ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Oil Active Fractions of Nigella Sativa and Their Activity Against Brest Cancer Cell Line MCF-7

Taif H. Al-Ameedy[1*], Rabab Omran[2]

Biology Department, Collage of Science, University of Babylon, Al-Hillah City, Babel, Iraq.

***Corresponding Author: Taif H. Al-Ameed**

Abstract

Objective: Seeds of *Nigella sativa* have been used as spice and food preservative, also these seeds have been used to promote health and fight disease especially in the Middle East. The aime of this study is to evaluate the effect of partially purified oil fractions extracted from *Nigella sativa* purified oil fractions against breast cancer cell line. Methods: The oil of black seed was extracted with 96% ethanol and partially purified by adsorption chromatographically using silica gel column with different solvents depending on the polarity of *N.sativa* compositions. Subsequently the resulted fractions (hexne, chloroform, ethyl acetate, acetone, ethanol, methanol and water fractions) were tested as anticancer against breast cancer cell line Michigan cancer foundation-7 (MCF-7) and compare with the human hepatic cell lineWRL 68 as a normal cell line. Results: The most effective oil fraction for killing MCF-7 cell was hexane with IC_{50}87.52µg/ml, followed ethyl acetate and ethanol fraction the cytotoxicity against WRL68 were significantly ($P \leq 0.0001$) less than for MCF7 and it was 203.1µg/ml and 218.3µg/ml respectively. Whereas the rest fractions including chloroform, acetone, methanol and water fractions had slightly anticancer activity. Conclusion: The black seed oil contain effective compounds act as antitumor agents and their were had no activity against normal cell, so we can use a chemotherapy drugs after perform another detailed research for other activities and study the chamical structure to modify for increase their anticancer activity.

Keywords: *Nigella sativa*, Oil seeds, Anticancer activity, Medicinal plants.

Introduction

The use of medicinal plants is very wide spread in many parts of the world because it is commonly considered that herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects [1]. Cancer remains leading cause of death worldwide. The International Agency for Research on Cancer (IARC) lately estimated that 7.6 million deaths worldwide were due to cancer with 12.7 million new cases per year being reported global [2]. A significant proportion of this trouble is borne by developing countries; 63% of cancer deaths are reported to be from developing countries [3, 4].

Nigella sativa (N. Sativa) is commonly known as black seed, *N. sativa* is a grassy plant belongs to the Ranunculaceae family, There are many reports concerning the biological and pharmacological activity of this plant, such as anti- inflammatory, immunomodulatory, antidiabetic, antibacterial, pain alleviating, antifungal, antioxidants, anticancer and anti-hypertensive effects [5]. The most important active compounds are thymoquinone (30 %-48%), thymohydroquinone, dithymoquinone, p-cymene (7 %-15 %), carvacrol , 4-terpineol, t-anethol ,sesquiterpene longifolene ,α-pinene and thymol *etc.* Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; *i.e.* isoquinoline alkaloids *e.g.* nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine.

Moreover, *N. sativa* seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent[6]. Aqueous and alcohol extracts of *N. sativa*were found to be effective

in vitroin inactivating MCF-7 breast cancer cells [7]. *N. sativa*, in combination with melatonin and retinoic acid reduced the carcinogenic effects of DMBA (7,12-di-methylbenz(a)anthracene) in mammary carcinoma of rats. Terpene-terminated 6-alkyl residues ofTQ were tested in MCF-7/Topo breast carcinoma by[8]. They found the derivatives inducing cell death by apoptosis. The aim of this study was to investigate the anticancer activity of *N. sativa* oil against breast cancer cell line.

Materials and Methodes

Plant Collection

The Black seeds as raw plant materials were purchased from a local market, in Babylon Province, Iraq, during February in 2018. The *N. sativa* seeds were imported from the Kingdom of Saudi Arabia. The seeds were washed with distilled water and dried in shade separately at room temperature. Most of the moisture has been removed, the plant material grounded in a mill to produce fine powdered. After that, the sample (1000-1500 g) was stored in dark glass containers at-20°C until extraction was performed.

