

Research Article



Anti-proliferative activity of *Vitex agnus castus* methanol leaves extract on Human ductal breast epithelial tumor cell line T47D

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ABSTRACT

Vitex agnus castus showed antiangiogenic activity in previous study. As angiogenesis is one of the approaches in cancer remedy. The aim of this study was to identify the antiproliferative activity of the methanol extract of *Vitex agnus castus* on T47D (Human ductal breast epithelial tumor cell line). MTT (3-(4, 5-Dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay was used as a measure of cell line proliferation. Cell line was purchased from ATCC (American Type Culture Collection). RPMI-1640, (Gibco.UK) were used to maintain T47D. 10% of heat inactivated foetal calf serum (HIFCS) was purchased from (Gibco, UK), and 1% pen/strep (Penicillin/streptomycin) (Sigma-Aldrich, Germany) was added to this medium to make complete growth medium. Serial dilutions of *Vitex agnus castus* methanol extract have been tested on 10^4 of T47D in each well of 96well plate. 1, 1-diphenyl -2-picrylhydrazyl (DPPH) assay used to measure the free radical scavenging activity of *Vitex agnus castus* methanol extract. The results showed that, the concentration that inhibits fifty percent of cell line after 48 hours of experiment (IC_{50}) was (92.82 μ g/ml). While the concentration that scavenge fifty percent of free radical was (126.79mg/ml). The antioxidant activity of *Vitex agnus castus* may elucidate the antiproliferative capability of *Vitex agnus castus* methanol extract. The study concluded that this extract may be of beneficial if use in combination with other anti-cancer drugs as adjuvant therapy.

Keywords: Anti-proliferative, *Vitex agnus castus*, Antioxidant, Breast cancer cell line.

INTRODUCTION

Vitex agnus castus, also called Vitex, Chaste Tree, Chasteberry, Abraham's Balm or Monk's Pepper, is a native of the Mediterranean region¹. It is one of the few temperate-zone species of Vitex, which is on the whole a genus of tropical and sub-tropical flowering plants^{2,3}. Vitex, its name in Pliny the Elder, is derived from the Latin vieo, meaning to weave or to tie up⁴; its macaronic specific name repeats "chaste" in both Greek and Latin. Now a day *Vitex agnus castus* is used to alleviate symptoms of various gynecological problems. All evidence is limited to standardised controlled extracts such as used in Germany, different extracts or herbal mixes may have significantly different properties and safety issues⁵. Some of the modern uses include premenstrual syndrome, abnormal uterine bleeding disorders and mastodynia⁶. Many scientists worked on this herb to isolate and identifying the active constituents. Flavonoids (vitexin, casticin), agnuside, p-hydroxybenzoic acidalkaloids, diterpenoids and steroidal hormone precursors have been identified in the chemical analysis of *Vitex agnus castus*⁷.

Breast cancer

The breast cancer is the second leading cause of death in women after cervical cancer⁸. Different types of treatment are available for patients with breast cancer. Some treatments are standard (the currently used treatment), and some are being tested in clinical trials. Herbal medicine uses plants, or mixtures of plant extracts, to treat illness and promote health. It aims to restore the body's ability to protect, regulate and heal itself⁹. It is a

whole body approach it is sometimes called phyto-medicine, phytotherapy or botanical medicine. Many modern drugs are made from plants. But herbalists don't extract plant substances in the way the drug industry does. Herbalists believe that the remedy works due to the delicate chemical balance of the whole plant, or mixtures of plants, not one particular active ingredient¹⁰.

MATERIALS AND METHODS

The leaves were collected from white flowered plant. Leaf specimen was labeled and annotated with date of collection and locality. Voucher specimen number (2) was deposited at the Herbarium, College of pharmacy, Karbala University. The plant was oven dried at 40oC. The dried leaves were separated and then ground into powder. The dried powder leaves (400gm) were extracted by adding 33gm in each of the twelve flasks with 200ml of methanol with continuous shaking by using the water bath for eight hours at 40°C, then filtration done by using filter paper watman 20cm¹¹.

