Assessment of Quinone Enriched Fraction in *Nigella sativa* Seed Extracts

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**ABSTRACT**

In recent days, the thirst for the identification of the potential bioactive compounds from the natural sources like medicinal plants is on continuous demand. Among scientists and academicians, it has created many interdisciplinary platforms for research in establishing new drugs from the natural sources. According to many recent studies, *Nigella sativa* is believed to be the rich source of quinone, an effective bioactive compound with lots of medicinal values. The purpose of this study was to isolate and estimate the quinone in *Nigella sativa* seed extracts (aqueous and ethanol). Based on the qualitative and quantitative determination, the extracts were further focused for isolation of quinone from both aqueous and ethanolic extracts of *Nigella sativa*. The isolated compound is identified by thin layer chromatography and purity is analyzed in High performance liquid chromatography. From the results we obtained, it was very clear that among the aqueous and ethanolic extracts of *Nigella sativa*, the ethanolic extract has been found with the highest quantity of quinine. This would be predicting that the ethanol extract of *Nigella sativa* may have good efficacy of pharmacological and therapeutic potentials like antidiabetic, anticancer, antimicrobial, anti-inflammatory properties when compared with the aqueous extract due to the presence of more quinone.

**INTRODUCTION**

Due to polluted environment & modernized culture, the causes of chronic illness are become quite common & thus may increase the mortality rate. The epidemiology studies implies Globally, the cancer is diagnosed as most common disease as third in males and the second in females in western countries (Schneider-Stock *et al.*, 2014). The plant are used as medicine due to the presence of natural bioactive constituent (or) salutary agent which would diminish (or) terminate the progression of cancer (Perše and Cerar, 2011). Several studies elucidates the secondary metabolites of the plants has ability for decreasing the risks of severity of disease (Lipkin *et al.*, 1999) & certain experimental in-vivo studies also shows that proliferation of tumors are inhibited by plant derivatives (Surh, 2003). *Nigella sativa* Linn is an annual herb plant has focused as important medicinal plant due to their beneficial effects over the health. There are more research evidence from different laboratories have provided about health promoting effects such as antioxidant activity, prevention of cancer in both animal and cell culture (Jayaprakasha *et al.*, 2008; Hasan *et al.*, 2011). Nigella seeds are regarded as the
stimulants for energy metabolism & it also involved in recovery from tiredness & depression, as Islamic prophet stated the black seed can heal all disease expect death (Jayaprakasha et al., 2007). The seeds of the plants are used as constringent, stimulant, diuretic, anthelmintic, dyspepsia, paralysis, piles & skin disease in medicinal field. Nigella sativa linn commonly known as Black caraway, Black seed, Black cumin, fennel flower, nigella, nutmeg flower, Roman coriander & Kalonji, belongs to Ranunculaceae family native to south & southwest Asia. It is used as traditional medicine for various disease from ancient period as a natural restorer (Harzallah et al., 2011) N.sativa essential oil are also contains essential compounds which has antioxidant & anti-inflammatory effects (Gali-Muhtasib et al., 2007; Kumar et al., 2012). In the previous decennary, the promising anti-tumor activity was investigated in Black cumin seeds (Musa et al., 2004). Many analytical methods such as HPLC, TLC & pulse polaro-graphic were done for determination of quinone & its derivatives in N.sativa seed oil.

The main focus of the present study is to highlight the quinone enriched fractions which would be a platform for the undiscovered potential of the plant. The study carried out qualitative & quantitative determination of quinone enriched fraction in Nigella sativa seed of aqueous & ethanol extract. Further, to assess the medicinal activity of the plant, the quinine bioactive compounds were isolated from plant both the extract & assessed its purity in HPLC technique.

**MATERIALS AND METHODS**

**Collection of plant materials**

The plant material of Nigella sativa used in this study was collected from a local market, Tambaram. Authentication of plant material is carried out by prof. P. JAYARAMAN, PLANT Anatomy Research Centre, Tambaram, Chennai 45.

Following identification of Voucher specimen, the plants were deposited in herbarium. Authentication number: PARC/2017/3364. Fresh seeds of the plant were collected, cleaned, washed, shade dried and used for further studies.

**Table 1: Yield of Nigella sativa seed Extracts**

| Solvent system | Powder weight (g) | Extract Weight (g) |
|----------------|-------------------|-------------------|
| Ethanol        | 100               | 9.8               |
| Aqueous        | 100               | 6.2               |

**Preparation of Ethanol extract**

The dried seeds of Nigella sativa were pulverized to a fine powder and stored in air tight containers separately. 30 g of dried seed powder were extracted with 250ml of ethanol for 18 cycles using Soxhlet apparatus. After the running time the extract was concentrated in a rotary vacuum evaporator with temperature ranging from 30 -40°C.

