Effect of *Vitex agnus castus* fruits methanol extract against murine mammary adenocarcinoma cell line (amm3) and rat embryonic fibroblast normal cell line (ref)

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**Article History:**
Received on: 10.03.2019
Revised on: 15.06.2019
Accepted on: 20.06.2019

**Keywords:**
*Vitex agnus castus*, mammmary gland adenocarcinoma cell line, rat embryonic fibroblast cell line, antiproliferative activity

**ABSTRACT**

*Vitex agnus castus* showed antiproliferative activity in several previous studies. Angiogenesis is one of the targets in the remediation of cancer. This study aimed to demonstrate the effect of methanol fruits extract of *Vitex agnus castus* on mouse mammary gland adenocarcinoma cell line and rat embryonic fibroblast cell line. The cell lines used in this study were obtained from tissue culture unit/ Iraqi Center for Cancer and Medical Genetic Researches, Al-Mustansiriyah University was maintained in RPMI- 1640 tissue media after preparing from 10% fetal calf serum, antibiotics solution and other materials to make complete growth medium. Serial solutions of *Vitex agnus castus*, methanol crude extract have been tested on 10⁴ of AMN3 and REF in each well of 96 well plates. The results of the current study showed that the concentration that inhibits fifty percent of cell line after 72 hours of the experiment (IC50) was 129ug/ml for AMN3 and 1324ug/ml for REF cell line. The antioxidant activity of *Vitex agnus castus* may indicate the proliferation inhibition activity of *Vitex agnus castus* methanol extract. The study concluded that this extract might be of benefit if used in combination with other anti-cancer drugs as adjuvant therapy.

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ISSN: 0975-7538
DOI: [https://doi.org/10.26452/ijrps.v10i3.1393](https://doi.org/10.26452/ijrps.v10i3.1393)

**INTRODUCTION**

*Vitex agnus castus*, also called Chaste Tree, Abraham’s balm, chaste berry, hemp tree, kaff Maryam, lilac chaste tree, monks pepper, shajerat Ebrahim, vitex, wild lavender (*W.H.O.*, 1999). Regional to the Mediterranean area, European and Asia; it is widely distributed in the Middle East, southern United States and Canada, it is plowed in warm temperate and sub-tropical regions of the world and gained mostly from Mediterranean countries especially Albania and Morocco, it prefers full sun to partial shade in a well-drained soil (*Rani and Sharma*, 2013). Historically, VAC found in the ancient as an official plant, and it is named in the works of Hippocrates, Dioscrides, Theophrastus, and others. The first detailed medicinal hints came from Hippocrates, mentioning its use not only for injuries, inflammation and spleen enlargement, but also the leaves in wine used for the flow of blood and for the expulsion after birth (*Gerhard and R.M.*, 1938). Several common uses include premenstrual syndrome (*Cerqueira et al.*, 2017), abnormal uterine bleeding disorders, and mastodynia (*Seidlova-Wuttke and Wuttke*, 2017). Many scientists worked on this herb to isolate and identify active constituents. Flavonoids (quercetin, rutin,
luteolin, kaempferol), agnuside, alkaloids, diterpenoids, and steroid hormone have been specified in the chemical analysis of Vitex agnus castus (R, 2011). Breast cancer is a folk of diseases where cells in the breast tissue expand and divide without standard control (Charan et al., 2018). Breast cancer is the one more leading cause of death in women (Mezher et al., 2017). Different types of treatment are available for patients with breast cancer (Gradishar et al., 2017). Herbal medicines have a pivotal role in the prevention and treatment of cancer by using plants, or hash of plant extracts, to cure the tumor and consolidate health. With advanced knowledge of molecular science and improvement in isolation and structure clarification techniques, we are in a much better position to identify various antitumor herbs and develop the remedy that might cure tumor. This curative works due to the accurate chemical balance of the whole plant, or mixtures of plants, not one specific active ingredient (Korrapati et al., 2016), (Alam et al., 2013).

MATERIALS AND METHODS

Plant materials

The herb included in this study was identified and authenticated in Al-Mustansira University, Pharmacognosy Department. The fruits were scoured and dried at room temperature, then ground into a fine powder. 470 gm of Dried fruit powder of plant will have extracted with 85% methanol in reflex apparatus until complete attrition. The alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40 c to give a dark greenish-yellow residue designated as a crude fraction (Harborne, 1998).

Cell Lines

Mouse mammary gland adenocarcinoma cell line (AMN3) and rat embryonic fibroblast normal cell line were provided by tissue culture unit, ICCMGR (Iraqi Centre for Cancer and Medical Genetic Researches), Baghdad, Iraq. The cells were preserved in RPMI-1640 media (Roswell Park Memorial Institute -1640 medium) with 10% fetal calf serum and incubated at 37 ºC in the humid atmosphere of 5% CO2. Cell line propagation measured according to Mosmann method (Mosmann, 1983). The cells were between passages 129-132. The cell viability for each cell line mentioned above respectively. The IC50 values for crude of each cell line were calculated by the logarithmic equation in Figure 1 and Figure 2 respectively. It was equal to 129 ug/ml for AMN3 and 1324 ug/ml for the REF cell line.

In the current study, the in vitro effect of methanol extract of Vitex agnus castus was evaluated against AMN3 and REF breast cancer cell line to discuss if this extract has any cell viability inhibition. The crude methanol extract of Vitex agnus castus fruits showed efficient antiproliferative activity against the cancer cell line and less toxic effect on the standard REF cell line. Selective cytotoxicity is required merit of a new candidate anticancer agent (Guzmán-Rodriguez et al., 2015). The cell viability for methanol extract was tested by the MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. The MTT assay is a colorimetric assay for determining cell proliferation. NAD (P) H-dependent cellular oxidoreductase enzymes may, under specific conditions, reflect the number of
existing fertile cells. These enzymes are capable for reduction the tetrazolium dye MTT 3-(4, 5-dimethylthiazol-2-yl)-2, diphenyltetrazolium bromide to its insoluble formazan, which has a purple colour (Hayder et al., 2015). Methanol extract reduces the viability of AMN3, which is a breast tumor cell line. To behold any agent as cytotoxic against cell lines, its IC50 should be less than 20 μg/ml (Hayder et al., 2015) ; (Tan et al., 2005). These returns showed that methanol crude extract had significant dose-dependent effectiveness against the growth of the cells AMN3. At the same time, this extract did not have any cytotoxic activity at the utilized dose. The extract had toxicity at high concentration, so no toxic effect against the above-named cell can be predictable in vitro.

**CONCLUSION**

The antioxidant activity of *Vitex agnus castus* may indicate the antiproliferative activity of *Vitex agnus castus* crude methanol extract. The study also concluded that this crude extract might be of benefit if used in combination with other anti-cancer drugs as adjuvant therapy.

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