Extraction of Bioactive Substances

Optimum extraction parameters will vary depending on the type of plant part and matrix. The plant sample may require different temperatures and solvent extraction mixtures. From previous experiments that optimize the extraction methods, 96 % ethanol (EtOH) was the best solvent to extract the active materials from black seeds; However the plant seed sample (50 g) was extracted twice with the 96% of ethanol (1000 ml) at the ratio of raw material to solvent 1:20 by soaking for 24 h at 30°C in the shaker incubator. The plant extract was decanted, filtered under vacuum, concentrated and the oil was recovered from oil-ethanol mixture by rotary evaporator at 45°C.The concentrated extract was stored in a dark container at −20°C for further purification [9].

Partially Purification of *N. sativa* oil using Adsorption Chromatography

The concentrated crude extract of black seeds (*Nigella sativa)* was partially purified using adsorption chromatography by silica gel column (mesh 60-120) to separate the components of the crude extract; the separation is accomplished because each component of the crude extract has a different polarity. More polar compounds will flow easily through the silica gel, while non-polar compounds will flow more slowly through the gel Silica gel Itself in non-polar, and thus is attracted to other non-polar molecules. The attraction of the non-polar molecules to the silica gel is what causes the non-polar components of a mixture to move slowly through the gel. Chemical separation relies on the relative speeds at which each component travels from the top of the column to the bottom when using a silica gel chromatography column. The slurry of silica gel was prepared by soaking with suitable solvent such as EtOH [20].

Subsequently poured into the column (2.5 × 25 cm) and washed with EtOH for one an hour to obtain better packing. Finally, it washed with hexane for one an hour to obtain hydrophobic conditions. Concentrated seed extract (5 ml) was loaded into the silica gel column and the active components were eluted successively with different polarity solvents using batch ways. These solvents include hexane, chloroform, ethylacetate, acetone, 95% EtOH, methanol (MeOH), and then distilled water (500-750 ml for each).

Each fraction drains out of the column and have a sharp ending point which was checked by absorbance at 275 nm using a spectrophotometer [20]. The oil was recovered from oil-solvent mixture by rotary evaporator at45°C and dried with anhydrous Na_2SO_4,as well as the water fraction was concentrated by rotary evaporator at 45°C and dried with anhydrous Na_2SO_4. Finally, the fractions of each solvent were stored in dark container at −20°C.

Anticancer activity of *Nigella Sativa* Oil-fractions

Cell Line

MCF-7 Cell Line

Michigan cancer foundation-7(MCF-7) cell line was derived from the pleural effusion from a 69 year old female suffering from a breast adenocarcinoma [10].

WRL 68 Cell Line bb

The human hepatic cell lineWRL 68 exhibits morphology similar to hepatocytes and hepatic primary cultures.

Cells have been shown to secrete albumin and alpha-feto protein and express liver specific enzymes such as alanine amino transferase [11].

The Cytotoxic Activity of Partially Purified *N. sativa* Fractions

This *in vitro* method was performed to investigate the possible cytotoxic effect of partially purified *N. sativa* fractions on cancer cell lines (MCF-7) and normal cell line WRL 68.

Cell Line Maintenance

When the cells in the vessel formed confluent monolayer, the following protocol was performed [11]:

- The growth medium was aspirated and the cell sheet washed with PBS.
- About 2 to 3 mL trypsin/versine solution was added to the cell. The vessel was turned over to cover the monolayer completely with gentle rocking. The vessel allowed incubation at37°Cfor 1 to 2min, until the cells were detached from the vessel.
- Fresh complete RPMI medium (15-20 mL) was added and cells were dispersed from the wedding surface into growth medium by pipetting.
- Cells were redistributed at required concentration into culture vessels, flasks or plates whatever needed and incubated at 37°Cin 5%CO_2 incubator.

Cell concentration was achieved by counting the cells using the hemocytometer and applying the formula:

Total Cell Count/mL =Cell Count x Dilution Factor (Sample Volume) x10^4

B. MTT Protocol

The cytotoxic effect of partially purified *N.sativa* fractions was performed using MTT ready to use kit which containing 10 vials of MTT solution (1 mL)and 2 bottles of Solubilization solution (50 mL).

Protocol

- Tumor cells ($1x10^4$ –$1x10^6$ cells/mL) were grown in 96 flat well micro-titer plates, in a final volume of 200µL complete culture medium per each well.

