ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Pharmacological, bioactive screening of medicinal plant Nigella *sativa* and the derived compound thymoquinone : An invitro study

Sandhya A, Gomathi Kannayiram*

Department of Biotechnology, Dr MGR Eduational and Research Institute, Maduravoyal, Chennai, Tamilnadu, India

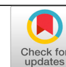
Article History:

Received on: 17 Mar 2020
Revised on: 14 Apr 2020
Accepted on: 29 Apr 2020

Keywords:

Nigella sativa,
Thymoquinone,
Anti-diabetic,
Anti-inflammatory,
Cholinesterase inhibitor

ABSTRACT



The richest biosource of drugs are from the medicinal plants which are the traditional medicine. The nutraceuticals, food supplements, Siddha, Ayurveda, pharamceutical medicines, synthetic drugs are from the plant sources. Inspite of large number of studies with herbal plants which have given good correlation in the phytochemical, anti-diabetic and anti-inflammatory content, Nigella *sativa*- a spice plant of Ranunculacea family showed significant properties than their counterparts. The seeds of Nigella *sativa* and the essential oil were found to exhibit various pharmacological activities like antianalgesic, antiulcer, anti-inflammatory, antibacterial, antimicrobial, anticancer and antidiabetic. Since there are no toxic effects or serious side effects observed using animal model and in the clinical trials, the study was carried out using Nigella *sativa* and Thymoquinone to find out the qualitative and quantitative phytochemical analysis, invitro - anti diabetic activity, anti inflammatory activity of Nigella *sativa* ethanolic extract and Thymoquinone effect on diabetes and Alzheimer's disease. The aim of the study is to prove that the Nigella sativa could be used as therapeutic agent and the compound Thymoquinone a potential cholienesterase inhibitor for the treatment of Alzheimer's disease.

*Corresponding Author

Name: Gomathi Kannayiram
Phone:
Email: gomes79@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i2.2239

Production and Hosted by

IJRPS | www.ijrps.com

© 2020 | All rights reserved.

INTRODUCTION

The richest biosource of drugs are from the medicinal plants which are the traditional medicine. The nutraceuticals, food supplements, Siddha, Ayurveda, pharamceutical medicines, synthetic drugs are from the plant sources. Based on the senses conducted by the WHO 80-90% of drugs used by the people are originated from the traditional medicine (Arunkumar and Muthuselvam, 2009).

The drugs formulated are derived from the plants stem, bark, flowers, leaves, roots, seeds, fruits. To design the drug the phytochemical constituents are very important (Mojab *et al.*, 2010; Parekh and Chanda, 2007). Similarly, various herbal extracts and essential oils are the richest source of phytochemicals such as tannins, carbohydrates, alkaloids, terpenoids, phenolics, flavonoids, steroids (Abbas *et al.*, 2012). Inspite of large number of studies with herbal plants which have given good correlation in the phytochemical, anti-diabetic and anti-inflammatory content, Nigella *sativa*- a spice plant of Ranunculacea family showed significant properties than their counterparts. The active compounds of Nigella *sativa* is used as a medicinal ailments and Nigella *sativa* seeds are used as a medicinal plant for thousand years (Toncer and Kizil, 2004).

The seeds of Nigella *sativa* and the essential oil were

found to exhibit various pharmacological activities like antianalgesic, antiulcer, anti-inflammatory, antibacterial, antimicrobial, anticancer and anti-diabetic (Hanafy and Hatem, 1991). Nigella *sativa* has many active compounds including Thymoquinone, Longifolene, Flavone, Tetracosanoic acid, Acetin, oleic acid and linoleic acid. The seed oil is used as local anaesthetic (Warrier *et al.*, 2004). Seeds and oil are the essential remedy for the treatment of various health issues including asthma, hypertension, diabetes, gastrointestinal problems, cough, jaundice, skin diseases, etc. Since there are no toxic effects or serious side effects observed using animal model and in the clinical trials, the study was carried out using Nigella *sativa* and Thymoquinone to find out the qualitative and quantitative phytochemical analysis, invitro - anti diabetic activity, anti inflammatory activity of Nigella *sativa* ethanolic extract and Thymoquinone effect on diabetes and Alzheimer's disease.

