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Phytoconstituents of Nigella Sativa and Quantitative Densitometric Analysis of its Bioactive Compound Thymoquinone

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Abstract

Herbal plants are a reservoir of potential phytochemical compounds and the richest bioresource of drugs for traditional systems of medicine, nutraceuticals, food supplements, modern medicines, pharmaceutical intermediates, folk medicines, and chemical entities for synthetic drugs. In the present study we find out phytoconstituents of Nigella sativa and quantitative densitometric analysis of its bioactive compound thymoquinone in the different solvent extracts. It was found that Nigella sativa seeds were extracted with ethanol, methanol, and benzene as solvents. Phytochemical analysis showed the presence of potent bioactive constituents such as alkaloids, phenols, tannins, trepenoid, saponins, and steroids in methanol extract. Benzene extracts have only alkaloids and steroids. While ethanol extract showed the presence of alkaloids, phenols, tannins, proteins, amino acids, flavonoids, terpenoids, saponins, and steroids. The high-performance thin layer chromatographic method (HPTLC) was employed to quantify and densitometrically analyze thymoquinone in methanol, ethanol, and benzene extract of Nigella sativa. The analysis was performed on an aluminum plate with a mobile phase of n-hexane: ethyl acetate: methanol (7:2:1 v/v/v) and a densitometric measurement using a TLC scanner (CAMAG) at 254 nm. The ethanol extract of N. Sativa exhibited single sharp peak of thymoquinone with 0.85 Rf value, the highest area of the band 8137.6, and a total recovery of was 98.08% which is nearly equal to the standard thymoquinone with Rf value (0.85), the highest area of the band 8789.4 and total recovery was obtained 100%. The present research indicated that purified thymoquinone from N. sativa is a potential source for therapeutic application.

Keywords: *Phytoconstituents, Herbal extract, Essential oil, Quantification, Chromatography.*

Introduction

Plants have been used for therapeutic purposes since the dawn of time. Plants have remained a valuable source of natural compounds for human health maintenance. Medicinal plants have been utilized to treat diseases for thousands of years across the world. Since before recorded history, plants have been utilised to treat or prevent illness. Many allusions to therapeutic plants can be

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found in the ancient Vedas, which date from 3500 B.C. to 800 B.C. Virikshayurveda is one of the oldest works in traditional herbal medicine, dating back to before the Christian period [1]. The use of medicinal plants to treat ailments has always been a prominent component of Islamic teaching and preaching. Islamic medicine began with Hazrat Adam and ended with Hazrat Muhammad, but the quest for and compilation of these remedies is currently ongoing [2, 3].

Plant-derived compounds have recently attracted a lot of attention due to their wide range of applications. Traditional medicines, modern medications, nutraceuticals, food supplements, folk remedies, pharmaceutical intermediates, and chemical entities for synthesised pharmaceuticals are all found in medicinal plants [4, 3]. *Nigella sativa* has been utilized by millions of people throughout Asia, the Middle East, and Africa for ages. Humans all across the world have known for thousands of years that this famous herb has incredible medicinal qualities. According to Paarakh [5], *Nigella sativa* is a commonly used medicinal plant in India and is popular in several indigenous medical systems, such as Ayurveda, Siddha, Unani, and Tibb. *Nigella sativa* has been used scientifically from the time of Prophet Muhammad, as well as for other historical purposes. It is the black seeds that Prophet Muhammad (PBUH) referred to when he said, "The black seed can cure every disease except death" [6, 3].

Fixed oil, alkaloids, saponins, and fatty acids make up 36-38 percent of the black seed. Steroids, tannins, flavonoids, coumarins, cardiac glycosides, saponins, and di-terpenes were found in the methanol and ethanol extracts of *N. sativa* [7]. [8] and [9] found that black seed extract or oil had antibacterial, antioxidant, and anti-tumor action, as well as a stimulatory effect on the immune system. *N. sativa* seeds are used as an astringent, bitter, stimulant, diuretic, emmenagogue, and anthelmintic. They can also be used to treat jaundice, intermittent fever, dyspepsia, paralysis, piles, and skin conditions. According to [10], the preventive and therapeutic powers are so extensive and diversified that it is regarded as a miracle remedy.

