. content

Identification of polyphenol content of *C. olitorius* extract was done by liquid chromatography high performance coupled with mass spectrometry (LC-MS/MS). In total, seven main polyphenolic compounds were identified. The results showed that the extract contains mainly derivatives of caffeoylquinic acid and quercetin such as 3-caffeoylquinic acid, quercetin glucoside, quercetin acetylglucoside, 3,5-dicaffeoylquinic acid (Table 1). Our results are coherent with the scientific literature. According to other studies *C. olitorius* was found containing nearly same content including caffeoylquinic acid and quercetin derivatives as well as antioxidant vitamins; ascorbic acid (Vitamin C) and *alpha*-tocopherol (Vitamin E) (Azuma et al. 1999; Handoussa et al. 2013). *C. olitorius* had shown to contain more Vitamin C and quercetin when compared with the richest sources for the reference component (Azuma et al. 1999; Bhagwat et al. 2011; Handoussa et al. 2013; Türkomp 2018). The identified compounds in the plant gives its strong antioxidant properties with the aid of free radical scavenging ability. Studies showed that *C. olitorius* was significantly able to reduce oxidative stress by increasing endogenous antioxidant enzymes; superoxide dismutase and catalase activities (Dawanjee et al. 2013; Boye et al. 2014,). Besides, *C. olitorius* due to its rich polyphenolic content showed reduction in Thiobarbituric acid reactive substances (TBARS) level which is known to increase oxidative stress due to lipid peroxidation (Dawanjee et al. 2013b).

Table 1. Identification of *Corchorus olitorius* L. extract content.

Rt	$[M-H]^-$	MS^2	Identified compounds
4.1	341	179, 161	Caffeoyl glucose
4.7	353	191, 179	3-Caffeoylquinic acid
9.9	463	299, 271, 255	Quercetin glucoside
10.9	505	299, 271, 255	Quercetin acetylglucoside
11.5	515	353, 191, 179, 173	3,5-Dicaffeoylquinic acid
12.1	515	353, 191, 179, 135	1,3-Dicaffeoylquinic acid
12.6	489	284, 255, 227	Luteolin / kaempferol acetylglucoside

**Adapted from Becer et al., 2020 (article ahead of publication)*

3.2. Cell viability and toxicity

MCF-7, breast adenocarcinoma cells were treated with five concentrations of *C. olitorius* EtOH extract at four different incubation period. Our results showed that the extract was only slightly effective in terms of reduction of cell viability at 50 and 100 µg/mL doses, incubated for 24 and 48 h. The maximum decline in cell viability of MCF-7 cells was observed at 100 µg/mL dose at 24 h (Figure 1). The decline was only about 10% which is not regarded as an efficient treatment dose. On the other hand, lesser concentrations and longer incubation periods not only cause any decrease in cell viability but increase breast cancer cell viability. Therefore, overall results suggest that the EtOH extract prepared from *C. olitorius* extract leaves is ineffective for reduction of cell viability and does not show cytotoxic effects in breast cancer cells *in vitro* condition.

There is a plausible mechanism behind the possibility of *C. olitorius* extract not showing sufficient effect in decreasing cell viability in breast cancer cells. It was stated earlier in the

article that the plant is rich in terms of phytochemical specifically quercetin and caffeoylquinic acid. Quercetin can be regarded as a phytoestrogen as it is an uncompetitive ligand for oestrogen receptors (Miodini et al. 1999). It is reported that at lower doses that can stimulate transcriptional activity, quercetin might promote cellular proliferation in MCF-7 cells through ERα domain (Maggiolini et al. 2001).