MATERIALS AND METHODS

Nigella *sativa* preparation

Nigella *sativa* seeds were purchased from local herbal store in Chennai. The seeds were authenticated and grounded to fine powder using electric grinder. The ground powder weighing 50 grams were soaked overnight in 150 ml of ethanol under shaking. The extracts were then filtered using whatmann no.1 filter paper and used the filtrate was used for further studies. The GCMS of Nigella *sativa* showed up various compounds out of which Thymoquinone (TQ) is used for further studies (Sandhya *et al.*, 2020).

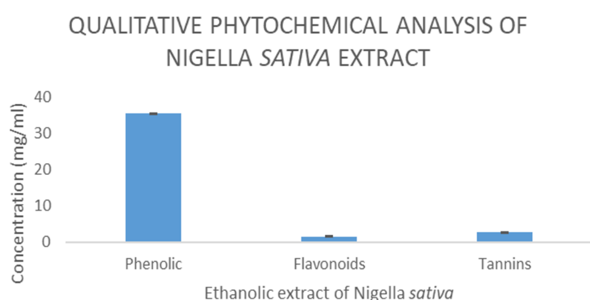


Figure 1: Qualitative analysis of Nigella *sativa* extract

Qualitative phytochemical analysis

Determinaton of total phenolic content

The determination of total phenolic content was studied using FolinCiocalteau (FC) method (Singleton *et al.*, 1999). The reaction mixture was prepared using 0.2 ml gallic acid (standard), 1.0 ml of FC

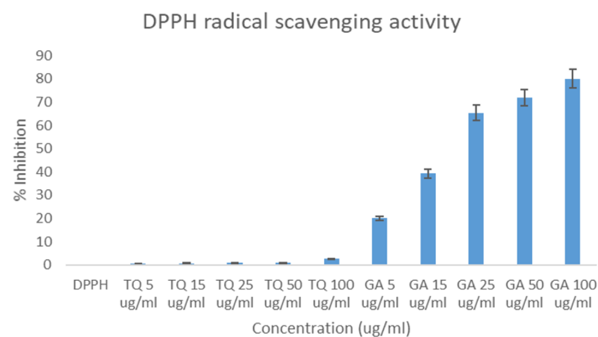


Figure 2: DPPH radical scavenging activity of Thymoquinone

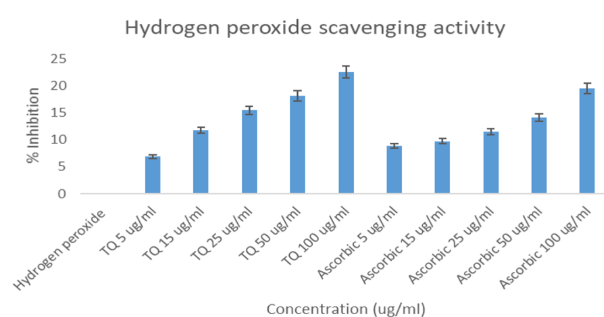


Figure 3: Hydrogen peroxide activity of Thymoquinone

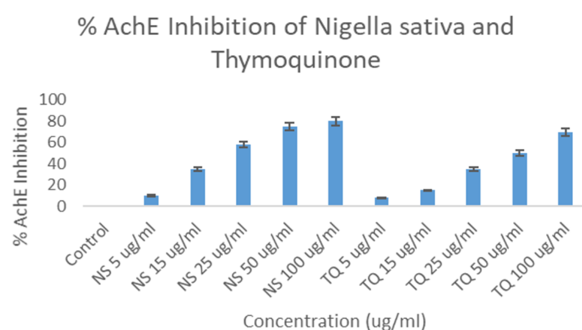


Figure 4: AchE inibitory Assay for Nigella *sativa* and Thymoquinone

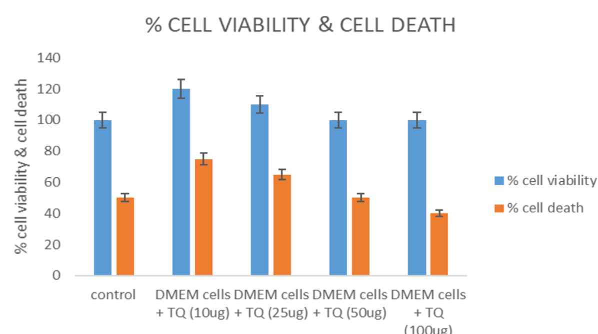


Figure 5: Cytotoxicity of Thymoquinone

Table 1: Phytochemicalscreening of Nigella *sativa*

S.no	Phytochemical analysis	Nigella sativa extract
1.	Alkaloids	+
2.	Steroids	+
3.	Phenol	+
4.	Terpenoids	-
5.	Flavonoids	+
6.	Tannins	-
7.	Saponins	-
8.	Cardiac Glycosides	+

