RESEARCH ARTICLE

Anti-oxidative and anti-proliferative activities of extracted phytochemical compound thymoquinone

Ayesha Siddique Butt,[1] Numrah Nisar,[2] Tahira Aziz Mughal,[3] Nadia Ghani,[4] Imran Altaf[5]

Abstract

Objective: To evaluate the anti-oxidant and anti-proliferative potential of Thymoquinone extracted from the essential oil of indigenous herbs of Nigella sativa and Thymus vulgaris.

Method: Extraction and quantification of Thymoquinone was carried out in July, 2017 in Department of Environmental Science, Lahore College for Women University (LCWU), Lahore. Thymoquinone was extracted from seeds of Nigella Sativa and aerial parts of Thymus vulgaris by employing soxhlet extraction with 1:4 ratios of n-hexane and methanol. High Performance Liquid Chromatography was used to quantify Thymoquinone from the methanolic extracted oil of sample by applying calibration curve method. Extracted Thymoquinone was identified by sample peaks obtained at retention time were compared with peak of standard Thymoquinone at respective time. The Thymoquinone obtained from both samples was then subjected to Fourier-transform infrared spectroscopy for confirmation by identifying its functional groups. Anti-oxidant activities of both samples were measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assay in Department of Environmental Science, LCWU. In-vitro anti-proliferative activities of extracted Thymoquinone were evaluated in HeLa cell cancer lines by cell proliferations Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay in Department of Microbiology, University of Veterinary and Animal Sciences (UVAS), Lahore. SPSS 18 and Graph pad prism 18 was used for data analysis.

Results: Soxhlet extraction with solvents ratios yielded 48.92% oil from Nigella sativa and 23.2 % from Thymus vulgaris. High Performance Liquid Chromatography peak of standard Thymoquinone was measured at retention time of 5.5 min which was then compared with the peak obtained from both samples at the similar retention time. The extracted Thymoquinone from both samples were quantified by calibration curve method showing 614.25 mg/L from Nigella sativa and 548.86 mg/L from Thymus vulgaris. The two anti-oxidant assays of both samples compared with standard Thymoquinone showed significant scavenging activities in dose amount manner. Cell proliferation of HeLa cancer significantly decreased with dose response manner ($p<0.01$), showing highest cell death in high concentration of Thymoquinone. Inhibitory concentration 50 (IC50) of cancer cell line treated with Nigella sativa oil was 0.5 µM and Thymus vulgaris was 18 µM compared to standard Thymoquinone, showing Inhibitory concentration50 (IC50) of 6 µM using Graph pad prism v.8.0.

Conclusion: Both Nigella sativa and Thymus vulgaris were found to be the best source of Thymoquinone as chemotherapeutic drug expressed potent anti-oxidant and anti-proliferative activities.

Keywords: Thymoquinone, Nigella sativa, Thymus vulgaris, Anti-oxidation, DPPH assay, FRAP assay, Anti-proliferation, Hela cancer cell line, MTT assay. (JPMA 69: 1479; 2019).**doi:10.5455/JPMA.302643156**

Introduction

The interest in phytochemical compounds with potent anti-cancer activities is growing as they are relatively environment-friendly, economical and because of their bioavailability.[1] Nigella sativa (N.S) is distinct to Mediterranean region, southern Europe, North Africa and southwest Asia, and it is cultivated in many countries in those regions.[2] It is widely studied for its potent potential against cancer proliferation, inflammation, hypertension, diabetes, gastrointestinal disease, as well as hepatic, cardiovascular, respiratory, immune and endocrine systems, and for its analgesic and microbial actions.[3] The major therapeutic properties of this plant are mainly due to the presence of Thymoquinone (TQ) comprising 25-45% of the essential oil of seed.[4,5]

Thymus vulgaris (T.V), native to Southern Europe and South East Asia regions, is known to be rich in phytochemical components, including TQ which contribute to its medicinal importance.[6] The reported beneficial properties of T.V include appetite stimulant effect, liver function improvement, treatment of cartilaginous tube, bronchial infection, inflammation, urinary infections and treatment of laryngitis. It has lately

[1-4]Department of Environmental Science, Lahore College for Women University, Lahore, [5]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore.

