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Cytotoxicity of *Vitex trifolia* leaf extracts on MCF-7 and Vero cell lines

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Abstract

Pharmacological and preventive properties of *Vitex trifolia* leaf extracts are well known, but the anticancer activity of methanol and petroleum ether extracts of *Vitex trifolia* leafs on human breast cancer cells have not been explored so far. Therefore, the present study was designed to investigate the cytotoxic activities of these extracts against MCF-7 and Vero cell line. Cells were exposed to 125 to 500 µg/ml of the extracts of *Vitex trifolia* for 72 h. Post-treatment, percent cell viability was studied by 3-(4, 5-dimethylthiazol-2yl)-2, 5-biphenyl tetrazolium bromide (MTT) assays. The results showed that petroleum ether and methanol extracts significantly reduced cell viability of MCF-7 cells in a concentration dependent manner. Concentrations of 125 µg/ml and above of petroleum ether and 500 µg/ml of methanol extract were found to be cytotoxic in MCF-7 cells. Cell mortality at 125, 250 and 500 µg/ml of petroleum ether extract was recorded as 98.80%, 93.40% and 87.75% respectively, whereas at 125, 250 and 500 of methanol extract values were 79.98%, 75.70% and 70.25%, respectively by MTT assay. MCF-7 and Vero cells exposed to 125, 250 and 500 µg/ml. The data revealed that the treatment with petroleum ether and methanol of *Vitex trifolia* leaf extract induced cell death in MCF-7 cells. Meanwhile the same extract showed a moderate inhibition against Vero cell lines. It may be concluded that *Vitex trifolia* can cause cell death in MCF-7 cancer cells, which can be considered as a promising chemotherapeutic agent in breast cancer treatment.

Keywords: *Vitex trifolia*, MCF-7, Vero, Cytotoxicity, MTT.

Introduction

Cancer is the major cause of mortality in the world and it claims more than 6 million lives each year.[1] Methods commonly used for the treatment of cancer, although possess some benefits, but still there is a significant need to improve current cancer therapies and search for novel compounds.[1] Breast cancer is one of the most common causes of the cancer in females in the whole world.[2] It has been observed that breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represents 13.7% of all cancer deaths due to the breast cancer in male and female.[3] It is the most frequent cancer in both developed and developing regions, but the rate of human breast cancer is higher in developing countries in compared to developed nations.[3]

Over the past several decades, there has been a particular interest in the role of medicinal plant extracts in cancer prevention. Plants are rich sources of chemically diverse compounds, many with beneficial properties to human health. Consequently, about 50% of the anticancer therapeutic agents known are derived from plants.[4] For example, compounds such as Taxol and Vinca alkaloids act to destabilize the microtubules of cancer cells, preventing the rapid proliferation of tumors.[5]

Vitex trifolia is basically a sea side shrub from the family Lamiaceae or Verbenaceae. The *Vitex* genus family is comprised of about 250 species of shrubs and trees; it's widely cultivated in warm temperate and subtropical regions.[6] *V. trifolia* L. is a shrub or shrubby tree that may grow up to 6 m. Its origin is unknown and several varieties have been described in distant countries as India and Mexico and Northern Sudan.[7] Several *Vitex* species are used as folk remedies in Mexico. *Vitex. mollis* is reported as a remedy to alleviate dysentery, as well as an analgesic and anti-inflammatory medicine; other folk uses include the treatment of scorpion stings, diarrhea and stomach ache.[8] Antimalarial, antimicrobial, and antifungal activity have been reported in *V. gaumeri*, *V. agnus-cas-tus* and *V. negundo*, respectively; *V. negundo* is also used as an anti-inflammatory agent, *Vitex negundo* (Family: Verbenaceae), is an

important medicinal plant found throughout India. *Vitex trifolia* extracts from the leafs and roots are the most important in the field of medicine and drug. Its leaves[9] and seeds[10], are widely used externally for rheumatism and inflammations of joints and are also reported to have insecticidal properties. Internally, decoction of its leaves is taken as diuretic, expectorant, vermifuge, tonic and febrifuge. The chemical components of the essential oil of leaf isolated from *Vitex negundo* and other species while *Vitex gaumeri* is used to treat colds and coughing spells.[11, 12] It is well known that a considerable number of plant species, besides their popular use as medicine in many countries, In India some species are present *Vitex glabrata, Vitex leucoxylon, Vitex penduncularis, Vitex pinnata,* and *Vitex trifolia*[13], possess insecticidal activities.