+ indicates the presence of the phytochemical constituents
- indicaes the absence of the phytochemical consituents

Table 2: Metal chelating assay of Thymoquinone and Nigella *sativa* extract

S.no	Parameters assessed	Thymoquinone	Nigella *sativa*
1.	Metal chelating assay	2.56±0.40	1.75±0.17

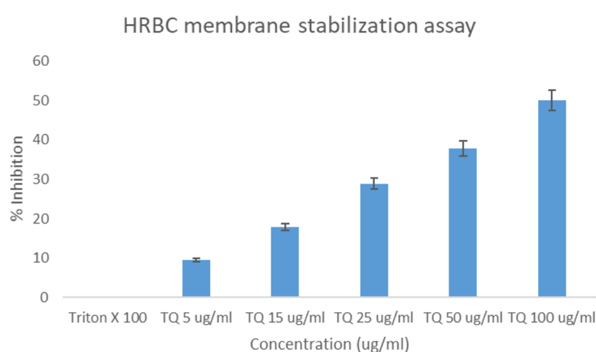


Figure 6: HRBC membrane stabilization assay of Thymoquinone

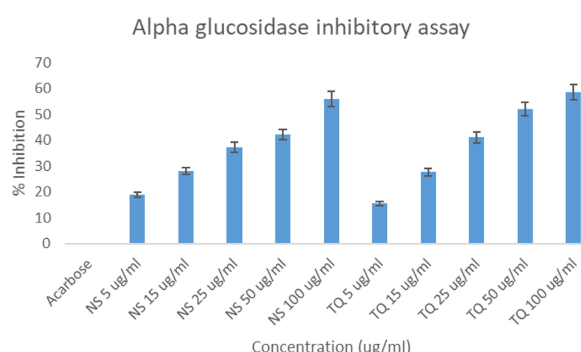


Figure 8: Alpha glucosidase inhibitory assay of Nigella *sativa* extract and Thymoquinone.

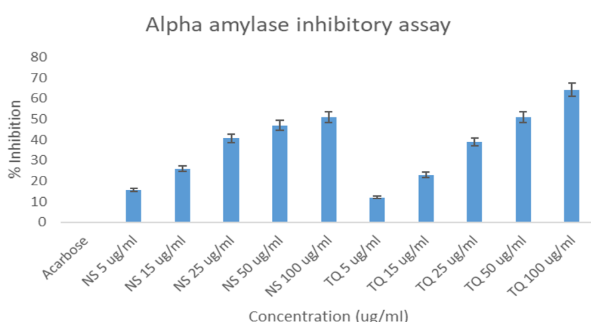


Figure 7: Alpha amylase inhibitory assay of Nigella *sativa* extract and Thymoquinone

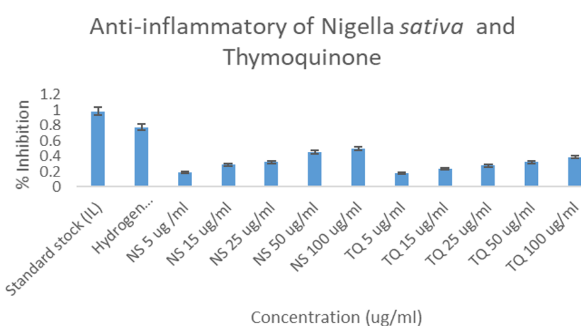


Figure 9: Anti - inflammatory activity of Nigella *sativa* and Thymoquinone

reagent, 0.8 ml of Na_2CO_3 solution were mixed well and allowed undisturbed for 2 hours at room temperature. The absorbance was measured using 750 nm using UV-VIS spectrophotometer against blank. The blank is prepared same as the reaction mixture but the extract is replaced with distilled water. The total phenolic content (Ye) was expressed in terms as mg/ml.