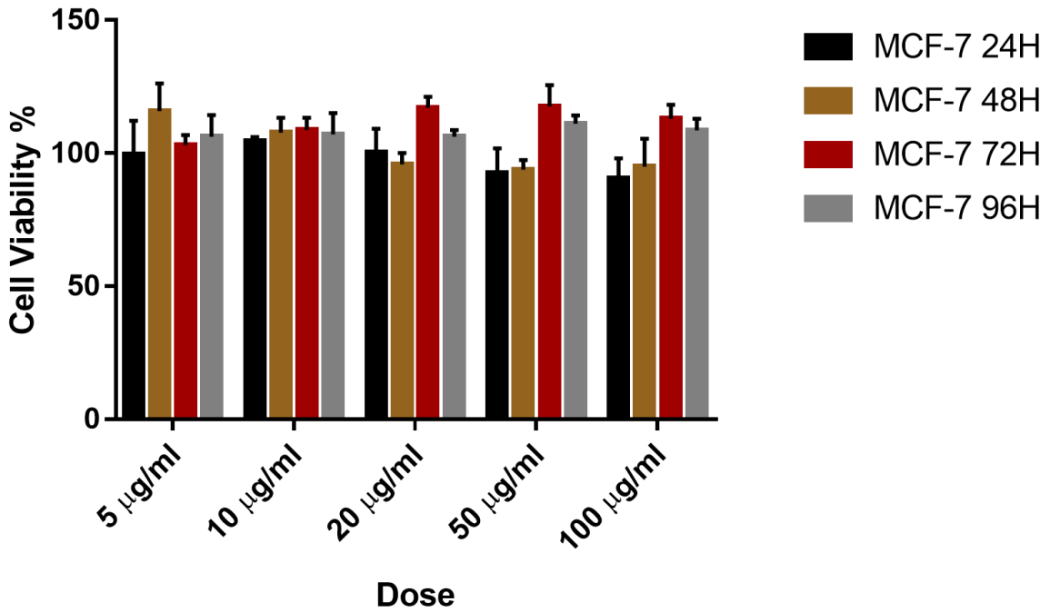

Figure 1. The effect of different concentrations of *C. olitorius* extract in MCF-7 breast adenocarcinoma cells at four incubation period (data are expressed as means ± SEM).

Conversely, at higher doses, quercetin can act as a cytotoxic agent that can kill MCF-7 cells in an ER independent way. Therefore, the dietary intake of quercetin should be carefully monitored in patients with breast cancer (Maggiolini et al. 2001). The quercetin derivatives may have different cytotoxic effects on MCF-7 cells. Therefore, the nature of the quercetin found in the nutrient content of foods should be established carefully (Kasiri et al. 2019). Additional to cytotoxic effects, quercetin may have apoptotic effects at 100µM concentration. This might be due to the generation of cellular reactive oxygen species (ROS) and increase in pro-oxidative state in MCF-7 cells. Quercetin may show phytotherapeutic effects through activating ROS dependent intrinsic apoptotic pathway (Wu et al. 2018). However, in our results, the EtOH extract of *C. olitorius* leaves did not exert an effective cytotoxic effect which further proves that its rich quercetin content inhibited MCF-7 cytotoxicity probably through the interaction of ER. Moreover, the extract increased cancer cell proliferation at longer incubation periods more specifically at 72 and 96 h. This might be due to quercetin increasing its activity during longer periods. One study discussed that quercetin might have the capacity to increase breast tissue carcinogenesis due to aiding the estrogen transformation of normal cells to malignant cells (Singh et al. 2010).

Another possible, suggested mechanism is quercetin in its glucoside form which was identified in our analysis, had shown lower affinity to estrogen receptors (Sørensen, 2018). Therefore, this can also reduce the potency of the extract for lowering the cell viability of MCF-7 cells. Contrary to other studies, which showed *C. olitorius* anti-cancer effects in different cell lines, our results stated increased cancer cell proliferation in

MCF-7 cells (Li et al. 2012; İşeri et al. 2013; Soykut et al. 2018).

4. Conclusion

Our results stated that *C. olitorius* plant contains strong phytochemicals which exert antioxidant properties such as quercetin and caffeoylquinic acid. These phytochemicals are usually effective at reduction in cancer cell proliferation. However, cell viability results stated that the EtOH extract of the leaves only showed a slight decrease in cell proliferation at 100 µg/ml dose, incubated for 24 h. Other doses were not effective at reducing cell proliferation and even caused increased in cancer cell proliferation. This effect might be due to quercetin found in plant extract interacting with ER positive MCF-7 cells by triggering transcriptional activity and promoting malignant cell proliferation. Therefore, people with ER positive breast carcinoadenoma should carefully monitor their quercetin intake as it might be a double-edged sword and might deteriorate the treatment process. However, it should be stated that more studies should be done for further clarification of the relationship between *C. olitorius*, quercetin and breast cancer.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Adegoke, A.A., Adeboye-Tayo, B.C., 2009. Phytochemical composition and antimicrobial effects of Corchorous olitorius leaf extracts on four bacterial isolates. Journal of Medicinal Plants Research, 3(3), 155-159.
2. Azuma, K., Nakayama, M., Koshioka, M., Ippoushi, K., Yamaguchi, Y., Kohata, K. et al., 1999. Phenolic antioxidants from the leaves of *Corchorus olitorius* L. Journal of Agricultural and Food Chemistry, 47, 10, 3963-3966. DOI 10.1021/jf990347p
3. Becer, E., Soykut, G., Kabadayı, H., Mammadov, E., Çalış, İ., Vatansever, S. 2020. Obtaining stem cell spheroids from foreskin tissue and the effect of *Corchorus olitorius* L. on spheroid proliferation. Turkish Journal of Phamaceutical Sciences. DOI: 10.4274/tjps.galenos.2019.05658 [article ahead of printing]
4. Bhagwat, S., Haytowitz, D.B., Holden J.M. 2011. USDA Database for the flavonoid content of selected foods (Report No: 3). Beltsville Human Nutrition Research Center: United States Department of Agriculture.
5. Boye, A., Barku, V.Y.A., Amoateng, P., Mbroh, L.A. 2014. Anti-nociceptive and antioxidant activities of an aqueous leaf extract of *Corchorus olitorius* L. (Tiliaceae). International Journal of Biological and Chemical Sciences, 8(6), 2395-2406. DOI 10.4314/ijbcs.v8i6.3
6. Dewanjee, S., Sahu, R., Karmakar, S. Gangopadhyay, M., 2013. Toxic effects of lead exposure in Wistar rats: Involvement of oxidative stress and the beneficial role of edible jute (*Corchorus olitorius*) leaves. Food Chemical Toxicology, 55, 78-91. DOI 10.1016/j.fct.2012.12.040
7. Duke, J.A. 1983 Handbook of Energy Crops; *Corchorus olitorus* L. Accessed online at 23.11.2015. https://hort.purdue.edu/newcrop/duke_energy/Corchorus_olitorius.html
8. Handoussa, H., Hanafi, R., Eddiasty, I., El-Gendy, M., El-Khatip, A., Linscheid, M. et al. 2013. Anti-inflammatory and cytotoxic activities of dietary phenolics isolated from *Corchorus olitorius* and *Vitis vinifera*. Journal of Functional Foods, 5, 1204-1216. DOI 10.1016/j.jff.2013.04.003
9. İşeri, Ö.D., Yurtcu, E., Sahin, F.İ., Mehmet, H., 2013. *Corchorus olitorius* (jute) extract induced cytotoxicity and genotoxicity on human multiple myeloma cells (ARH-77). Pharmaceutical Biology, 51(6), 766-770. DOI 10.3109/13880209.2013.765897
10. Kasiri, N., Rahmati, M., Ahmedi, L., Eskandari, N., Motedayyen, H. 2019. Therapeutic potential of quercetin on human breast cancer in different dimensions. Inflammopharmacology. DOI 10.1007/s10787-019-00660-y. [Epub ahead of print]
11. Li, G.J., Huang, S.Y., Wu, M.Y., Chen, Y.C., Tsang, S.F., Chyuan, J.H., et al. 2012. Induction of apoptosis by ethanolic extract of *Corchorus olitorius* leaf in human hepatocellular carcinoma (HepG2) cells