Assessment of proliferation inhibition of cancer cell line T47D

The (3-(4, 5-Dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) MTT assay was used as a measure of cell line proliferation according to Mosmann method. All of the cells were between passages 4-7. The cells were treated with several concentrations of Vitex agnus castus methanol extract for 48 hrs. MTT was prepared by adding 5mg/ml in PBS (phosphate buffer saline). 20 μ l of MTT was used per well and the plates were incubated at 37°C, in 5% CO₂ for 5hrs. The plates were removed from the



incubator and the supernatant was aspirated. DMSO (200 μ l) was added to each well. The plates were shaken vigorously 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 micro-plate 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 quadruplicate, and the experiment was repeated twice. The concentration of the cells in each well was 1×10^4 , the percentage of cell line inhibition was determined as the mean \pm SD, using the following equation.

$$1-(A0-A1)/(A2-A1)$$

A0 = Absorbance of sample

A1 = Absorbance of blank

A2 = Absorbance of control

IC₅₀ values were calculated by the linear equation¹².

1, 1-diphenyl -2-picrylhydrazyl (DPPH) free radical scavenging activity

The free radical scavenging activity of the *Vitex agnus castus* methanol extract was measured by DPPH methods. One ml of 0.1 mM solution of DPPH in methanol was added to 2ml *Vitex agnus castus* methanol extracts with the following concentrations (0.5, 0.25, 0.12, 0.062, 0.031, 0.015, and 0.007mg/ml); after 30min, absorbance was measured at 517nm. All concentrations of *Vitex agnus castus* extract were tested in triplicate. Percentage reduction of DPPH (Q) was calculated according to the formula below¹³.

$$Q=100 \times (A0-AC) / A0$$

Where

A0= Absorbance of control

AC=Absorbance of the two samples after 30 min incubation.

RESULTS

Activity of *Vitex agnus castus* methanol leaves extracts on T47D breast cancer cell line

In vitro screening of *Vitex agnus castus* methanol extract on T47D hormonal dependant breast cancer cell line, which were in passage 7 the results showed a dose-dependent inhibition on the cell growth after 48hr. The extract concentrations used were 200, 100, 50, 25, 12.5 and 6.25 μ g/ml, with each concentration in quadruplicate and the experiments were repeated twice. The data is represented as the mean \pm SD. The percentages of the T47D cell proliferation inhibition were 92.2 \pm 0.01%, 78.27 \pm 0.01%, 33 \pm 0.05%, 8.7 \pm 0.1%, 4.3 \pm 0.02% and 1.1 \pm 0.06% for methanol extract at each concentration mentioned above respectively. The IC₅₀ value was deduced from the graph for the methanolic extract of

Vitex agnus castus, was calculated by using the following linear regression equation below: $Y=0.5064(X) + 3.02$, where Y= the percentage of inhibition and X= concentration. The IC₅₀ value for ME was 92.82 μ g/ml. Figure 1 shows the dose response curve.

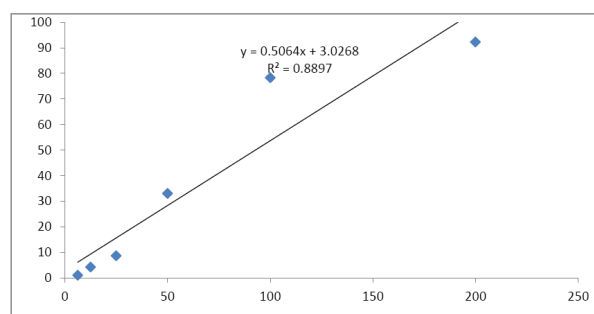


Figure 1: Dose response curve of *Vitex agnus castus* methanol extract on T47D

DPPH Assay for *Vitex agnus castus* methanol extract

Figure 2 shows the dose response curve of methanol extract of *Vitex agnus castus* on DPPH scavenging activity. The data is represented as mean \pm SD. Serial dilution of the concentrations ranged of 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0156 and 0.0078mg/ml was used throughout. Methanol was used as a solvent, each concentration was triplicate, IC₅₀ of the DPPH scavenging activity, of ME, was calculated by the linear regression equation.