  The microplate was covered by sterilized parafilm and shacked gently.
- The plates were incubated at 37 °C, 5 % CO_2 for 24hrs.
- After incubation, the medium was removed and two fold serial dilutions of the desired oil-fraction (12.5, 25, 50, 100, 200, 400 µg/mL) were added to the wells.
- Triplicates were used per each concentration as well as the controls (cells treated with serum free medium).Plates were incubated at 37°C, 5 % CO_2 for selected exposure time (24 hrs).
- After exposure, 10 µl of the MTT solution was added to each well. Plates were further incubated at 37°C, 5% CO_2 for 4 hrs.
- The media were carefully removed and100 µL of solubilization solution was added per each well for 5min.
- The absorbance was determined by using an ELISA reader at a wavelength of 575nm.The data of optical density was subjected to statistical analysis in order to calculate the concentration of compounds required to cause 50% reduction in cell viability for each cell line.

Results And Discussion

Nigella sativa L. seeds or Black cumin or black seeds are generally applied in traditional medicine. *Nigella* seed oil (NSO) composition is identified to be location-dependent. In the present study, the oil of *Nigella* seed was extracted by solvent.

Oil extract yield was 40% when 96% ethanol was used for extraction and the oil was recovered from the oil-ethanol mixture by rotary evaporator at 45°C. This result was similar to previous studies which reported the *Nigella*-seed oil composed about 27-37% of the seeds depending on the extraction methods.

Whereas the oil quality of the *Nigella sativa* seeds cultivated in the Kingdom of Saudi-Arabia was similar to that other origin, such as the Mediterranean and western countries 21 The extracted oil was partially purified using batch wise adsorption chromatography method by silica gel.

The results appeared the presence seven fractions depending on the polarity of solvents (hexane, chloroform, ethyl acetate, acetone, ethanol, methanol and water). These oil fractions were different in polarity, color and density (Fig.1).