Vitex trifolia was selected to evaluate the activity of petroleum ether and methanol crude extracts against human breast cancer MCF-7 cell line also the cytotoxicity against Vero cell line was evaluated.

Materials and Methods

Plant materials

Vitex trifolia study was collected from, West Sudan, collected on February 2012. The taxonomic identification of the plant was carried out at Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Center for Research by W.E.A/Alla. A voucher specimen was deposited at the herbarium of the institute. The (Leafs) were air-dried at room temperature (28-30^{o}C) for three weeks and coarsely ground to powder by a mechanical grinder.

Table (1): *Vitex trifolia* selected to be investigated against MCF-7 and Vero cell line

Scientific Name	Family name	Part Used	Yield percentage		Traditional medicine
			Petroleum ether	Mrthanol	
Vitex trifolia	Verbenaceae	Leaf	3.8	23.9	Used to treat colds, diarrhea, dysentery, inflammation, itch, measles, sore throat, wounds and sexually transmitted diseases, antimalarial, antimicrobial.

This table indicates the scientific names, families, parts used, yield percentage based on the dried weight of methanol and petroleum ether extracts and traditional uses of *Vitex trifolia.*

Preparation of crude extracts

30 grams of the coarsely ground material of the leaf were successively extracted for by soxhlet apparatus using petroleum ether, and methanol. The extracts were then filtered and evaporated under reduced pressure using rotatory evaporator apparatus.

Chemicals and consumables

MEM culture medium, RPMI medium, antibiotics- atimycotic solution, fetal bovine serum (FBS) and trypsin were purchased from Invitogen, Life Sciences, USA. Consumables and culture wares used in the study were procured from Nunc, Denmark. Ethanol and all other specified reagents and solvents were purchased from Sigma Chemical Company Pvt. Ltd. St. Louis, MO, USA.

Cell lines

The cell lines MCF-7 (human breast adenocarcinoma) and Vero cells (Normal, African green monkey kidney) were provided from Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), National Centre for Research Khartoum. Sudan. Each cell line was cultured in a suitable medium to obtain the desired growth and the growth curve of each cell line was plotted.

Cytotoxicity Screening

Microculture tetrazolium MTT assay was utilized to evaluate the cytotoxicity of the studied plants.

Microculture Tetrazolium (MTT) Assay

Principle of MTT assay

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl) -2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.[14]

Preparation of *Vitex trifolia* extracts for MTT assay

Using a sensitive balance 5 mg of each extracts was weighed and put in eppendorf tubes. 50 µl of DMSO were added to the extract and the volume was completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by a magnetic stirrer to obtain a homogenous solution.

Cell Line and Culturing Medium

Vero (Normal, African green monkey kidney) and MCF-7 (human Breast Cancer) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37^{o}C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passage weekly, and the culture medium was changed twice a week.

Cell counting

Cell counts were done using the improved Neubauer chamber. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with equal volume of 0.4% Trypan blue in a small tube. The chamber was charged with cell suspension. After the cells had settled, the chamber was placed under light microscope. Using 10 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. The following formula was used for calculating cells:

$$\text{(Cells/ml) } N = \frac{\text{Number of cells counted X dilution factor X } 10^4}{4}$$