via a mitochondria-dependent pathway. Molecules, 17, 9348-9360. DOI 10.3390/molecules17089348.
12. Maggiolini, M., Bonofiglio, D., Marsico, S., Panno, M.L., Bruno Cenni, B., Picard, D., And`O, S., 2001. Estrogen receptor α mediates the proliferative but not the cytotoxic dose-dependent effects of two major phytoestrogens on human breast cancer cells. Molecular Pharmacology, 60(3), 595-602.
13. Miodini, P., Fioravanti L., Di Fronzo G., Cappelletti V., 1999. The two phyto-oestrogens genistein and quercetin exert different effects on oestrogen receptor function. British Journal of Cancer, 80(8), 1150-1155. DOI 10.1038/sj.bjc.6690479
14. Pal, D.K., Mandal, M., Senthilkumar, G.P., Padhiari, A, 2006. Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed. Fitoterapia, 77, 589-591. DOI 10.1016/j.fitote.2006.06.015
15. Singh, B., Mense, S.M., Bhat, N.K., Putty, S., Guthiel, W.A., Remotti, F., Bhat, H.K., 2010. Dietary quercetin exacerbates the development of estrogen-induced breast tumors in female ACI rats. Toxicology and Applied Pharmacology, 247(2), 83–90. DOI 10.1016/j.taap.2010.06.011
16. Soykut, G., Becer, E., Calis, I., Yucecan, S., Vatansever, S. 2018. Apoptotic effects of Corchorus olitorius L. leaf extracts in colon adenocarcinoma cell lines. Progress in Nutrition, 20, 4, 689-698. DOI 0.23751/pn.v20i4.6892
17. Sørensen, B.G., 2018. Quercetin - creating an estrogenic response. University of Copenhagen. Accessed online at 27.11.2019 https://natoxaq.ku.dk/toxin-of-the-week/quercetin/
18. Türkomp. 2018. Ulusal Gıda Kompozisyon Veri Tabanı, C Vitamini. Accesed online at 17.06.2018. http://www.turkomp.gov.tr/component_result-c-vitamini-32
19. WCRF, World Cancer Research Fund/American Institute for Cancer Research (2018). Diet, Nutrition, Physical Activity and Breast Cancer: A Global Perspective (Report No: 2018 revised). Dietandcancerreport.org: World Cancer Research Fund/American Institute for Cancer Research.
20. WHO World Health Organization. 2019. Breast Globacan 2018. Accessed online at 27.11.2019 https://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheet.pdf
21. Wu, Q., Needs, P.W., Lu, Y., Kroon, P.A., Daoyuan Rena, D., Yang, X., 2018. Different antitumor effects of quercetin, quercetin-3'-sulfate and quercetin-3-glucuronide in human breast cancer MCF-7 cells. Food & Function, 9, 1736–1746. DOI 10.1039/c7fo01964e