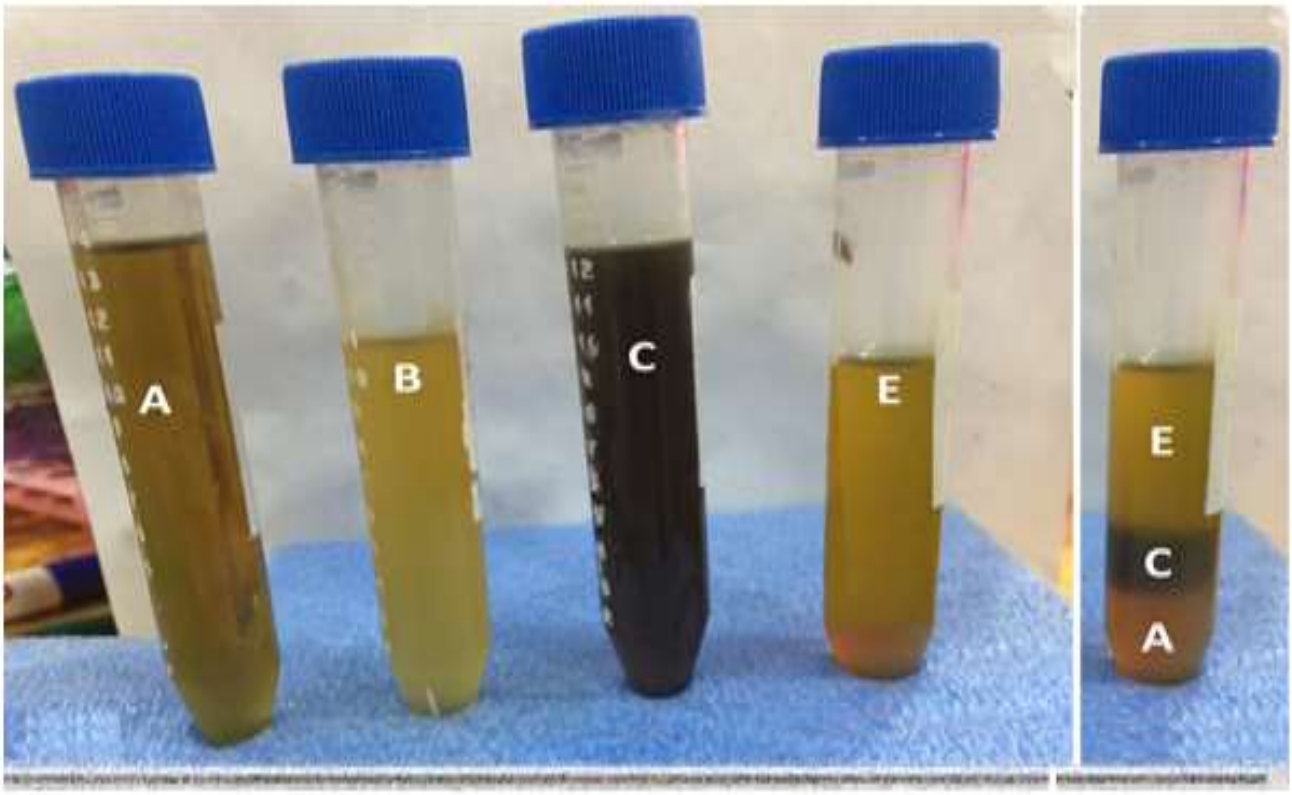

Fig. 1: Partially purified *N. sativa* oil Fractions A: chloroform fraction; B: Hexane fraction; C: Ethyl acetate fraction; E: Ethanol fraction n; E+C+A mixture which different in oil density. All oil fractions were recovered by rotary evaporator until all solvents elimenated

The Cytotoxic Effect of Partially Purified *N. Sativa* Oil Fractions

MTT was used to measure the MCF-7cell viability after exposure of the cells to different concentrations from each oil fraction tested for 24 h at 37°C (Table 1). The results showed an inhibition in the cell viability in a different rates based on the concentrations and the type of the fractions, which showed a decreasing effect on the MCF-7 cell viability in different rates and cytotoxicity was improved by accumulative the *N. sativa* oil concentration.

The most effective oil fraction for killing MCF-7 cell was hexane with IC_{50}87.52µg/ml, when MCF7 cells treated with different concentrations (400, 200, 100, 50, 25µg/ml) which inhibit growth of cell line in (63%±7.6, 44%±3.2, 85.4%±3.5, 95.1%±1.1, 95.6%±1.8) respectively as shown in Table (1). The hexane fraction had more significant ($P\leq$ 0.0001) cytotoxic activity against cancer cell line MCF7 in comparing with normal cell line WRL68 with IC_{50} 167.5µg/ml. The cytotoxic effect of both ethyl acetate and ethanol against cancer cell line was higher than the rest oil fractions (chloroform, acetone, methanol and water) with IC_{50} (97.95 µg/ml and 109.9 µg/ml) respectively, the viable inhibition rate for ethyl acetate was ranged from (59.6%±5.9-5.1%±1.1) in concentration (400-25 µg/ml). Ethanol fraction inhibited MCF7 in the range between (54%±4.3 - 6.3%±1.3 µg/ml) in concentrations (400-25 µg/ml). For both ethyl acetate and ethanol fraction the cytotoxicity against WRL68 were significantly ($P\leq$ 0.0001) less than for MCF7 and it was 203.1µg/ml and 218.3µg/ml respectively.

The extract of *N. sativa* oil fractions at all concentrations for the other fractions (chloroform, acetone, methanol and water) had a slightly significant cytotoxic effect on the MCF7 cell line and less cytotoxic effect for WRL68 cell line that there were no significance between cancer cell line and normal cell line ($P\geq$ 0.05) comparing with another fraction as shown in Table (1). The chloroform fraction had an inhibition rate 33.7% in the concentration of 400µg/ml with IC_{50} about 130.1µg/ml while the WRL68 was 149.6µg/ml that mean that there was no significance between the two cell lines.

Table 1: Cytotoxic effect of *N. sativa* fraction oil against cancer cell line.