MTT procedure

The monolayer cell culture formed in the culturing flasks was trypsinized and the cells were put in centrifuging tube and centrifuged for 5 minutes separating the cells from the supernatant that flicked out. 1 ml complete medium was added to the cells and all the cell suspension was contained in a basin. In a 96- well microtitre plate, serial dilutions of each extracts were prepared. 3 duplicated concentrations for each extracts i.e. 6 wells for each of 8 extracts. All wells in rows A, B and C were used in addition to first 4 wells from each rows D, E and F. The first 2 wells of row G were used for the negative control and the first 2 wells of row H were used for the positive control (Triton X). 20 µl complete medium pipetted in all wells in rows B, C and mentioned wells of rows E and F. Then 20 µl from each extracts were pipetted in rows A and B and first 4 wells of rows E and F. 20 µl taken from row B were pipetted and mixed well in row C from which 20 µl were taken and flicked out. The same was done from E to F. After that 80 µl complete medium were added to all used wells. Then adjusting the cell account to 3000 cell/well, 100 µl of cell suspension were added completing all wells to the volume 200 µl. Now, we have duplicated three concentrations 500, 250, 125 µg/ml for each extract. Then the plate was covered and incubated at 37°C for 96 hours.

On the fourth day, the supernatant was removed from each well without detaching the cells. MTT suspension stock (5 mg/ml) prepared earlier in 100 ml phosphate buffer solution (PBS) was diluted (1:3.5) in a culture medium. To each well of the 96-well plate, 50 µl of diluted MTT were added. The plate was incubated for further 4 hours at 37°C. MTT was removed carefully without detaching cells, and 100 µl of DMSO were added to each well. The plate was agitated at room temperature for 10 minutes then read at 540 nm using microplate reader. The percentage growth inhibition was calculated using the formula below:

% Cell inhibition = 100-{(Ac-At)/Ac} × 100

Where, **At** = Absorbance value of test compound; **Ac** =Absorbance value of control.

Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program Student t test was used to determine significant difference between control and plant extracts at level of $P< 0.05$.

Results

Identification of medicinal plants with significant cytotoxic potential useful for the development of cancer therapeutics has gained increasing importance in the last decade, and research in this field is still expanding. In the present study, the yield % of *Vitex trifolia* leafs petroleum ether, methanol extract was 3.8, 23.9 respectively, the cytotoxic effect of *Vitex trifolia* extracts against human cancer cell lines MCF-7, and also the normal cell line Vero, was determined using the MTT assay. The results have been summarized in tables 1 and 2.

The extracts from *Vitex trifolia* showed significant anticancerous activity against MCF-7 cell line in all concentrations. Anticancer activity of *Vitex trifolia* petroleum ether extract against breast cancer cell line MCF-7 through MTT assay reveals that maximum inhibition of 96.80%, 93.40% and 87.75% was found at 500 ppm, 250 ppm, and 125 ppm respectively with IC_{50} 0.41 µg/ml. Meanwhile the methanol extract showed 79.89%, 75.70% and 70.25% at concentrations 500 ppm, 250 ppm, and 125 ppm respectively with IC_{50} 6.72 µg/ml. *Vitex trifolia* extracts had moderate cytotoxicity with 59.20% Inhibition in 500 ppm and IC_{50} >100 µg/ml with methanolic extract and 54.55% Inhibition in 500 ppm and IC_{50} >100 µg/ml in the experiment for their cytotoxicity activity against Vero cells by using MTT assay table (1). The results of cytotoxicity evaluation of extract was ranging from (500 to 125) µg/ml as shown in Figure (1-2) and table (2-3)

Table 2: Inhibition percentage and IC_{50} of *Vitex trifolia* MTT assay against MCF-7 cell line

Name of plant (part)	Concentration (µg/ml)	Petroleum ether		Methanol	
		Inhibition (%) ± SD	IC_{50} (µg/ml)	Inhibition (%) ± SD	IC_{50} (µg/ml)
Vitex trifolia (leafs)	500	98.80 ± 0.00	0.41	79.89 ± 0.04	6.72
	250	93.40 ± 0.01		75.70 ± 0.04	
	125	87.75 ± 0.04		70.25 ± 0.16	
*Control		98.3 ± 0.01		98.3 ± 0.01	

Table 3: Inhibition percentage and IC_{50} of of *Vitex trifolia* MTT assay against Vero cell line