The majority of the biological activities of black seed are now known to be related to thymoquinone, the primary, secondary metabolite found in the seeds of N. sativa, as evidenced by scientific investigations [11, 12]. Because of these characteristics, thymoquinone derived from N. sativa is a useful tool in the pharmaceutical and cosmetic sectors today. As a result of the current discovery that ethanol extract of N. Sativa had a total recovery of thymoquinone of 98.08 percent, extracted thymoquinone from the seed of N. Sativa is a potential source for the treatment of various disorders.

Material and Methods

Collection and Identification of Plant Material

Seeds of *Nigella sativa* were purchased from the local market and were authenticated by Dr. F. Shah, Department of Botany, Saifia Science College Bhopal. The voucher specimen no (J/R562) was deposited at the Herbarium of the Faculty of Botany, Saifia Science College Bhopal (M.P.) India.

Preparation of Nigella Sativa Extract

The seed of *Nigella sativa* were extracted in the soxhlet apparatus using ethanol (Soxhlet extraction is used for separating components based on the difference in the solubility in the solvent. The seed of *Nigella sativa* (200gm) was placed in the soxhlet extractor flask. 200 ml of each solvent, ethanol, methanol, benzene, and aqueous, was separately taken in the round bottom flask. The soxhlet extraction was carried out continuously at an appropriate temperature for 6-8 hrs till the extract was collected in the extractor flask. The extract thus obtained was collected in collection bottles and was further subjected to concentration using a Rotary

vacuum evaporator. After soxhlet extraction, the extract was filtered and then concentrated using a rotary vacuum evaporator. The extract was taken in a round bottom flask which was heated at an appropriate temperature on a water bath. The vapors of the solvent rise in the condenser, and after condensation, the solvent droplets were collected in the collecting flask and were lyophilized [13, 14].

Phytochemical Analysis

Phytochemical analysis was carried out to using the standard method of Trease and Evans [15], 1 mL of the plant extract was used, along with a few drops of Mayer reagents. The presence of alkaloids was confirmed by the formation of a cream-colored precipitate. 1 mL of the extract was obtained, and a few drops of the Fehling solution were added to it. The presence of carbohydrates and glycosides is confirmed by the formation of a red precipitate. We took 1 mL of extract and mixed it with a few drops of ferric chloride solution. The presence of phenolic compounds and tannins is confirmed by the formation of a bluish-black precipitate. The presence of proteins and amino acids in extracts was confirmed by adding a few drops of ninhydrin solution to 1 ml of extract. The purplish-pink colour verifies the presence of proteins and amino acids in extracts. A few drops of sodium hydroxide solution were added to 1 mL of extract. The presence of flavonoids is confirmed by the intense yellow colour. 1 mL of the extract was combined with a few drops of chloroform and sulphuric acid. The presence of terpenoids is indicated by a reddish brown colour. 1 mL of the extract was diluted to 10 mL with distilled water. The presence of saponins is confirmed by the formation of stable foam. 1 ml of the extract was mixed with 5 ml of distilled water, boiled, and then 5 ml of 1 percent HCl was added. The presence of phlobatanins is confirmed by the formation of a crimson precipitate. 1 ml of the extract was taken, and 2 ml of chloroform and 2 ml of strong sulphuric acid were added to it. The presence of steroids is confirmed by the formation of a reddish-brown coating at the contact.

Characterization of Thymoquinone

The lyophilized extract of *Nigella sativa*; ethanol, methanol, benzene, and aqueous was subjected to the analysis of its active ingredient by using high-performance thin-layer chromatography (HPTLC) densitometry according to the method of Sharma et al. [16] with some modifications. A winCATS Planar Chromatography Manager equipped with an automatic TLC sampler (Linomat 5) and a CAMAG TLC Scanner with a UV cabinet were used for qualitative as well as quantitative analyses. Chromatography was performed on a Merck silica gel 60F 254 HPTLC plate of 20.0 ×10.0 cm size and 0.2mm thickness, with n-hexane: ethyl acetate: methanol (7:2:1 v/v/v) as the mobile phase after saturation for 5–7min. The development distance was 90 mm. thymoquinone was quantified at 254 nm, its wavelength of maximum absorbance. The different concentrations 2, 4, 6, 8, 10 and 12 µL of thymoquinone were applied on 6 tracks of the TLC plate, and then developed using the above solvent system and the optical density scanned. Relative comparison of thymoquinone concentration in different samples using UV-Vis spectrometry.