Correspondence: Ayesha Siddique Butt. Email: aqua_feb_13@hotmail.com

shown anti-proliferative effects.[7,8]

TQ (2-isopropyl-5-methyl-benzoquinone) is proved to be effective as anti-cancer by its mode of action of apoptosis such as chromatin condensation, translocation of phosphatidyl serine across the plasma membrane and deoxyribonucleic acid (DNA) fragmentation in TQ-treated cells.[9] Literature has assessed anticancer activities of TQ, disclosing that it displays multi-targeted effects related with chemo-sensitisation and chemo-prevention of different cancers like breast, colon, leukaemia, squamous cell carcinoma (SCC) and cervical cancer.[10] The expected mechanism of TQ against different cancer cell development in vitro and in vivo specify the contribution of TQ in different cell death, signalling pathways including proliferation, apoptosis, increase reactive oxygen specie (ROS) generation, angiogenesis and tumour-induced immunosuppression.[8] Many researchers emphasised additional exploration concerning anti-oxidant and anti-proliferative activities of TQ because it is a safe and promising anti-cancer component with target specificity up to the nano level.[11]

The current study was planned to extract maximum bioactive TQ from samples of N.S and T.V to achieve potent anti-proliferative and anti-oxidant activities.

Materials and Methods

The seeds of N.S (wild type) were purchased from the herbal medicinal shops Anarkali, Lahore, in the month of February, 2017. Plants of T.V were collected from Changa Manga in months of March - April, 2017. Both samples were identified by Prof. Dr. Tahira Aziz Mughal (LCWU, Lahore).

Reagents used included TQ (99% pure analytical standard, Sigma Aldrich, Germany), 95% Methanol of High Performance Liquid Chromatography (HPLC) grade (Sigma Aldrich, Germany), n-Hexane (98% pure, Sigma Aldrich, Germany) and isopropyl alcohol HPLC grade (Sigma Aldrich, Germany), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Ascorbic acid, 95%, 1% potassium ferricyanide [K3Fe (CN)6], 0.1 % ferric chloride [FeCl3], Dimethyl Sulfoxide (DMSO reagent >99.9% pure, molecular weight (Mol.Wt.) =78.13 g/mol, Sigma Aldrich, Germany), Dulbecco's Modified Eagle's medium (DMEM) (prepared by mixing solid DMEM 3.4g in 100ml distilled water and sodium bicarbonate), Methylthiazolyldiphenyl-tetrazolium bromide (MTT) cell proliferation assay kit (Trevigen, USA), and Henrietta Lacks (HeLa) cell lines (human cervical adenocarcinoma) (Quality Operation Laboratory (QOL), Microbiology Section, UVAS, Lahore).

The extraction and purification on TQ was carried out in July, 2017 in Department of Environmental Science, LCWU. For the extraction of TQ, the collected seeds of N.S (100g) were washed with deionised water to remove any dirt, and then left for drying at room temperature; 33°C. The aerial part of T.V (1kg) was dried in the air for a week. These dried samples were finely ground in the grinding laboratory mill (Food mixer; National, Japan) sieved through the sieve of mesh size 0.6mm.

The finely ground samples were subjected to the soxhlet extraction following the procedure with modification.[12] The samples were weighed about 25g and placed in cotton cellulose extraction thimble of size 25×80mm in a soxhlet with solvents combinations of n-hexane: Methanol (H:M) 50:200ml (1:4) separately for 6-hours. The extract from both the samples were collected and further re-extracted with 30ml methanol three times in separatory funnel. Extra solvent was removed by rotary evaporation at 40°C under vacuum for 5 minutes. Each sample was then centrifuged at 4000rpm for 30 minutes. The upper oily layer was separated and stored at 4°C until analysis. The yield (%) of oil was calculated by equation 1.[13]

$$\text{Yield of extracted oil (\%)} = \frac{\text{Mass of extracted oil (g)}}{\text{(Mass of raw material (g)}} \times 100 \quad (1)$$

For HPLC Analysis: TQ standard (98% pure, Sigma Aldrich, Germany) was prepared in Methanol 100ppm by adding 100mg/L. HPLC analysis was carried out with Waters (600) HPLC controlled by computer-based Empower software (Quick start) with personal work station hardware system. The reversed phase column used was with octadecylsilyl groups with 18 carbon atoms (ODS C-18), particle size 5µm, 4.6 x 250nm in length. The mobile phase was eluted with isocratic elution mode using acetonitrile and methanol (30: 70) at flow rate of 1.5 mL/min. The sample total run time was 25 minutes. Temperature of column was kept at 25°C. The injection volume of the sample was 20µL at the detection of 254nm with resolution of 1.2nm. The column was washed with acetonitrile with 25 minute run to remove any impurities before the run of the other sample. Retention time of standard TQ obtained from the HPLC chromatogram was compared with the extracted sample of N.S and T.V to identify TQ.