Determination of total tannin content

Using Aluminium Chloride method (Ribereau-Gayon *et al.*, 1998) the total tannin content (g/l) was determined. This methods involves heating of tannin in acidic medium which leads to the formation of cyanidins. Two of the reaction tube

 © International Journal of Research in Pharmaceutical Sciences

contains, 1ml of sample, 0.5 ml water and 1.5 ml of 12 N HCl. The tube 1 was mixed and heated in water bath at 100°C for 30 minutes. The tube 2 is kept at room temperature. The tubes were cooled and 0.25 ml of ethanol was added to the mixture and the absorbance was measured at 520 nm.

Determination of total flavonoids

The total flavonoids content was studied using (Zhuang *et al.*, 2007). 1 ml of extract was added to 4 ml of water in 10 ml volumetric flask. After 5 mins of incubation, 5% of $NaNO_2$ (0.3 ml), 2% of $AlCl_3$ (1.5 ml) was added to the 10 ml volumetric flask. The mixture was then kept at constant shaking for 5 mins followed by the addition of 2 ml of NaOH (1M) with constant shaking. The absorbance was measured at 510 nm using blank. The content of total flavonoid was expressed in terms of mg of catechin equivalent / 1 g of content. The samples were analysed in duplicates and calculated based on the caliberation curve of catechin.

Phytochemical screening of Nigella *sativa*

The crude extract of Nigella *sativa* was analysed to determine the bioactive components present in the extract as per the standard protocol described in (Hamburger and Hostettmann, 1991; Madhukar, 2013; Kamal, 2014).

Test for alkaloids

1.5 ml of 1% HCl was added to 2 ml of extract. The mixture was then heated in water bath followed by the addition of wanger's reagent (6 drops). Orange precipitate formation indicates the presence of alkaloids.

Test for steroids

5 ml of chloroform and 2 ml of acetic anyhdride was added to 2 ml of extract followed by the addition of concentrated sulphuric acid. Reddish brown colouration formed at the interface shows the presence of steroids.

Test for phenol

To 2 ml of extract, ferric chloride solution (5%) was added. The presence of phenol is identified by deep blue black colour.

Test for terpenoids

The extract is treated with choloroform along with few drops of concentrated sulphuric acid. The mixture is shaken well and allowed to stand undisturbed for few minutes. Yellow coloured layer formed at the lower region indicates the presence of terpenoids.

Test for flavonoids

To the extract ferric chloride solution was added in drops. The presence of flavonoid were detected using the intense green colour formed.

Test for Tannins

The extract was mixed with distilled water to make diluted test sample. To the sample 5% ferric chloride (2ml) was added. Blue green colour indicates the presence of tannins.

Test for saponins

The diluted extract was continuously shaken in a graduated cylinder for few mins. The foam layer which is formed on the top indicates the presence of saponins.

Test for cardiac glycosides

To the test sample (2ml), 3ml of glacial acetic acid followed by a drop of 5% ferric chloride was added. To the reaction mixture 0.5 ml of concentrated sulphuric acid was added along the sides of the test tube. The blue color formation in the acetic acid layer shows the presence of cardiac glycosides.

Anti-oxidant activity

DPPH (2,2 Diphenyl - 1 - picrylhydrazyl) radical scavenging activity of Thymoquinone

The compound of various concentration was added to DPPH (0.1mM). The DPPH alone serves as control. The thymoquinone was prepared using methanol. The samples were then incubated for half an hour in dark. The absorbance was measured 517 nm using UV - Vis spectrophotometry. Gallic acid was used as standard. The triplicates obtained was used to calculate the percentage of incubation (Gyamfi *et al.*, 1999).