Results and Discussion

The different solvent of varying polarity were used for the extraction of the seed of *Nigella sativa* for phytochemical analysis, and the result of the extraction revealed the presence of various phytochemical constituents. Methanol extract showed the presence of alkaloids, phenols, tannins, trepenoid, saponins and steroids. At the same time, the benzene extracts has only alkaloids and steroids. While ethanol extract showed the presence of alkaloids, phenols, tannins, proteins, amino acids, flavonoids, terpenoids, saponins, and steroids (Table 1). The present findings regarding phytochemical analysis of *N. sativa*

are also in corroboration with the work of Tiwari et al. [17], who reported *N.sativa* phytochemistry, pharmacology, and its therapeutic potential. It was observed alkaloids, phenols, flavonoids, glycosides, terpenoids, and steroids are the important phytoconstituents of *N. sativa*. Our results were in full agreement with [18], who observed that flavonoids, tannins, coumarines, saponins and sterols were found present in the leaf, stem, and seed extracts of *N. sativa.* The present findings suggest that Phytoconstituents of N. sativa are well supported by earlier classic work of [9], who reported *Nigella plants'* traditional uses, bioactive phytoconstituents, preclinical and clinical studies. It was found it is constituted of alkaloids, terpenes, and phenolic compounds.

Table: 1. Phytochemical Constituents Present in Ethanol Extract of Nigella Sativa (Seeds)

Plant Constituents	**Ethanol**	**Benzene**	**Methanol**
Alkaloids	+	+	+
Carbohydrates and Glycosides	-	-	-
Phenolics compounds and Tannins	+	-	+
Proteins and Amino Acids	+	-	+
Flavonoids	+	-	-
Terpenoids	+	-	+
Saponins	+	-	+
Phlobatannins	-	-	-
Steroids	+	+	+

It is well known that the substance which is responsible for the diverse biological activity of *Nigella sativa* is thymoquinone. To develop a suitable and accurate densitometric HPTLC method for thymoquinone analysis, the mobile phase composition was improved. The mobile phase n-hexane: ethyl acetate: methanol (7:2:1 v/v/v) resulted in a sharp, symmetrical, and well-resolved single peak of standard thymoquinone with R_f value (0.85), highest area of the band 8789.4 and total recovery was obtained 100%.

The most preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs used is HPTLC. This technique has shown accuracy, sensitivity, suitability for high-throughput screening, and reliability in quantifying analytes from nanogram to microgram levels [19, 20].

Methanol extract of *Nigella sativa* showed one sharp peak with 0.86 R_f value, the highest area of the band 5398.1, and total recovery was 85.43% of thymoquinone. Benzene extract revealed 0.85 R_f values, the highest area of the band 3321.6 with a recovery of 62.59 of thymoquinone, while ethanol extract exhibited the highest area of the band 8137.6, with 0.85 R_f value and total recovery of thymoquinone was 98.08% which is nearly equal to the standard thymoquinone procure by sigma (Table 2 and Figure 1). In the present study, extraction of thymoquinone of *N. sativa* are in full validation with those of [21], where they have reported quantitative determination of thymoquinone in *N. Sativa* extract and its formulation using HPTLC densiometric method. It was found to give compact spots for thymoquinone (R_f 0.77). The mean recoveries measured at three concentrations were more than 95%.

The developed TLC method for quantification of thymoquinone showed a correlation coefficient which is clearly seen in the Figure 2 polynomial regression curve graph via height (r = 0.99907) which was highly significant (P<0.05). The calibration plot of the peak area against the amount of thymoquinone was linear in the range of 2-12 µg/spot. The linear regression equation was Y=1672.852+8.355, where Y is the response

and x is the amount of thymoquinone. The present findings for extraction of thymoquinone *N. sativa* are in corroboration with the work of [22], who reported the HPTLC densitometric method for analysis of thymoquinone in *N. sativa* extracts and marketed formulations. It was obtained for thymoquinone at an R_f value of 0.48± 0.04. The calibration plot was linear in the range of 50-700 ng/spot of thymoquinone, and the correlation coefficient of 0.998 was indicative of good linear dependence of peak area on concentration, and total recovery was 98.39.