TQ extracted from both the samples was quantified by calibration curve method of standard TQ dilution. Dilutions of TQ pure standard were prepared from 100mg/L (100, 80, 60, 40 and 20mg/L). Each dilution was injected separately and peak area was calculated. The calibration curve was made and TQ was quantified by applying regression equation 2.[14] $y=mx+c$ (2)

Fourier-transform infrared (FTIR) spectrum of standard TQ and extracted samples N.S and T.V were recorded. For this purposes IR Tracer - 100 Fourier Transform Infrared Spectrophotometer (Shimadzu) with attenuated total reflection (ATR) accessory and mercury cadmium telluride (MCT) detector was used. The resolution was set at 400-4000 cm-1 with a resolution 4 cm-1 at 100 scans. TQ extracted from both plants was analysed by FTIR, and IR spectra were compared with the IR of pure TQ (98% pure, Sigma Aldrich, Germany).

For the evaluation of anti-inflammatory activities of significant anti-oxidant tests Ferric Reducing Antioxidant Power (FRAP) assay and DPPH radical Scavenging Activities Assay were performed.

For FRAP assay, the reduction of ferric (Fe3+) to ferrous (Fe2+) was determined by the absorbance of Perl's Persian blue complex following the FRAP assay protocol.[15] Different concentrations of TQ (600, 400, 200, 100, 50, 25 µg/mL) were prepared. The quantified TQ in extracted oil of both samples i.e., N.S and T.V was also evaluated for anti-oxidant activities by observing absorbance at 700nm in ultraviolet (UV) spectrometer. Ascorbic acid was used as the reference. Increased absorbance of different samples indicated decreased reducing power.[16] FRAP inhibition activity was calculated by equation 3.[17]

FRAP inhibition activity (%) = (A0-A1)/A0× 100 (3)

Where

A0= absorbance of control; and

A1= absorbance of sample.

Reduction of DPPH purple colour for measuring free radical scavenging activity was observed in samples of different dilutions of TQ standard and extracted oil of N.S and T.V by following DPPH assay protocol.[18] Different concentrations (600, 400, 200, 100, 50, 25 µg/mL) of TQ standard were prepared. Absorbance was taken at 517nm, lower absorbance of reaction mixture results in higher inhibition activity. Inhibitory concentration50 (IC50) of standard TQ was determined by nonlinear regression curve. The DPPH activity was calculated by formula equation 4.[19]

DPPH inhibition activity (%) = (A0-A1)/A0× 100 (4)

Where A0= absorbance of control; and A1= absorbance of sample

Further, HeLa cell lines were grown in medium of DMEM for 24-hour incubation to make a monolayer adherent cell line culture. A 96-well plate was used for cell culture and efficacy of TQ against this cancer cell culture. TQ standard was made of concentration 100 µM in <0.1% DMSO and media. Serial dilutions of TQ 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM were prepared in well plate. The groups were designed according to dilutions and treatment with different agents (Table). Experimental groups I, II and III were treated with pure TQ and quantified TQ in samples of N.S and T.V respectively. Negative control groups were designed according to the solvent of experimental groups. Group IV was negative control of group I in which cells were treated with 0.1% DMSO. The cancer cells in group V, negative control for group II and III, was treated with methanol. The well plate was then incubated for 24 hours and MTT assay was performed by following the available protocol with Trevigen MTT cell proliferation kit assay. The cell proliferation was observed after 24-hour incubation by observing optical density of cell culture plate calculated by enzyme-linked immunosorbent assay (ELISA) plate reader Multi Skan Ex. (Thermo Electron Corporation, USA). IC50 of HeLa cancer cell line treated with standard TQ and extracted TQ of both samples was determined. Cell proliferation percentage of cancer cell line was calculated by equation 5.[20]

Table: Different treatments groups for MTT assay against HeLa cancer cell line.

Groups	Treatment	Experimental/Control
I	TQ pure+0.1%DMSO DMEM Media + cancer cell culture	Experimental
II	Extracted TQ Nigella sativa + 0.1% DMSO DMEM media +cancer cell culture	Experimental
III	Extracted TQ Thymus vulgaris + 0.1% DMSO DMEM media +cancer cell culture	Experimental
IV	0.1% DMSO DMEM Media + cancer cell culture	Negative Control
V	Methanol+ 0.1% DMSO+DMEM media + cancer cell culture	Negative control
VI	Untreated cancer cell culture	Positive Control

MTT: Methylthiazolyldiphenyl-tetrazolium bromide
TQ: Thymoquinone
DMSO: Dimethyl Sulfoxide
DMEM: Dulbecco's Modified Eagle's medium.

$$\text{Cell proliferation (\%)} = \frac{\text{OD of experimental-OD of negative control}}{\text{OD of positive control}} \times 100 \quad (5)$$

All data was presented as mean±standard error of the mean (SEM) taking $p<0.05$ as statistically significant using Microsoft Excel 2010. Nonlinear regression curve was applied for measuring IC50 wherever needed by Graph pad prism v. 8.0. SPSS 18 was used for mean, standard deviation (SD) and SEM values.