**Preparation of Aqueous extract**

20 g of dried seed powder were defatted by soaking the seed with petroleum ether for one day &

All chemicals including solvents used were of highest purity and analytical grade purchased from Sigma Aldrich, USA and Karthick Enterprises Pvt. Ltd., Chennai – 45.

**Sources of fine chemicals**

Graph 1: The Quinone content of extracts of N.sativa

**Figure 1: Identification of isolated compound on TLC Plate**

**Figure 2: HPLC Chromatogram of isolated compound of aqueous extract**

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Table 2: Qualitative analysis of Quinone in crude extracts of *Nigella sativa*

| S.No | Phytochemical compound | Reagents          | Ethanol extract | Aqueous extract | Observation          |
|------|------------------------|-------------------|-----------------|-----------------|----------------------|
| 1    | Quinone                | Extract+H2So4 (or) HCl | +++            | +++             | Red or Yellow precipitate |

4-quinone (16.4 mg) was dissolved in 100 ml of distilled ethanol. This solution can be stored in the dark for at least a week. Suitable dilutions of this stock solution were made to obtain working standards of varying concentrations.

2. **8-Hydroxyquinoline** A 2.5% (W/V) solution of 8-hydroxyquinoline tilled ethanol was used.

3. **Ammonium hydroxide** Liquor ammonia (Sp. gr. 0.88) was diluted with null amount of water and used throughout the studies.

**Procedure**

To 1 ml of a thymoquinone solution in ethanol were added as standard solution, the aqueous extract & ethanol extract were added in test tube marked as T1 & T2. In all the test tube, 0.2 ml of 8-hydroxyquinoline reagent, 0.8 ml of ammonium hydroxide and 1 ml of water in the given order. After mixing, the tubes were heated for 10 min, on a water-bath maintained at 60 °C. The contents were diluted with water to make up to 6 ml and kept at room temperature (25-30 °C) for 30 min. The absorbance of the blue-green complex developed was measured at 685 nm.

**Isolation of Active compound**

**Column chromatography**

The 5 gm of aqueous extract & 9gm of ethanol extract was dissolved in respective solvent and adsorbed into silica gel 100 ~ 200 mesh. After evaporation of the solvent, the adsorbed material was loaded in to silica gel column (100 ~ 200), prepared in hexane. The column was eluted with hexane followed by gradually increasing polarity with Hexane: ethyl acetate (95:5; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80) and finally with 100% Ethylacetate. The column further eluted with ethyl acetate: chloroform (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80) and finally with 100% chloroform. The column was further eluted with Chloroform: Methanol (98:2; 94:6; 92:8; 90:10; 85:15; 80:20) and finally with 50% methanol Fractions were monitored under TLC for identification of active compound fractions for both the extracts. The resulting material was purified by using activated charcoal in hot ethyl acetate and the fractions are kept at room temperature for overnight.

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Thin layer chromatography

The fractionated compound of Plant extracts was subjected to thin layer chromatography (TLC) as per conventional method using silica gel 60F254, 5x3 cm (Merck) were cut using TLC cut Glass capillary tubes were used to spot the extract in TLC plates. Different solvent systems ranging from lower to higher polarities were tested for the separation of bioactive components. In the TLC chamber the solvent system viz hexane: ethyl acetate (90:10) was used. After pre-saturation with mobile phase for 30 min the plates were kept inside the chamber and the elution was performed using above mentioned solvent systems. After completion of the elution the plates were dried and subjected to visualized under UV chamber and sprayed using different spray reagents. Rf values determined by using following formula

\[ R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}} \]

HPLC Analysis For purpose of the HPLC analysis, the isolated compound of aqueous extracts and ethanolic extract were re-dissolved in distilled water; 80% methanol, respectively (20 mg/ml concentration). After filtration through 0.45 μm, 13-mm Millipore filters, 20 μl of each sample were injected into an Intertsil ODS-3 C18, (250 x 4.0 mm, 5 μm) reversed phase column of a High-Performance Liquid Chromatography System (Schimadzu) connected to a UV-Vis detector (Model-UFLC). The HPLC analysis was performed using a linear gradient of 80% water in methanol to 100% methanol for 30 min, followed by 100% methanol for 50 min with a flow rate of 0.5 ml / min, detection 254 nm. The peak areas and peak heights were analyzed by the software package (CLASS-VP) provided with the HPLC system. For determination of HPLC profile of thymoquinone, the chemical purchased from Sigma Aldrich, USA was re-dissolved in DMSO (1 mg/ml concentration) and 10 μl of the sample was injected into the HPLC system. HPLC analysis was performed as described above for the isolated compound of aqueous and ethanol extracts.