Hydrogen peroxide scavenging activity of Thymoquinone

The modified method of Dehpour was used to analyse and determine the scavenging activity of thymoquinone. Hydrogen peroxide (40mM) was prepared using phosphate buffer (pH 7.4) and various concentrations of compound was added and incubated for 30 mins. The absorbance was measured using UV.Vis spectrophotometry at 560 nm. Phosphate buffer alone serves as blank. Ascorbic acid was used as standard. The percentage of scavenging was calculated using the formula given below (Ngonda, 2013).

$$\%scavenged\ (H_2O_2) = \\ 1.\ Abs(std)/Abs(cntrl) \times 100$$

Metal chelating assay

500μM of freshly prepared $FeSO_4$ (150μL) was added to a mixture containing 0.1M of Tris - HCl

(168μL), 0.8% w/v sodium chloride (218μL). To the mixture the extract of various concentration was added. 0.25% of 1,10-Phenanthroline (3μL) was added to the mixture (Puntel *et al.*, 2005). The absorbance was measured at 510 nm. The chelating activity of Fe^{2+} was calculated using the equation:

$$(\%) Inhibition = (Abscon - Abssam)/Abscon \times 100$$

Acetyl - cholinesterase activity assay

The evaluation of acetylcholinesterase (AChE) activity of the extracts (Ellman *et al.*, 1961). To each well of the microplate 40 microliter of enzyme (AChE) (0.28U/ml), 140μL of 5,5- dithiobis-(2-nitrobenzoic) acid (3.3mM) which was prepared in 0.1 M phosphate buffered solution of pH 7.0, which also contains 6mM of $NaHCO_3$). The extract of various concentration and 80 μL phosphate buffer of pH8.0 was added to the mixture. The solution is incubated for about 20 mins at 25°C. Acetylthiocholine iodide (0.05mM) 40μL, was added to each well after the addition of substrate and the absorbance was measured at 412 nm. The enzyme inhibitiory activity was expressed in terms of inhibition.

Cytotoxicity assay

The isolated lymphocyte cells were cultured in humidified 5% (v/v) CO_2 / air at 37°C in dulbecco's modified eagle medium supplemented with 10% Fetal bovine serum and 100 U/ml penicillin. 5 x 10^4 cells/ml of cells were placed in 96 well plates. Insulin fibril was prepared by agitating the Insulin (Human recombinant insulin (10 mg/ml)) at 65°Cfor 5 hours (Jayamani and Shanmugam, 2014). The insulin fibril pre-formed with and without Thymoquinone was diluted with freshly prepared medium and added to the wells of final concentration 2μm ol/L. The same volume of medium is added to the control well. The plates were incubated at 37°C for 48 hrs. Cell viability was determined by using MTT toxicity assay. The MTT (5 mg/ml) was added to each well and incubated at 37°C for 3 hrs. The medium was removed and DMSO was added to each well. The plates were mixed well and the absorbance was measured at 490 nm using microplate reader (Khan *et al.*, 2017).

HRBC membrane stabilization assay of Thymoquinone

Blood was drawn in a tube which contains EDTA which prevents coagulation. Various concentrations of the compound was added to 100μl of blood. Triton x 100, along with blood (positive control), and blood alone serves as negative control. The sample was incubated for 30 mins. The sample is then centrifuged at 3000 rpm for 20 mins and the supernatant was then removed for analysis. The absorbance was measured at 517 nm using UV-Vis spectrophotometer.

Anti - diabetic activity of Nigella *sativa* extract and Thymoquinone

a -amylase inhibitory assay

According to (Zhang *et al.*, 2010), the alpha amylase inhibitory studies were carried out. The Nigella *sativa* extract and the Thymoquinone compound were used for the studies. The various concentrations of the extracts and the compound was mixed with di-methyl sulfoxide solution. The alpha amylase was dissolved in phosphate buffer of pH 6.8. After incubation, starch solution (1%) was added to all the tubes. The reaction mixture was incubated again for 15 minutes. After the incubation time the reaction was stopped using 1 ml of di-nitro salicylic acid (DNS) reagent and the tubes were boiled in water bath for about 10 minutes. The contents were then cooled, to which 10 ml of distilled water was added. Absorbance was measured at 540 nm. Acarbose serves to be the positive control.

$$Percentage\ of\ inhibition = (Absorbance\ of\ control - Absorbance\ of\ test\ sample) / (Absorbance\ of\ control) \times 100$$

a- glucosidase inhibitory assay

α- glucosidase inhibitory assay was determined based on the protocol (Ting *et al.*, 2005). 112μ L of potassium phosphate buffer (pH 6.8), 20 μL of enzyme solution, 8μL of the extract and compound TQ were mixed and incubated at 37°C for 15 minutes. Following 20 μL of p-NPG was added and further incubated for another 15 minutes at 37°C. The reaction was terminated with 80μL of Na_2CO_3 solution. Absorbance was measured at 405 nm. 8μL of DMSO was added to control and blank incase of extract and compound. Acarbose serves as positive control.

$$Inhibitory\ activity\ (\%) = [1 - (ODsample - OD\ sample\ blank) / (ODcontrol - OD\ blank)] \times 100$$

Anti inflammatory activity of Nigella *sativa* extract Thymoquinone

Interleukin -1 beta (IL-1β) were used to analyse using lymphocyte cell culture supernatant using ELISA kit which is available commercially. Based on the bioassay technology protocol the lymphocyte sample was processed and the triplicates were obtained (Quillin *et al.*, 2006).