The three-dimensional CAMAG HPTLC image also shows quantification of unknown concentrations of the ethanol extract of *N. Sativa* in triplet; unknown concentrations of the methanol extract of *N. Sativa* in triplet; Benzene extract in triplet; different concentrations of thymoquinone (2 -12µL) (Figure 3). The present findings are in full agreement with those of Belete, and Dagne [23], who reported that HPTLC assay of thymoquinone in black seed and black seed oil (*Nigella Sativa* Linn) and identification of thymoquinone conversion with UV-VIS. It was found thymoquinone spot from 80% in the methanol extract of the seed was confirmed by Nuclear Magnetic Spectroscopy (NMR). The solvent system consisted of hexane and dichloromethane (1:1) were used and all the spots were visualized and quantitated at 254 nm. The thymoquinone content of freshly pressed black seed oil was 1.3%, while that of the seed was 1%.

Table: 2. Quantification of Thymoquinone

Sample	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
Standard Thymoquinone	0.79	1.8	0.85	339.9	100.00	0.93	3.8	8789.4	100.00
Methanol Extract	0.80	9.0	0.86	199.1	82.46	0.90	4.02	5398.1	85.43
Benzene Extract	0.79	13.5	0.85	98.7	59.06	0.88	56.1	3321.6	62.59
Ethanol Extract	0.79	5.3	0.85	307.6	96.64	0.93	8.5	8137.6	98.08

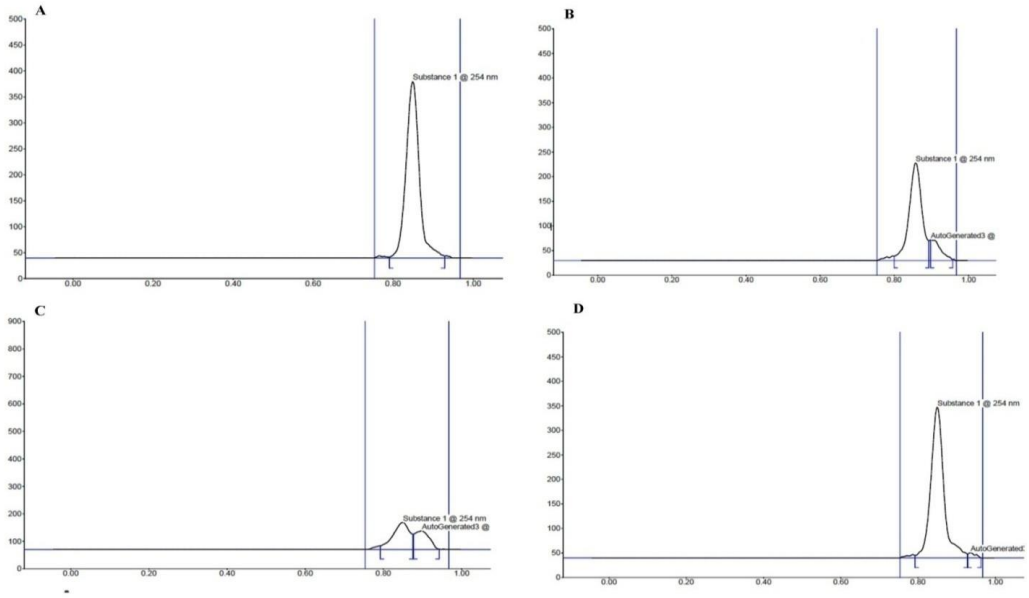

Figure: 1. HPTLC chromatogram: [A] Thymoquinone [B] Methanol Extract [C] Benzene Extract [D] Ethanol Extract