Results

Methanolic extraction by employing soxhlet in which n-hexane was in lower ratio than methanol (1:4) extracted significantly higher ($p<0.05$) volume of oil from N.S and

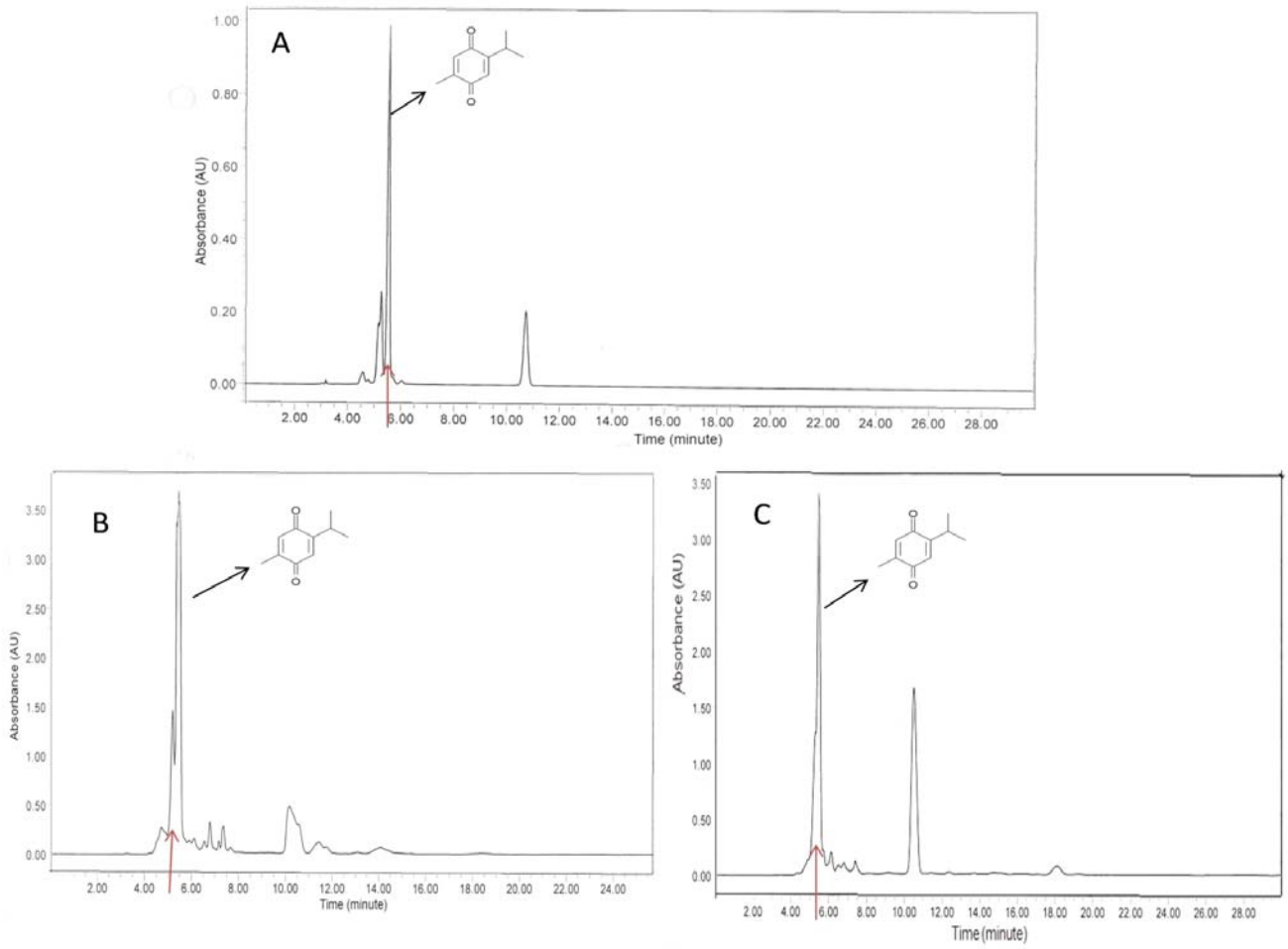

Figure-1: High Performance Liquid Chromatography (HPLC) chromatograms representing retention peak of (a) extracted Thymoquinone (TQ) from Nigella sativa (b) extracted TQ from Thymus vulgaris and (c) standard TQ.