RESULTS AND DISCUSSION

Medicinal plants play main role by treating or protecting human health in dreadful disease due its potential activity of phytochemicals of secondary metabolites. Thereby large numbers of medicinal plants constituents are showing beneficial therapeautic effects (Salim and Fukushima, 2003). The successive extraction of Black cumin seed using different solvent, its yield are shown in the Table 1.

The secondary metabolites (or) phytochemical active compounds of Nigella sativa extracts show the result in Table 2. Quinone is one of the most active component in plant extracts, here both the solvent extract shows the presence of quinone which possess biological properties such as anti-aging, anti-inflammation, anti-cardiovascular disease, anti-tumor activity, antimicrobial & anti-parasitic activity (Zu et al., 2010).

The quantitative determination are carried out for assessing the quinone rich fraction among the two extracts by the colorimeter method (Rani et al., 1978). The Nigella sativa aqueous extract shows the value about 1.58 ± 0.63, whereas in ethanol extract shows about 8.96 ± 1.59. Hence the result of ethanol extract are found to be quinone enrich fraction than the aqueous extract are depicted in Graph 1. This quinone of extract are substances that neutralize harmful free radicals & prevent oxidative damage to cells, & also involved in other factors help in reducing both total & LDL (bad) cholesterol, anti-bacterial, anti-inflammatory, & protect the liver by enhancing the removal of toxins, metabolize, drugs which are crucial to health (Ahmad et al., 2013). (Paarak, 2010), were reported that the Nigella sativa Linn has pharmacological activity such as anti-tumor, anti-diabetic activity, Gastro protective activity, pulmonary activity, Nephroprotective activity, Hepatoprotective activity.

The Nigella sativa seeds are used as oral traditional medicine by people due to medicinal value. Many studies reveals that Nigella sativa plant extract has cytotoxicity effect over various types of cancer cell lines in vivo & in vitro (Salomi et al., 1991). Further to confirm the activity of quinone enrich fraction of ethanol extract & aqueous extract, the extract were subjected to column chromatography for isolation of quinone fraction. For aqueous extract, the fractions were monitored under TLC and similar fractions were observed and fractions number 53 - 59 (compound-1) and 60-129 (mixture of compounds).In the ethanol extract, the Fractions were monitored under TLC and similar fractions were observed and fractions number 54 - 65 (compound -1) and 80 - 112 (mixture of compounds). The isolated fraction of single compound was identified by TLC with various spraying reagent (Figure 1) depicts with similar Rf value (0.52) & as single spot obtained for both the extract shows that it could be the same bioactive compound. The other report in identification of the compounds in Nigella sativa sample extract was based on retention times & comparison of UV spectra (Avula et al., 2013).
The method analyze the nine compound in *Nigella sativa* extract as compared with commercial product of *Nigella* which are vary in the concentration of both the samples. (El-Najjar et al., 2011) has reported that the active component of the plant was extracted & assessed the binding capacity in the serum was done using HPLC-MS methods. A comparative study of polyherbal mixture of *Nigella* sativa seeds Hemidesmus indicus roots & *Smilax glabra* rhizome would suggest that the crude ethanol extract of *N. sativa* has more potential than the other were proved by HPLC data (Samarakoon et al., 2010).

Further HPLC analysis of isolated fraction with standard thymoquinone helps to understand about the presence of maximum percentage of quinone among the two extracts. Thus the result of HPLC chromatogram(Figure 2 &Figure 3) demonstrates that isolated compound of ethanol extract has more quantity of quinone found at the retention time (4.025 with the 88% of purity), whereas the aqueous isolated fraction containing quinone found at the retention time (4.015 with the 75% of purity).These were compared by using standard thymoquinone which were found at the retention time (4.257 with the purity of 98%) as standard chromatogram (Figure 4).As with the above result, it confirms that the quinone is the active compound of extracts & also the HPLC data suggest that the isolated compound of ethanol extract are quinone enriched fraction than the aqueous extract.

**CONCLUSIONS**

In our study, the *Nigella* sativa extracts were isolated & analysed for quinone enriched fraction to assess its maximum potential activity. The result concludes that the ethanolic extract has higher bioactive compound of quinone as compared with aqueous extract by the evident from all basic experiments & HPLC profiles. The report of this study would be the substantial platform for preparation of therapeutic agents to explore the potential activity of *Nigella* sativa against various disease. Further, extensive research of *Nigella* sativa will be greater part in exploring its medicinal properties.

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