Effect of *Vitex agnus castus* fruits methanol extract against murine mammary adenocarcinoma cell line (AMN3) and rat embryonic fibroblast normal cell line (REF)

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**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 10∗4 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.

**INTRODUCTION**

*Vitex agnus castus*, also called Chaste Tree, Abrahams 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 State 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 *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-Mustansirya 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°C 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 cells were exposed with serial concentrations of crude methanol extract of *vitex agnus castus* for 72 hrs. 20μl of MTT was used per well, and the plates were incubated at 37°C, in 5% CO₂ for 5hrs. The plates were taken away from the incubator, and the supernatant was aspirated. DMSO (200μl) was added to each well. The plates were shaken forcibly for one minute at room temperature to dissolve the dark blue crystals. The absorbance reading was taken at 570nm and the reference at 650nm by using microplate reader. The absorbance of cells cultured in control media was taken to represent 100% viability. The viability of treated cells was determined as a percentage of that for the untreated control. Each concentration was tested in triplicate, and the experiment was refined twice. The concentration of the cells in each well was 1x10⁴. The percentage of cell line inhibition was determined as the mean ± SD using the following equation.

\[ 1-(A0-A1)/(A2-A1) \]

A1 = Absorbance of sample A0 = Absorbance of control IC50 values was calculated by the logarithmic correlation equation.

**RESULTS AND DISCUSSION**

In vitro experience of *Vitex agnus castus* methanol extract on AMN3 and REF cell lines, which were in passage 129-132 the results demonstrated a dose-dependent inhibition on the cell growth after 72hr. The crud extract concentrations used were 400, 200, 100, 50, 25, and 12.5μg/ml, with each concentration in triplicate, and the trials were repeated twice for each cell line. The data is explained as the mean ± SD. The percentages of the AMN3 and REF cell proliferation inhibition were (73.56 ± 2.92%, 55.33 ± 2.85%, 44.83 ± 2.53%, 30.44 ± 1.76%, 20.33 ± 1.92% and 9.10 ± 1.53%) (7.53 ± 0.53%, 7.13 ± 0.58%, 6.4 ± 0.34%, 5.56 ± 0.56%, 4.13 ± 0.23%, 3.63 ± 0.31%) for crude methanol extract at each concentration and cell line mentioned above respectively. The IC50 values for crud 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-Rodríguez 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
Figure 1: Cell proliferation effect of serial concentration of methanol crude extract on the REF cell line

Figure 2: Cell proliferation effect of serial concentration of methanol crude extract on AMN3 cell line

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 20μg/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.

REFERENCES