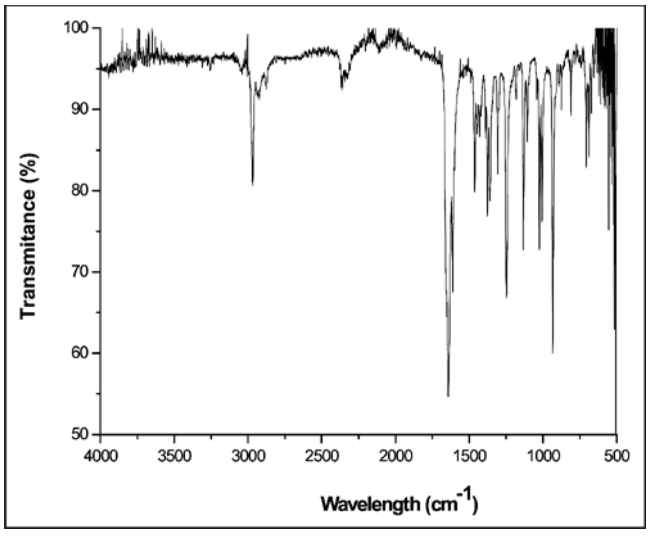

Figure-2: Infrared (IR) spectrum of standard Thymoquinone (TQ) obtained by Fourier-transform infrared (FTIR) spectroscopy analysis.

T.V (15.8±0.18 mL and 9.7±0.02 mL respectively). Total mass of extracted oils was found significantly high ($p<0.05$) from N.S (48.92%) and lower from T.V (23.2%).

Standard TQ was subjected to HPLC and showed its peak at retention time of 5.5 minute. Both samples showed respective peak of TQ at 5.5 minute identifying that TQ was successfully extracted from both samples (Figure-1 A-C). TQ quantified from N.S was 614.25 mg/L and from T.V it was 548.8 mg/L.

FTIR analysis showed similar pattern in the spectra of TQ standard and extracted TQ. Peaks at 3498 cm^{-1} represents primary amines -NH2 groups, 3023 and 2897 cm^{-1} for aliphatic C-H stretching (CH3), 1655 cm^{-1} for Ester C=O stretching, 1460 cm^{-1} aliphatic C-H bending CH2, 1200 for Ester C-O stretching and 943 and 727 cm^{-1} for trans -CH=CH- (Figure-2).

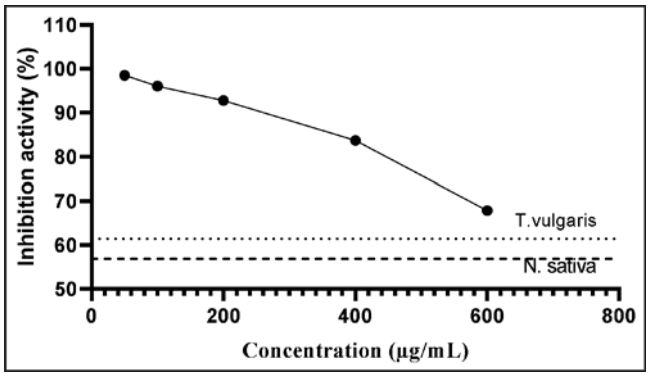

Figure-3: Inhibition (%) of standard Thymoquinone (TQ) and extracted TQ of Nigella sativa and Thymus vulgaris by Ferric Reducing Antioxidant Power (FRAP) assay.

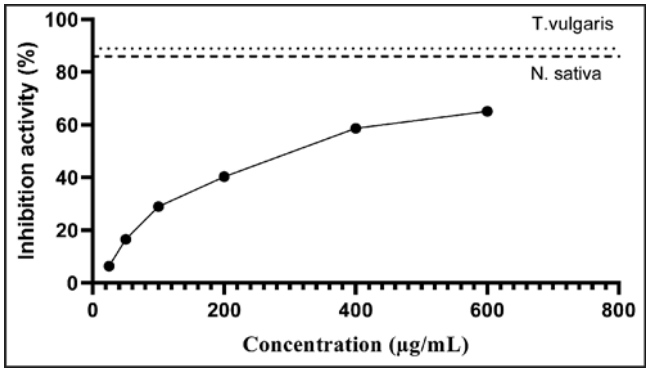

Figure-4: Inhibition activity (%) of standard Thymoquinone (TQ) and extracted TQ of Nigella sativa and Thymus vulgaris by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay,

It was determined that reduction of Fe3+ to Fe2+ decreased with increase in concentration of TQ (Figure-3). N.S and T.V had potent reduction activities i.e., 58% and 62% respectively as compared to standard TQ. DPPH assay for standard TQ showed dose-response manner i.e., with increasing TQ concentration, scavenging activity increased. N.S and T.V showed increased inhibition activity i.e. 86% and 89% as compared to TQ standard (Figure-4). IC50 for TQ in DPPH assay was 146.8µg/mL calculated by nonlinear regression curve with Graph pad prism 8. T.V. showed potent anti-oxidant activities compared to standard TQ and N.S.