Name of plant (part)	Concentration (µg/ml)	Petroleum ether		Methanol	
		Inhibition (%) ± SD	IC_{50} (µg/ml)	Inhibition (%) ± SD	IC_{50} (µg/ml)
Vitex trifolia (leafs)	500	54.55 ± 0.21	369,77	59.20 ± 0.31	349.07
	250	43.52 ± 0.65		43.61 ± 0.15	
	125	-3.04 ± 0.16		10.48 ± 0.12	
*Control		95.3 ± 0.01		95.3 ± 0.01	

Key : *Control = Triton-x100 was used as the control positive at 0.2 µg/mL.
The maximum concentration used was 500 µg/mL. When this concentration produced less than 50% inhibition, the IC_{50} cannot be calculated.
This table indicates the % inhibition of Vero cell line growth *in vitro* by methanolic extract and Petroleum ether extract of the *Vitex trifolia* (leafs). MTT colorimetric assay was used. Reading in triplicate for different concentrations 500-125 µg/mL.

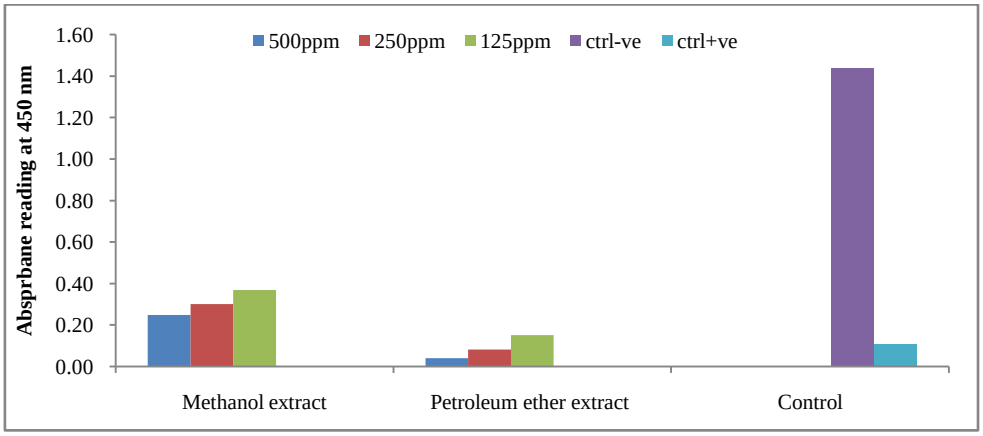

Figure 1: MTT reduction cytotoxic assay for evaluation of *Vitex trifolia* extracts against MCF-7 cell lines

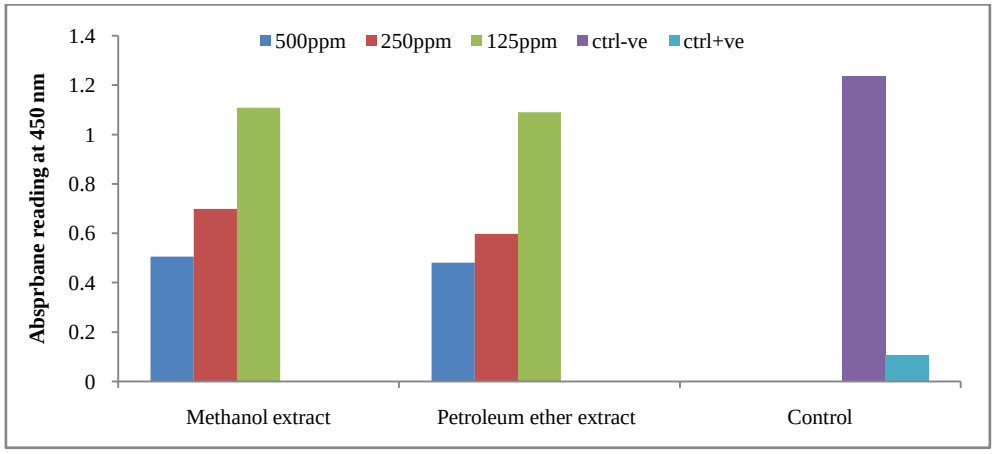

Figure 2: MTT reduction cytotoxic assay for evaluation of *Vitex trifolia* extracts against Vero cell lines