		Viability%							
Concentration(µg/ml)		**400**	**200**	**100**	**50**	**25**	**12.5**	**IC50**	***P*-value**
Hexane	**MCF7**	37.2±7.6	56.1±3.2	85.4±3.5	95.1±1.1	95.6±1.8	95.3±0.9	87.52	0.0001
	WRL68	71.9±0.8	84.4±1.2	39.6±2.1	95.3±1.1	95.2±1	94.8±2.3	167.5	
Chloroform	**MCF7**	66.3±2.2	89.8±4.7	94.6±1.8	95.7±2.5	96.9±1.1	96.3±0.9	130.1	0.6500
	WRL68	85±0.7	87.9±3.5	93.6±3.1	96.2±1.2	95.5±4.1	95.7±2.7	149.6	
Ethyl acetate	**MCF7**	40.4±5.9	57.2±3.2	67.6±2.6	80.9±1.9	94.9±1.1.	95.3±2.4	97.95	0.0001
	WRL68	69.9±2.9	86.8±1.5	94.2±1.8	95.8±0.4	95.3±0.9	95.3±2.9	203.1	
Acetone	**MCF7**	76.5±3.3	85.7±1.9	92.4±1.8	95.4±0.8	97.1±1.3	95.8±3.2	144.6	0.5110
	WRL68	84.2±1.9	90.2±3.7	92.2±2.7	96.4±1.2	96.9±1.1	94.2±2.9	113.0	
Ethanol	**MCF7**	46.8±4.3	63.7±2.1	77.6±2.4	90.5±1.8	94.7±1.3	95±2.6	109.9	0.0001
	WRL68	77.2±3.6	86±0.8	92.1±1.5	96.1±1.2	94.9±2.1	96±2.6	218.3	
Methanol	**MCF7**	73.9±4.9	79.4±1.5	96.3±1.4	95.3±2.1	94.7±3.2	96±2	134.1	0.165
	WRL68	83.5±0.8	88.2±2.6	93.6±2.1	95.2±1.4	95.5±0.8	96.8±4.4	164.8	
H_2O	**MCF7**	73.3±4.4	80.6±1.9	93.6±3.9	94.2±0.8	95.9±0.8	96.1±1.1	172.9	0.0995
	WRL68	71.9±0.8	84.8±1.2	93.6±2.1	95.3±1.1	95.2±0.9	97±2.4	222.2	

* (P≤ 0.005). MCF7: a breast cancer cell line Michigan Cancer Foundation-7 ; WRL68: a normal cell line of non-tumorigenic fetal hepatic cell line

Acetone fraction gave the same result the viability of cell after treatment ranged from 73.9%±4.9 to 94.7%±3.2 with IC_{50} about 144.6µg/ml for MCF7 cell line and 113.0 µg/ml for WRL68 cell line, that gave slightly effect on both cell lines and there were no significance between them. Finally methanol and water fractions showed the same results with IC_{50} about 134.1µg/ml and 172.9µg/ml respectively, and there was no significance between them.

The present results indicate that the effect of different fractions of black seed oil can vary in cytotoxicity due to the difference in their chemical contents. These results consist with the previous studies that reported the *N. sativa* oil extracts had variable effect against cancer cell lines due to the differences in cell line and oil compositions depending on the extraction methods [12]. From our results the hexane, ethyl acetate and ethanol-oil fractions exhibited a significant cytotoxic effect against breast cancer MCF7 cell line used, while the chloroform, acetone, methanol and water fractions showed only limited cytotoxic activity, and that may be due to most active compounds were more soluble and separated in the hexane, ethyl acetate and ethanol solvents.

Many reports mention that the differential cytotoxic effect of these extracts was related not only to their chemical composition, but also to the nature of the tumor cell lines [13]. This activity may be due to the presence of two essential components of the fixed oil of *N. sativa* thymoquinone and dithymoquinone that have a cytotoxic effect of cancer cell line and these results agreed with another study that refer to the same action of these two components and their effect on cancer cells [14]. While the activity of ethyl acetate fraction may be due to the presence of terpenes as major components that may explain its high cytotoxic activity [15].

Actually, the anti-cancer potential of *N. sativa* oil fractions has been recently reported [16;17;18] The results of the study agreed with previous research that reported the hexane, ethyl acetate and ethanol fractions were found to exhibit a strong growth inhibitory effect on all malignant cells tested [19].Results that gained from this study also agreed with Mbarek *et al.* [21].

Study that showed that essential oils and the ethylacetate fraction of black cumin seeds had anticancer activity in a variety of different tumor cells (P815, Vero, BSR, and ICO1 cell line MCF7). The results showed ethyl acetate extracts and essential oils have a cytotoxic effect on P815 cells (IC50 (% v/v) 0.6, 0.75), on Vero cells (IC50 (% v/v) 0.22; 0.25), on BSR cells (IC50 (% v/v) 0.2; 1.2%). This research with *N. sativa* seed showed differences in IC_{50} values because of the differences of the target cell [22]. At the same time the data from this study did not agree with other researches that showed the chloroform fraction of volatile oil of black cumin have cytotoxic activity against breast cancer cell [23].