 © International Journal of Research in Pharmaceutical Sciences

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of Nigella *sativa* extract

The Figure 1, shows the highest concentration of total phenolic content was recorded in the ethanolic extract of Nigella *sativa*. The amount of polyphenols in ethanolic extract was 35.6 mg/ml. Similarly, in flavonoids and tannins it is obtained as 1.5 mg/ml and 2.6 mg/ml. These results are based on the study made by (Cheikh-Rouhou *et al.*, 2007) which states that Nigella *sativa* is the potential source of phenolic compounds.

Phytochemical screening of Nigella *sativa*

In the analysed results it is evident that the phytochemical screening of the Nigella *sativa* extract showed the presence of secondary metabolities like Alkaloids, Steroids, Flavonoids, Glycosides (Table 1). These compounds are with medicinal as well as physiological benefits. This isolation of bioactive compounds helps in the formulation of new drugs for various diseases and disorders.

Anti - oxidant activity

DPPH radical scavenging activity of Thymoquinone

DPPH radicals are reduced due to the donation of hydrogen molecules which is more stable and estimates free radicals. The DPPH activity of the Thymoquinone (IC 50 value 121.56$\pm$0.66μg/ml) is showed in the Figure 2. The DMSO dissolved compound thymoquinone showed low scavenging activity when compared with gallic acid (IC 50 value = 2.46$\pm$0.76μg/ml). Nigella *sativa* extract exhibited IC 50 value 11.26$\pm$0.93 μg/ml. Based on this result it is evident that thymoquinone has a low potential effect towards the antioxidant activity whereas Nigella sativa exhibited good potential towards antioxidant activity (Sandhya *et al.*, 2020).

Hydrogen peroxide scavenging activity of Thymoquinone

The compound Thymoquinone was analyzed by measuring decrement of hydrogen peroxide at 230 nm. The compound is found to be more effective whose IC 50 value is 12.31 μg/ml and dose dependent, as similar to Nigella sativa (Sandhya *et al.*, 2020) when compared to the standards ascorbic acid whose IC 50 value is 17.50μg/ml, Figure 3.

Metal chelating assay of Thymoquinone and Nigella *sativa* extract

As shown in the results the extract Nigella *sativa* chelates the metal Fe(II) at various concentrations whose mean$\pm$SD values are found to be 1.75$\pm$0.17 when compared to thymoquinone (2.56$\pm$0.40).The result revealed that the extract has high scavenging ability of OH when compared to TQ which is less efficient in scavenging the OH molecules (Table 2).

Acetyl - Cholinesterase activity of Thymoquinone and Nigella *sativa* extract

Cholinesterase study reveals the role of TQ as a cholinesterase inhibitor. The inhibition of the enzyme cholinesterase by TQ showed higher inhibition when compared to Nigella *sativa* extract. The highest cholinesterase activity of TQ was 53.7 $\pm$1.1 μg/ml, in which Nigella *sativa* extract exhibited 84.7 $\pm$4.3μg/ml. This indicates that the TQ is effective in preventing the enzyme cholinesterase's role in neurotransmitter AchE hydrolysis (Figure 4).

Cytotoxicity assay of Thymoquinone

The isolated lymphocytes were treated with Thymoquinone (TQ) compound to study the cytotoxicity of the cell. The assay showed statistical decrease in cell death at the concentration of 100μg (40%) when compared with the control. This showed a less effect on cell proliferation (Figure 5).

HRBC membrane stabilization assay of Thymoquinone

The hemolysis prevention using Thymoquinone compound is determined using the stabilization studies. Triton x 100 destabilizes the RBC membrane and makes the hemoglobin to leak. Different concentrations of the compound were analysed and it was found that Thymoquinone prevents the leak of hemoglobin from the RBC by stabilizing the membrane. This even suggests that Thymoquinone is non toxic and it can be used for biological studies Figure 6.