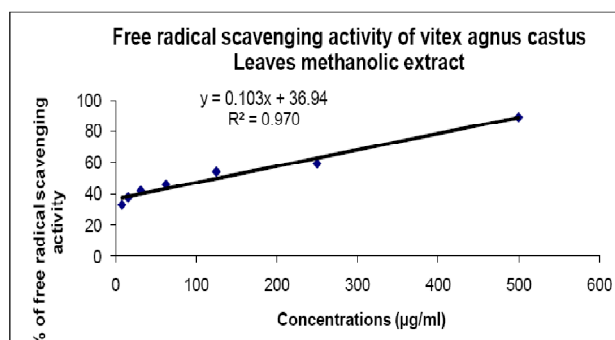


Figure 2: The dose response curve of serial dilutions of *Vitex agnus castus* methanol leaves extract on DPPH free radical scavenging activity

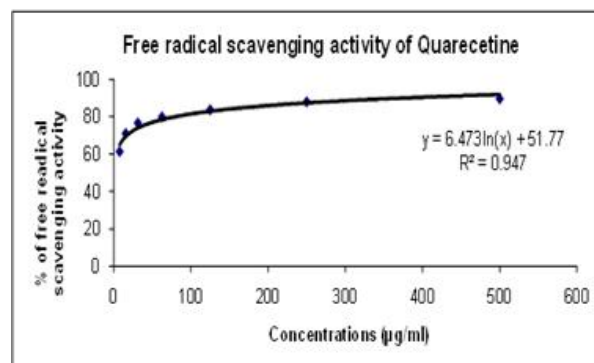


Figure 3: The dose response curve of serial dilutions of quercetin on DPPH free radical scavenging activity

The equation were as follows $Y=0.103X+36.94$, where Y= Percentage of DPPH scavenging activity and

X=concentration. Y is the percentage of scavenging and it is set to be 50%. The IC₅₀ of DPPH scavenging activity for ME 126.79mg/ml. Figure 3 showed the dose response curve of the positive control, Quercetin used as positive control and it showed scavenging activity through the equation $6.47\ln(x) + 51.77$ the IC₅₀ was 1.30mg/ml.

DISCUSSION

The methanol extract of *Vitex agnus castus* showed potent anti-angiogenesis activity against the rat aorta; and the anti-angiogenic agents may have anti-tumour activity⁷. Most of the clinically used anti-tumour agents possess significant cytotoxic activity in cell culture systems¹⁴. In the present study, the *in vitro* effect of methanol extract of *Vitex agnus castus* was evaluated against T47D breast cancer cell line to investigate if this extract has any cytotoxicity. Selective cytotoxicity is a desired feature of a new candidate anticancer agent. The cytotoxicity 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 assessing cell viability. NAD (P) H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present.¹⁵ These enzymes are capable of reducing the tetrazolium dye MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple colour. Other closely related tetrazolium dyes including XTT, MTS¹⁵. Methanol extract decreases the viability of T47D which is breast cancer cell line. To consider any agent as cytotoxic against cell lines, its IC₅₀ should be less than 20µg/ml¹⁶.

These finding showed that ME had a significant dose-dependent efficacy against the growth of the cells T47D. At the same time, this extract did not have any cytotoxic activity at the applied dose. The extract had toxicity at high concentration, so no toxic effect against the above named cell can be expected *in vitro*¹⁶.

Free radical scavenging activity of *Vitex agnus castus* leaves extracts (DPPH Assay)

Free radical scavenging activity for the methanol extract was important to be test to understand the mechanism of action. The presence of flavonoids and terpenes in *Vitex agnus castus* may elucidate the cell line proliferation inhibition mechanism, as both groups may show their pharmacological effect through antioxidant properties.¹⁷ Possible mechanisms of action were suggested. Polyphenols decreased Nitric oxide (NO) secretion. This effect correlates with their antiproliferative action and the inhibition of inducible NO synthase. It is therefore proposed that the antiproliferative effect of polyphenols is mediated through the modulation of NO production. In conclusion, the data show a direct inhibitory effect of high concentrations of methanol extract on the proliferation of human T47D Breast cancer cell lines mediated by the production of NO¹⁸.

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