Discussion

World Health Organization investigation shows that 80% of world populations relies on traditional medicines.[15] Of these, at least 30% utilized medicinal plants from clinical indication.[16] Available literatures on medicinal plants indicate that promising phytochemicals can be developed for many health problems.[17-19] Plant extracts as a traditional remedies are already being used to treat a variety of diseases, including cancer.[20-24] The utilization of medicinal plants is more common in underdeveloped countries[25], and experimental studies showed that the extracts of the various plants can also protect against breast cancer cells.[19]

Vitex trifolia leaf extracts were tested for their 72 hours effect on MCF-7 human breast cancer cell lines and Vero normal, African green monkey kidney using the MTT bioassay and the results are presented in Tabel 1 and 2. Among all the extracts tested, petroleum ether extracts showed a strong inhibition against the MCF-7 cell lines proliferation with the IC_{50} of 0.41 µg/ml methanolic extracts had IC_{50} values of 6.71 µg/ml, the same extracts showed higher IC_{50} values with the Vero cell line which were regarded weak cytotoxicity. With the high prevalence of cancer cases, searching for naturally occurring agents that may inhibit cancer development is becoming an important objective for scientists. Primates, anatomically and physiologically similar to human, are a potential source of new drugs or lead compounds for chemoprevention or chemotherapy of human diseases. So, the search for anticancer agents on the basis of follow-up of primate uses of plants is a new approach that is highly possible to get new anticancer drugs or lead compounds of plant origin. In this study, we showed that the extracts of plants ingested by primates inhibited the growth of MCF-7 breast cancer cell lines and had strong cytotoxicity in a concentration-dependent manner. As shown in Table 2 that all tested extracts showed a variety of IC_{50} values in inhibiting MCF-7 cancer cells proliferation. These values indicated the cytotoxicity level of the extracts, the lower IC_{50} values the higher toxicity. So, based on the IC_{50} values, the cytotoxicity level of the extracts might be divided into strong (<100 µg/ml), moderate (101-200 µg/ml), and weak (>200µg/ml). The extracts of *Vitex trifolia* leafs which showed a strong inhibition against the MCF-7 cell lines and weak inhibition against the Vero cell lines. In addition, the *Vitex trifolia* extracts were non-toxic to the Vero cell lines this result agree with Zullies Ikawati who studied the acute toxicity of the extract combination of *V. trifolia* leaves in rats and the LD_{50} value showed that the highest dose can be administered without lethal effect, indicating that the extract has became safe.[26]

Conclusion

In conclusion *Vitex trifolia* can be a better candidate for isolation of cytotoxic and anticancer compounds, specially petroleum ether, extract and fraction of *Vitex trifolia.* On the basis of present investigation this plant species can be further investigated for pharmaceutical applications and achievement of novel anticancer compounds.

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References

1. Chermahini SH, Adibah F, Majid A, Sarmidi MR, Taghizadeh E and Salehnezhad S. Impact of saffron as an anti-cancer and anti-tumor herb. Afri. J. Pharm. Pharmacol., 2010; 4: 834-840.

2. World Cancer Report (WCR). International agency for research on cancer. Retrieved 2008; 02-26.

3. Ferlay JBF, Pisani P, Parkin DM. GLOBOCAN 2000: Cancer incidence, mortality and prevalence worldwide, Version 2000; 1.0. 2001.

4. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. Life Sci.2005; 78: 431-441.

5. Prasain, J.K., and Barnes, S. Metabolism and bioavailability of flavonoids in chemoprevention: current analytical strategies and future prospectus. Mol. Pharm. 2007; 4(6):846-864.

6. EL-Kousy S, Mohamed M, Mohamed S. Phenolic and biological activities of *Vitex trifolia* aerials parts. Life Sci J, 2012; 9(2):670-677.

7. McMillan X. A concise dictionary of plants cultivated in the United States and Canada. In: Bayley, L.H. (Ed.). Hortorium. Cornell University, New York, pp. 1976; 1161-1162.