Anti - diabetic activity of Thymoquinone and Nigella *sativa* extract

α- amylase inhibitory assay

The alpha amylase inhibitory assay of Nigella *sativa* showed the highest inhibition rate of about 50.84% at 100 μg/ml concentration whereas for TQ the highest inhibition rate 64.09% at 100 μg/ml concentration and the lowest inhibition rate of about 15.49 % 10 μg/ml concentration while TQ showed inhibition rate of 12.02% at 10 μg/ml concentration as shown in Figure 7. Acarbose serves as positive control. The result showed that the extract and the compound TQ is dose dependent and revealed the increase in inhibitory activity against amylase enzyme.TQ showed potential activity towards inhibition when compared to Nigella *sativa* extract.

α- glucosidase inhibitory assay

Alpha glucosidase inhibitory activity of Nigella

sativa extract and TQ was used to analyse the inhibitory activity of the samples. The inhibitory activity was determined by using p-Nitrophenyl - α - D- glucopyranoside (p-NPG) which is a substrate and they are compared with the control Acarbose. The extract of concentration 5, 15, 25, 50 μg/ml showed less than 50% of inhibition whereas 100 μg/ml showed more than 50% of inhibition when compared to TQ. TQ inhibited more than 50 % at concentrations 50 and 100 μg/ml. Based on the results, TQ showed an effective inhibitory activity when compared with the extract Figure 8.

Anti-inflammatory activity of Thymoquinone and Nigella *sativa* extract

Increased level of IL-1β cytokine is the major cause in various metabolic disorders. The inhibition of these will prevent the pathogenic effects which leads to metabolic disorders (Bang *et al.*, 2009). The down regulation of these is an important goal in pharmacology for the inflammatory related disorders. The extract and compound TQ for the inhibition of IL-1β were analysed and the effect was expressed in Figure 9. It is evident that TQ is more potent than extract in the inhibition of IL-1 β. This is compared with the standard IL-1β, and also with hydrogen peroxide treated cells. Based the results the TQ showed inhibition, it has a potent anti-inflammatory property.

CONCLUSIONS

The invitro studies revealed that the crude extract of Nigella *sativa* have many phytochemical constituents that are medicinally significant. Crude extract of Nigella *sativa* and the isolated compound Thymoquinone, have anti-inflammatory and anti-diabetic activities which was evident from IL-1 beta assay, Alpha amylase and alpha glucosidase inhibitory assay. Though Nigella *sativa* is more effective in DPPH radical scavenging and metal chelating assay than Thymoquinone, the latter (TQ) showed more efficacy in hydrogen peroxide scavenging activity and AchE inhibitory assay. Such properties make Thymoquinone a potential and promising compound in the treatment of Alzheimer's disease.

ACKNOWLEDGEMENT

I thank Dr. K. Gomathi, Professor and Deputy Head, Department of Biotechnology, Dr. M.G.R Educational and Research Institute, Chennai for supporting my work and for guiding me. I feel thankful to acknowledge that this research is funded by the Founder chancellor's Doctoral Fellowship, Dr. M.G.R Educational and Research Institute (Deemed University).

REFERENCES

Abbas, M., Mehmood, T., Bashir, A., Zafar, M., Afzal, A. 2012. Economics of Lallemantia royleana (tukham-e-balangoo) production in the low intensity cropping zone of the Punjab, Pakistan. *Pakistan Journal of Agricultural Research*, 25(2).

Arunkumar, S., Muthuselvam, M. 2009. Analysis of phytochemical constituents and antimicrobial activities of Aloe vera L. against clinical pathogens. *World Journal of Agricultural Sciences*, 5(5):572–576.

Bang, J. S., Choi, H. M., Sur, B. J., Lim, S. J., Kim, J. Y., Yang, H. I., Kim, K. S. 2009. Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1β-stimulated fibroblast-like synoviocytes and in rat arthritis models. *Arthritis research & therapy*, 11(2).

Cheikh-Rouhou, S., Besbes, S., Hentati, B., Blecker, C., Deroanne, C., Attia, H. 2007. Nigella sativa L.: Chemical composition and physicochemical characteristics of lipid fraction. *Food Chemistry*, 101(2):673–681.