Extracts of *N. sativa* contain volatile and non-volatile oils, amino acids, proteins, carbohydrates, alkaloids, nitrogen compounds, saponnins, and minerals such as sodium, calcium, iron, and potassium; generally, more than 100 compounds have been isolated from *N. sativa* and their structure illuminated.

Several studies have recognized the majority of the pharmacological activity of *N. sativa* for its quinone content, which includes thymoquinone (TQ) and its dimmer dithymoquinone, thymohydroquinone (THQ), and thymol [24; 25]. The cytotoxic activity against breast cancer cells of *N. sativa* its mostly due to thymoquinone compounds of volatile oil that contained, which is responsible of anticancer, Thymoquinone have a certain role in inhibiting the growth of cancer cells and induce apoptosis in cancer cells [26; 27]. Thymoquinone (TQ) and its dimmer thymohydroquinone (THQ), dithymoquinone and thymol, are recognized for their specific antitumor special effects in varied human cancer cell lines.

This specificity is because activation of NF-κB is considerably higher in carcinogenic cells than in normal cells, in addition to that TQ can inhibit breast cancer by deactivate PI3K/AKT signaling pathway and trigger PTEN expression [28]. TQ also play its anti-cancer effect by modifying tumor suppressor genes such as P53, P21 and P27. TQ can induce cancer cell apoptosis by reducing Bcl-2 and increasing Bax and increasing the release of cytochrome C.

In human breast cancer, TQ acts pro-apoptotic effect of deactivating STAT3 and its block genes such as Bcl-2 and VEGF (vascular endothelial growth factor, and since the mechanism of action of TQ involves NF-κB, the effect of TQ on cancer cells is greatly more reflective than on normal cells [29]. Studies comparing the anticancer activity and toxicity of several quinone compounds found variation in the degree of their effectiveness against various cancer cell lines[30].

Conclusion

The black seed oil that separated with hexane contain most active compound for antitumor, then ethyl acetate and ethanol-oil fractions respectively. The black seed oil contain effective compounds act as antitumor agents and their were had no activity against normal cell, so we can use a chemotherapy drugs after perform another detailed research for other activities and study the chamical structure to modify for increase their anticancer activity.

Acknowledgment

The authors are thankful to Dr.Ahmad Fadial for his help to accomplish anticancer activity of the oil fraction at Al-Nahrain University, Baghdad. This research which was done at the Laboratory of Biotechnology and Genetic Engineering, Biology Department, College of Science, University of Babylon, Iraq.

References

1. Philomena G (2011) Concerns regarding the safety and toxicity of medicinal plants - An overview. J. Appl. Pharmaceut. Sci., 1(6):40-4.
2. International Agency for Research on Cancer (IARC) (2010) GLOBOCAN 2008, Cancer incidence and mortality worldwide. Lyon, France: IARC.

3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global stics. CA Cancer J Clin, 61: 69

4. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int. J. Cancer, 127: 2893-917.

5. Al-Jassir MS (1992) Chemical composition and microflora of black cumin (Nigella sativa L.) seeds growing in Saudi Arabia. Food Chem., 45: 239-42.

6. Lei X, Lv X, Liu M, Yang Z, Ji M, Guo X et al (2012) Thymoquinone inhibits growth and augments 5-fluorouracil-induced apoptosis in gastric cancer cells both in vitro and in vivo. Biochem Biophys Res Commun., 417(2): 864-68.

7. Hadjzadeh MA, Keshavarzi Z, Yazdi TSA, Ghasem SM, Rajaei Z, Khajavi Rad A (2012) Effect of alcoholic extract of Nigella sativa on cisplatin-induced toxicity in rat. Iran J. Kidney Dis., 6(2):99-104.

8. Mahmoud SS, Torchilin VP (2012) Hormetic/cytotoxic effects of Nigella sativa seed alcoholic and aqueous extracts on MCF-7 breast cancer cells alone or in combination with doxorubicin. Cell Biochem Biophys, 25(7): 1392-1398.

9. Effenberger K, Breyer S, Schobert R (2010) Terpene conjugates of the Nigella sativa seed-oil constituent thymoquinone with enhanced efficacy in cancer cells. Chem Biodive, 7(1): 129-139.

10. Cheng L, Xia TS, Wang YF, Zhou W, Liang XQ, Xue JQ (2014) The anticancer effect and mechanism of α-hederin on breast cancer cells. Int. J. Oncol., 45(2):757-63.