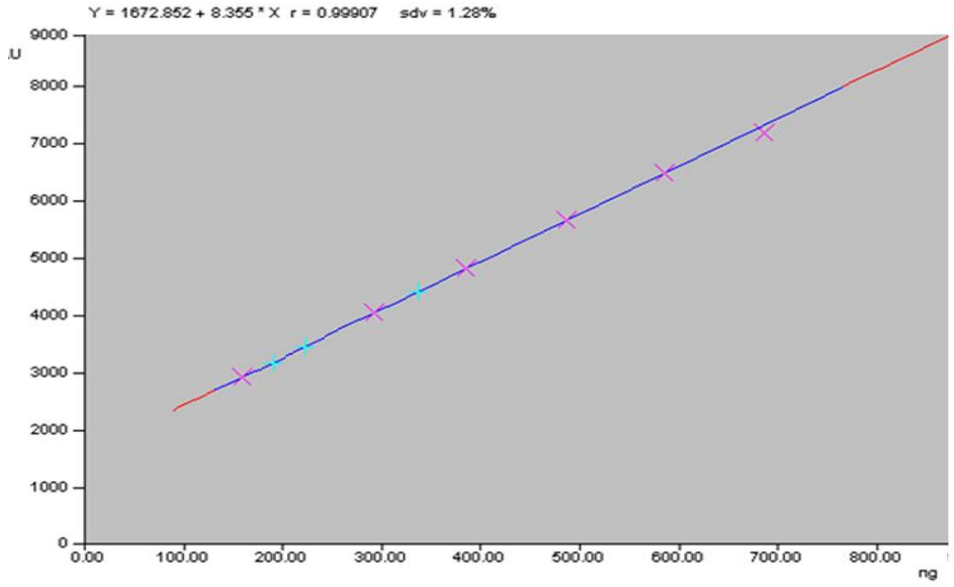

Figure 2. Polynomial Regression Curve Graph of Thymoquinone (1-12µL)

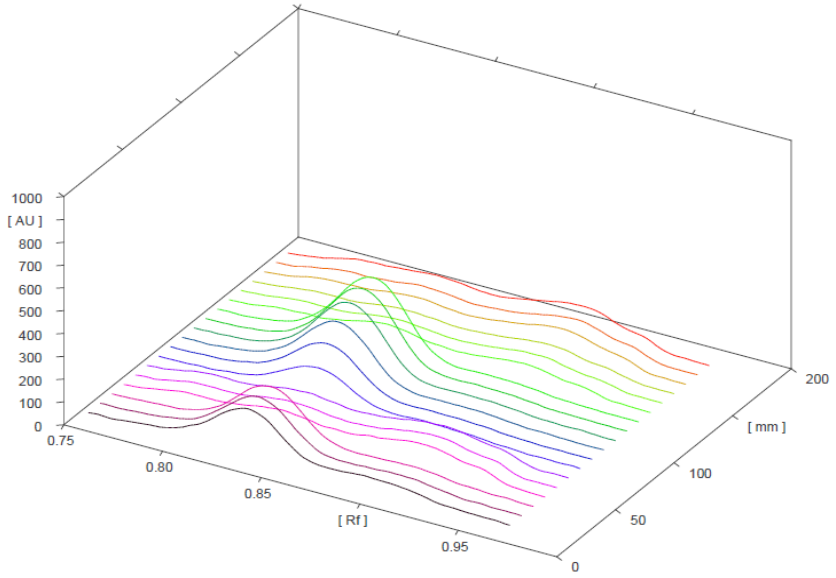

Figure 3. The Three-Dimensional CAMAG HPTLC Image at 254 nm

Conclusion

Phytochemical evaluation of *N. Sativa* seed provides information about the number of medicinally important secondary metabolites, which impart anti-microbial, anti-diabetic, antioxidant, and anti-tumor activity and a stimulatory effect on the immune system. Phytochemical profiling of plants and quantification of compounds present in plants, with increasing demand for herbal products as medicines and cosmetics there is an urgent need for standardization of plant products. In the present study, a high-performance thin layer chromatographic method was used for the quantification of phytoconstituents.

It is clearly giving evidence of the bioactive thymoquinone in ethanol extracts of *Nigella Sativa.* It was found the characterized thymoquinone showed very high similarities compared to standard thymoquinone procured by sigma. This indicated that characterized thymoquinone can be a prosperous source of *N.sativa* for therapeutic use.

Conflict of Interest

The authors state that the publishing of this paper does not include any conflicts of interest.

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