Ellman, G. L., Courtney, K. D., Andres, V., Featherstone, R. M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7(2):88–95.

Gyamfi, M. A., Yonamine, M., Aniya, Y. 1999. Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries. *General Pharmacology: The Vascular System*, 32(6):661–667.

Hamburger, M., Hostettmann, K. 1991. 7. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry*, 30(12):3864–3874.

Hanafy, M. S. M., Hatem, M. E. 1991. Studies on the antimicrobial activity of Nigella sativa seed (black cumin). *Journal of Ethnopharmacology*, 34(2-3):275–278.

Jayamani, J., Shanmugam, G. 2014. Gallic acid, one of the components in many plant tissues, is a potential inhibitor for insulin amyloid fibril formation. *European Journal of Medicinal Chemistry*, 85:352–358.

Kamal, A. 2014. Phytochemical screening of Syzygium Cumini Seeds. *Indian Journal of Plant Sciences*, 3(4):2319–3824.

Khan, M. S., Siddiqui, S. P., Tarannum, N. 2017. A systematic review on the synthesis and biological activity of hydrazide derivatives. *Hygeia Journal*

 © International Journal of Research in Pharmaceutical Sciences

for Drugs and Medicine, 9(1):61–79.

Madhukar, C. 2013. Phytochemical screening of cumin seeds extract. *Rep. Opin*, 5(1):57–58.

Mojab, F., Kamalinejad, M., Ghaderi, N., Vahidipour, H. R. 2010. Phytochemical screening of some species of Iranian plants. *Iranian Journal of Pharmaceutical Research*, pages 77–82.

Ngonda, F. 2013. In-vitro antioxidant activity and free radical scavenging potential of roots of Malawian Trichodesma Zeylanicumm (burm.f.). *Asian Journal of Biomedical and Pharmaceutical Sciences*, 3(20):21–25.

Parekh, J., Chanda, S. 2007. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, 10(2).

Puntel, R. L., Nogueira, C. W., Rocha, J. B. T. 2005. Krebs Cycle Intermediates Modulate Thiobarbituric Acid Reactive Species (TBARS) Production in Rat Brain In Vitro. *Neurochemical Research*, 30(2):225–235.

Quillin, M. L., Wingfield, P. T., Matthews, B. W. 2006. Determination of solvent content in cavities in IL-1beta using experimentally phased electron density. *Proceedings of the National Academy of Sciences*, 103(52):19749–19753.

Ribereau-Gayon, P., Dubourdieu, D., Doneche, B., Lonvaud-Funel, A. 1998. Handbook of enology. I: The microbiology of wine and vinification. pages 410–419, New York. Wiley.

Sandhya, A., Kanayiram, G., Kiruthika, L., Afroz, S. A. 2020. Nigella Sativa : A Potential Inhibitor for Insulin Fibril Formation. *International Journal of Research in Pharmaceutical Sciences*, 11(1):765–774.

Singleton, V. L., Orthofer, R., Lamuela-Raventós, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *In Methods in enzymology*, 14:152–178.

Ting, L., Zhang, X., Song, Y., Liu, J. 2005. A microplate-based screening method for alpha-glucosidase inhibitors. *Chinese Journal of Clinical Pharmacology and Therapeutics*, 10(10):1128–1134.

Toncer, O., Kizil, S. 2004. Effect of seed rate on agronomic and technologic characters of Nigella sativa L. *International Journal of Agriculture and Biology*, 6(3):529–532.

Warrier, P. K., Nambiar, V. P. K., Ramankutty 2004. Indian medicinal plants - A compendium of 500 species. *Orient Longman Pvt Ltd*, pages 4–4.

Zhang, L., Li, J., Hogan, S., Chung, H., Welbaum, G. E., Zhou, K. 2010. Inhibitory effect of raspberries on starch digestive enzyme and their antioxidant properties and phenolic composition. *Food Chemistry*, 119(2):592–599.

Zhuang, P., Yang, Q. W., Wang, H. B., Shu, W. S. 2007. Phytoextraction of Heavy Metals by Eight Plant Species in the Field. *Water, Air, and Soil Pollution*, 184(1-4):235–242.