The HeLa cancer cell treated with standard TQ showed dose-dependent manner of TQ as minimum cell death occurred in the lowest dilution of 3.12 µM and highly significant cell deaths ($p<0.01$) in higher dilution of 100 µM after 24 hours. Similar pattern of dose-response was observed in the treatment of HeLa cancer cell line with purified extracted TQ (Figure-5). The cell viability percentage significantly decreased ($p<0.01$) with increase in the amount of TQ. IC50 of HeLa cancer cell line in the experimental group was highest in group III i.e., 18.2 µM, for group I it was 3.102 µM, and the lowest in group II at 0.5 µM.

Discussion

Current study was designed to extract phytochemical compounds Thymoquinone (TQ) for utilizing as natural

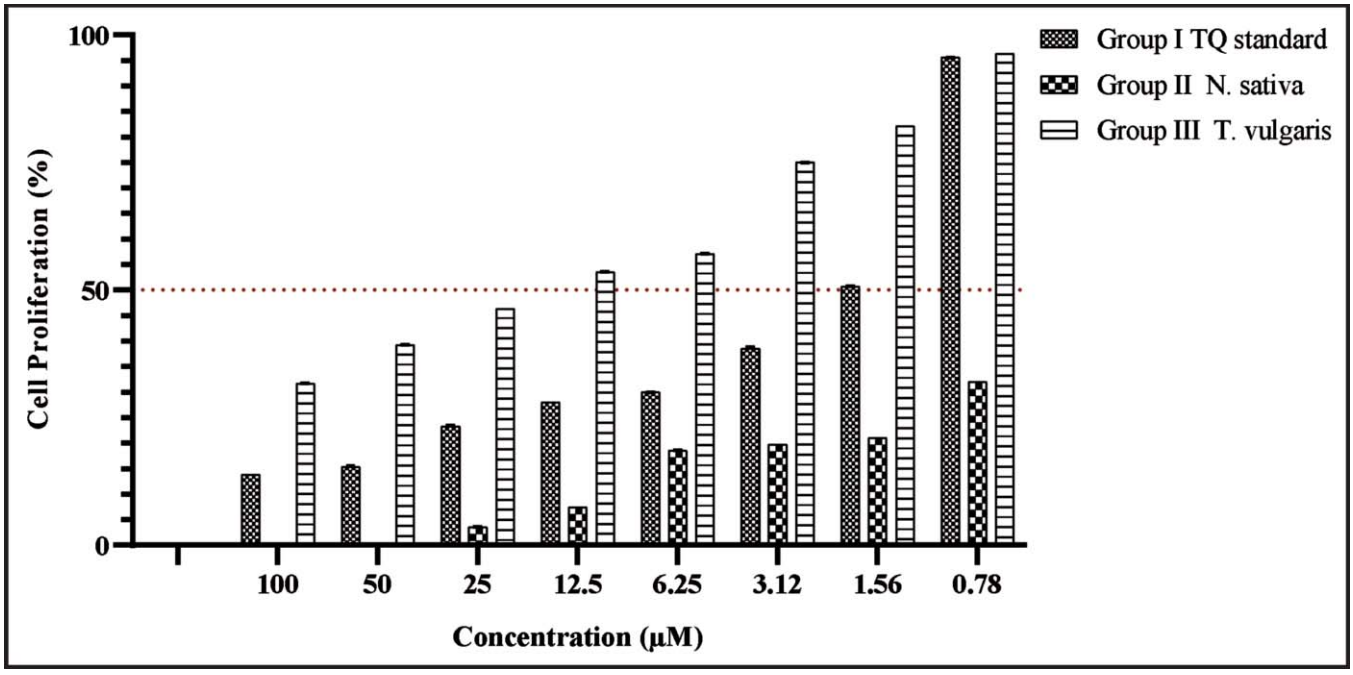

Figure-5: Cell proliferation (%) of HeLa cancer cell line by Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay treated with standard Thymoquinone (TQ) and extracted TQ from Nigella sativa (N. sativa) and Thymus vulgaris (T. vulgaris).

drug for anti-oxidative and anti-proliferative activities. Plant derived medicines are preferable over synthetic drugs due to their less reported side effects.[4] TQ from both samples was precisely extracted by employing soxhlet extraction and quantified by HPLC analysis. TQ was quantified in significantly higher amount from seed of N. S as compared to T. V aerial parts. This result was in agreement with the earlier findings in showing comparatively high amount of TQ from N.S as compared to T.V.[21,22] The amount of TQ yield from fats of plant species depends on the plant species, extraction methods, solvents, time and temperature.[23] N.S oil had high amount of extracted TQ compared to the T.V which is secondary to earlier studies showing N.S as effective source of TQ.[24,25] The quantity of TQ as obtained from the current study, however, is lesser than the reported ones, which may be because of the geographical area and quality of seeds/plants. Seed fat also varies with the region where they are grown and this affects the quantity of bioactive compounds in it.[8]