11. Bourgou S, Pichette A, Marzouk B, Legault J (2010) Bioactivities of black cumin essential oil and its main terpenes from Tunisia. South African J. Botany, 76: (2) 210-16.

12. Soule HD, et al (1973) A human cell line from a pleural effusion derived from a breast carcinoma. J. Natl. Cancer Inst., 51(5):1409-416.

13. Asita, Salehhuddin (2013) Heat Sensitivity between Human Normal Liver (WRL-68) and Breast Cancer (MDA-MB 231) Cell lines. Int. J. Chem., Enviro. & Bio.Sci., 2320-4087.

14. Kumar N, Mueen A K K, Dang R, Husain A (2008) Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone. J. Med. Plants Res., 2: 361-64.

15. Freshney RI (2010) Culture of animal cells. A manual of basic technique and specialized applications. 6th (ed.), John Wiley and Sons, Inc. USA.

16. Gharby S, Harhar H, Guillaume D, Roudani A, Boulbaroud S, Ibrahimi M, et al (2015) Chemical investigation of Nigella sativa L. seed oil produced in Morocco. J. Saudi Soci. Agri. Sci., 14(2): 172-77.

17. Gali-Muhtasib H, Diab-Assaf M, Boltze C, Al-Hmaira J, Hartig R, Roessner A, et al (2004) Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a 53-dependent mechanism. Int. J. Oncol., 25: 857-866.

18. Swamy SM, Tan BK (2000) Cytotoxic and immunopotentiating effects of ethanolic extract of Nigella sativa L. seeds. J. Ethnopharmacol., 70: 1-7.

19. Ekowati H, Prasasti E, Rastuti U (2011) The active fraction from Nigella sativaand its activity against T47D cell line. Indones J. Chem.,11: 217-22.

20. Omran R, Zahraa M Al-taee, Hayder O Hashim, Mohammed J Al-jassini (2017) Extraction of phenolic compound as antioxidants from some plants and their cytotoxic activity against breast cancer cell line. Asian J Pharm Clinic Res, 10 (7): 349-55

21. Ait Mbarek L, Ait Mouse H, Elabbadi N (2007) Anti-tumor properties of blackseed (Nigella sativa L.) extracts. Braz. J. Med. Biol. Res., 40: 839-47.

22. Tabasi N, Khajavi-Rad A, Mahmoudi M (2010) The effects of Nigella sativa ethanolic extract on proliferation and apoptosis of renal cell carcinoma ACHN cell line. J. Shahrekord Univ. Med. Sci., 12:7-14.

23. Fukuyama Y, Minoshima Y, Kishimoto Y, Chen IS, Takahashi H, Esumi T (2004) Iridoid glucosides and p-coumaroyl iridoids from Viburnum luzonicum and their cytotoxicity. J. Nat. Prod., 67: 1833-838.

24. Ali BH, G Blunden (2003) Pharmacological and toxicological properties of Nigella sativa. Phytother Res, 17: 299-305.

25. Gali-Muhtasib H, Roessner A, Schneider-Stock R (2006) Thymoquinone: a promising anti-cancer drug from natural sources. Int. J. Biochem Cell Biol., 38: 1249-53.

26. Arafa ESA, Zhu Q, Shah ZI (2011) Thymoquinone up-regulates PTEN expression and induces apoptosis in doxorubicin-resistant human breast cancer cells. Mutat. Res, 706: 28-35.

27. Agbaria, Riad et al (2015) Anticancer Activity of Nigella sativa (black Seed) and Its Relationship with the Thermal Processing and Quinone Composition of the Seed. Drug D/es. Development and Therapy, 9: 3119-3124.

28. Kensara, Osama Adnan et al (2016) Thymoquinone Subdues Tumor Growth and Potentiates the Chemopreventive Effect of 5-Fluorouracil on the Early Stages of Colorectal Carcinogenesis in Rats. Drug Design Develop Therapy, 10: 2239-53.

29. Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS et al (2004) Free radicals and antioxidants in human health: current status and future prospects. J. Assoc. Physicians India, 52: 794-804.

30. Aljabre SHM, Alakloby OM, Randhawa MA (2015) Dermatological effects of Nigella sativa. J. Derm Derm. Sur., 10(2): 92-8.