8. Argueta A, Cano LM, Rodarte ME. Atlas de las Plantas de la Medicina TradicionalIy III. Instituto Nacional Indigenista; 1994;537-538.

9. Dharmasiri MG, Jayakody JRAC, Galhena G. Anti-inflammatory and analgesic activity of mature fresh leaves of *Vitex negundo*. Journal of Ethnopharmacology, 2003;87(2-3):199-206.

10. Chawla AS, Sharma AK, Handa SS, Dhar KL. Chemical investigation and inflammatory activity of *Vitex negundo* seeds. J. Nat. Prod, 1992; 55 (2): 163-167.

11. Ekundayo O, Laakso I, Holopainen M, Hiltunen R, Oguntimein B, Kauppinen V. The chemical composition and antimicrobial activity of the leaf oil of *Vitex agnus*-castus L. J. Essent. Oil Res. 1990;2(3):115-119.

12. Damayanti M, Susheela M, Sharma GJ. Effect of plant extracts and systemic fungicide on the pineapple fruit-rotting fungus, *Ceratocystis paradoxa*. Cytobios; 1996; 86(346):155-165.

13. Wealth of India, 1976. Raw Materials. CISR, New Delhi, 1976, 10, pp. 522.

14. Patel S., Gheewala N., Suthar A., and Shah A. *In-vitro* cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and Vero cell line. International Journal of Pharmacy and Pharmaceutical Sciences, 2009;1(1):38-46.

15. WHO. Regional Office for Western Pacific, research guidelines for evaluating the safety and efficacy of herbal medicines. Manila, Philippines: 1993; WITO.

16. Martins ER, Mitsugui SY, Silvia AV. Da colheita a comercialização. In: Martins, E.R. (org) Plantas Medicinais. 1aed. Viçosa, Departamento de Fitotecnia/Universidade Federal de Vicosa,1992; 1-27.

17. Gupta SS. Prospects and perspectives of natural plant products in medicine. Indian J Pharmacol, 1994;26:1-12.

18. Rodeiro I, Donato MT, Martinez I. Potential hepatoprotective effects of new Cuban natural products in rat hepatocytes culture. Toxicol in Vitro, 2008; 22, 1242-1249.

19. Farshori NN, Al-Sheddi ES, Al-Oqail MM. Hepatoprotective potential of *Lavandula coronopifolia* extracts against ethanol induced oxidative stress-mediated cytotoxicity in HepG2 cells. Toxicol Ind Health, Epub ahead of print 2013.

20. Zheng GQ, Kenney PM, Zhang J. Inhibition of benzo a pyrene-induced tumorigenesis by myristicin, a volatile aroma constituent of parsley leaf oil. Carcinogenesis, 1992;13,1921-1923.

21. Svejda B, Aguiriano-Moser V, Sturm S. Anticancer activity of novel plant extracts from trailliaedoxa gracilis (W. W. Smith & Forrest) in human carcinoid KRJ-I cells. Anticancer Res, 2010;30:55-64.

22. Khan MA, Chen HC, Tania M. Anticancer activities of *Nigella sativa* (Black Cumin). Afr J Tradit Complement Altern Med, 2011;8:226-232.

23. Randhawa MA, Alghamdi MS. Anticancer activity of *Nigella sativa* (Black Seed) - a review. Am J Chinese Med, 2011;39:1075-1091.

24. Sharma JVC, Pitchaiah G, Satyavati D. *In vitro* anticancer activity of methanolic extract of roots of *Glochidion zeylanicum* (Gaertn). IJRPBS, 2011;2:760-764.

25. Nida Nayyar Farshori, Ebtesam Saad Al-Sheddi, Mai Mohammad Al-Oqail, Javed Musarrat, Abdulaziz Ali Al-Khedhairy, Maqsood Ahmed Siddiqui. Anticancer activity of *Petroselinum sativum* seed extracts on MCF-7 human breast cancer cells. Asian Pacific Journal of Cancer Prevention, 2013;14:5719-5723.

26. Kulkari L.A. Pharmacological review on *Vitex trifolia* (Verbaeaceae) Pharmacology online 2011; 3:858-863.