Antioxidants inhibit with the generation of reactive oxygen specie (ROS) and also play a crucial role in their inactivation. ROS are source impairment to cellular biomolecules such as proteins, nucleic acids, carbohydrates and lipids.[26] FRAP assay for evaluation of anti-oxidant activities showed influential anti-oxidant nature of TQ from both samples seconds the studies showing potent FRAP reducing activity of TQ.[27] The radical scavenging activities DPPH assay was determined on the basis of IC50 of TQ which was 146.8 µg/mL supporting the results of an earlier study that needed 2.26 mg/ mL of TQ for 50% inhibition activity DPPH.[28]

Extensive researches are being carried out for prevention and control of carcinogenic process by using naturally occurring phyto-component. The current study showed that in-vitro cell proliferation of HeLa cancer cell line had significant cell death with dose-dependent manner of TQ. Studies showed anti-cancer effect of TQ on different type of cancer cells through in-vitro and in-vivo which indicate the involvement of TQ in different cell death signalling pathways, including apoptosis, proliferation, angiogenesis and tumour-induced immunosuppression.[29] MTT assay is a standardised and recognised test to screen possible cytotoxic effects in cancer cell lines though it does not give any comprehensive information other than membrane stability of the cell, but help in determination of potent activity of drug-dose and time-dependent manner.[30] Although complete inhibition action mechanism of TQ is still unclear[31] studies are suggested to reveal the TQ cytotoxicity and apoptotic induction for tumour inhibition in animal models.

Conclusion

The precise method for extraction and quantification of TQ from N.S and T.V was successfully modified by employing soxhlet extraction and HPLC analysis. N.S was found to be good enough to be used as the best source for economical production of TQ, which can be enhanced by modifying extraction procedure or solvent used. T.V showed strong anti-oxidant activities when compared to N.S whereas N.S had showed more potent anti-proliferative activities. Anti-proliferative activities of TQ revealed the dose-dependent relationship of drug and disease by resulting in decreased cell viability of HeLa cancer cell with increasing concentration of TQ without any further side reactions due to overdosing. This extracted phyto-chemical component can be used as safe and cheaper drug for uplifting the economic conditions of pharmaceutical companies worldwide in cancer treatment.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Bhadoriya SS, Mangal A, Madoriya N. Bioavailability and bioactivity enhancement of herbal drugs by "Nanotechnology": a review. J Curr Pharm Res 2011; 8: 1-7
2. Darakhshan S, Bidmeshki Pour A, Hosseinzadeh CA, Sisakhtnezhad S. Thymoquinone and its therapeutic potentials. Pharmacol Res 2015; 95: 138-58.
3. Abdel-Zaher AO, Abdel-Rahman MS, Elwasei FM. Protective effect of Nigella sativa oil against tramadol-induced tolerance and dependence in mice: role of nitric oxide and oxidative stress. Neurotoxicol 2011; 32: 725–33.
4. Ahmad A, Husain A, Mujeeb M, Khan S, Najmi, AK, Siddique NA, et al. Review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed 2011; 1691: 1-75.
5. Woo CC, Loo SY, Gee V, Yap CW, Sethi G, Kumar AP et al. Anticancer activity of thymoquinone in breast cancer cells: Possible involvement of PPAR-γ pathway. Biochem Pharmacol 2011; 82: 464–75.
6. Prasanth RV, Kandisa RV, Varsha PV, Satyam S. Review on Thymus vulgaris Traditional Uses and Pharmacological Properties. Med Aromat Plants 2014; 3: 164.
7. Monira A, El KA, Naima Z. Evaluation of protective and antioxidant activity of thyme (thymus vulgaris) extract on paracetamol-induced toxicity in rats. Aus J Basic App Sci 2012; 6: 467-74.
8. Butt, SA, Nisar N, Mughal AT. A review: Therapeutics potentials of phytochemical drugs and their loading in pH specific degradable Nano-drug carrier targeting colorectal cancer. J Pak Med Assoc 2018; 68: 607-14.
9. Grosso C, Figueiredo A, Burillo J, Mainar AM, Urieta JS, Barroso JG, et al. Composition and antioxidant activity of Thymus vulgaris volatiles: Comparison between supercritical fluid extraction and hydro distillation. J Sep Sci 2010; 33: 2211-8.
10. Attoub S, Sperandio O, Raza H, Arafat K, Al-Salam S, Al-Sultan, MA, et al. Thymoquinone as an anticancer agent: Evidence from inhibition of cancer cells viability and invasion in vitro and tumor

growth in vivo. Fundam Clin Pharmacol 2012; 27: 557-69.
11. Bourzac K. Nanotechnology: carrying drugs. Nature 2012; 491: S58–60.
12. Yusufi M, Banerjee S, Mohammad M, Khatal S, Venkateswara SK, Khan EM, et al. Synthesis, characterization and anti-tumor activity of novel thymoquinone analogs against pancreatic cancer. Bio Org Med Chem Lett 2013; 23: 3101–4.
13. Kusuma S, Mahfud M. The extraction of essential oils from patchouli leaves (Pogostemon cablin Benth) using a microwave air-hydro distillation method as a new green technique. RSC Adv 2017;7:1336.
14. Kayesh R, Rahman A, Sultan ZM, Uddin GM, Akhtar F, Rashid AM. Development and Validation of a RP-HPLC Method for the Quantification of Omeprazole in Pharmaceutical Dosage Form. J Sci Res 2013; 5: 335-42.
15. Vijayalakshmi M, Ruckmani K. Ferric reducing anti-oxidant power assay in plant extract. Bangladesh J Pharmacol 2016; 11: 570-572.
16. Afroze MS, Ahmed T, Nasir uddin M, Majumder, S. Phytochemical screening and in vitro determination of antioxidant potential of methanolic extract of Streospermum chelonoides. JAPS 2013; 3:117-21.
17. Pracheta V S, Ritu P, Sharma S. Preliminary Phytochemical Screening and in vitro Antioxidant Potential of Hydro-Ethanolic extract of Euphorbia neriifolia Linn. Int J Pharm Tech Res 2011;3:124-32.
18. Singh SS, Singh DG, Schuff C, de Lampasona MP, Catalán CAN. Composition, in vitro antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black cumin seeds (Nigella sativa L.). BioMed Res Int 2014; 2014: 918209.
19. Najafabad M A, Jamei R. Free radical scavenging capacity and antioxidant activity of methanolic and ethanolic extracts of plum (Prunus domestica L.) in both fresh and dried samples. AJP 2014; 4:343-53.
20. Zang S, Tang Q, Liu H, Li L, Guo F, Pan X, et al. Curcumol inhibits the proliferation of gastric adenocarcinoma MGC-803 cells via downregulation of IDH1. Oncol Report 2017; 38: 3583-91.
21. Chaudhry H, Fatima N, Ahmad ZI. Evaluation of Antioxidant and Antibacterial Potentials of Nigella sativa L. Suspension Cultures under Elicitation. Bio Med Res Int 2015; 2015: 1-13.
22. Ashraf S, Rao M, Kaneez F, Qadri S, Al-Marzouqi, Chandranath I, et al. Nigella sativa Extract as a potent Antioxidant for Petrochemical-Induced oxidative Stress. J Chromatogr Sci 2011; 49:221-228.
23. Taborsky J, Kunt M, Kloucek P, Lachman J, Zeleny V, Kokoska L. Identification of potential sources of thymoquinone and related compounds in Asteraceae, Cupressaceae, Lamiaceae, and Ranunculaceae families. Cent Eur J Chem 2012; 10:1899-906.
24. Khan A, Chen H, Tania M, Zhang D. Anticancer Activities of Nigella Sativa (Black Cumin). Afr J Tradit Complement Altern Med 2011; 8(5 Suppl): 226–32.
25. Aziz S, Rehman H, Irshad M, Farina S, Asghar F, Hussain H. Phytotoxic and Antifungal Activities of Essential Oils of Thymus serpyllum Grown in the State of Jammu and Kashmir. J Essent Oil Bear Plants 2010; 13: 224.
26. Adjimani JP, Asare P. Antioxidant and free radical scavenging activity of iron chelators. Toxicol Rep 2015; 2: 721-8.
27. Mohammad KN, Manap YAZ, Tan PC, Muhialdin JB, Alheli MA, Hussin MSA. The Effects of Different Extraction Methods on Antioxidant Properties, Chemical Composition, and Thermal Behavior of Black Seed (Nigella sativa L.) Oil. Evi-Based Comp Alter Med 2016;2016:1-10.
28. Solati Z, Baharin BS, Bagheri H. Antioxidant property, thymoquinone content and chemical characteristics of different extracts from Nigella sativa L. seeds. J Am Oil Chem So 2014; 91: 295-300.
29. Marjaneh, M, Al-hassan, FM, Shahrul H. Thymoquinone regulates gene expression levels in the estrogen metabolic and interferon pathways in MCF7 breast cancer cells. Int J Mol Med 2014; 33: 8-16.
30. Yazan SL, Ng KW, Al-Naqeeb G, Ismail M. Cytotoxicity of Thymoquinone (TQ) from Nigella sativa Towards Human Cervical Carcinoma Cells (HeLa). J Pharm Res 2009;2:585-9.
31. Salim, ZAL, Mohan S, Othman R. Thymoquinone Induces Mitochondria-Mediated Apoptosis in Acute Lymphoblastic Leukaemia in Vitro. Molecules. 2013;18: 11219-40