



## An Overview of Isolation Techniques, Pharmacological Activities, and Analytical Approaches for Thymoquinone

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### ABSTRACT

Herbal medicine has received a lot of interest in the last few years, and it is being utilized as a substitute for pharmaceutical medications. *Nigella Sativa* seeds produce an oil constituent that is volatile in nature and contains Thymoquinone. Thymoquinone is a naturally occurring chemical of the benzoquinone family. The pharmacological properties of Thymoquinone including anti-inflammatory, antioxidant, immunomodulatory, antimicrobial, antihistaminic, and antitumor activities were studied. As a result of its medical and industrial value, several extraction methods have been developed, including distillation, maceration, percolation, and Soxhlet extraction, etc. To identify and quantify Thymoquinone, many analytical and chromatographic techniques have been developed, including HPLC, RP-HPLC, UPLC, LC-MS, HPTLC, and the X-ray diffraction method. The HPTLC technique is a reliable method for measuring its active components within a sample at concentrations ranging from nanograms to micrograms.

**KEYWORDS:** Thymoquinone, Extraction, HPLC, HPTLC, UPLC

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### INTRODUCTION

The active ingredient of *Nigella Sativa* seeds is Thymoquinone, which is volatile in nature. Kalonji and black seeds are other names for *Nigella Sativa* seeds. It comes to the Ranunculaceae (buttercup) family and the genus *Nigella* L. *Nigella Sativa* has several active ingredients, including thymoquinone, saponins, proteins, alkaloids, fatty acids, flavonoids, all of which have benefits in the treatment of various medical conditions. Thymoquinone (TQ) is the most common component in the volatile oil of *N. Sativa* seeds, accounting for the majority of the herb's characteristics.

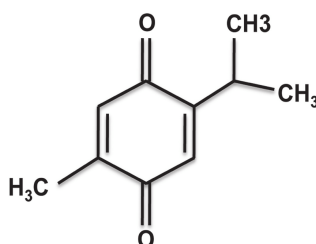


Figure 1: Structure of Thymoquinone

*N. Sativa* L. is one of the earliest cultivated plants referenced in several religious and medicinal books. It grows naturally in the region of the Mediterranean and West Asian countries. Thymoquinone is a common ingredient in the volatile oil of *N. Sativa* seeds and is responsible for the pharmacological effects. *N. Sativa* is primarily investigated for medicinal reasons because it has pharmacological qualities, including antihypertensive, anticancer, and immunomodulatory effects. Thymoquinone was studied for various types of pharmacological effects, like anti-inflammatory, antioxidant, anti-histaminic, immunomodulatory, and antimicrobial properties.[1]

### PHYTOCHEMICAL PROFILE [2]

The phytochemical properties of Thymoquinone were presented in Table 1.

### **SOURCE OF THYMOQUINONE: - [3]**

The sources of Thymoquinone were described in Table 2.

### **PHARMACOLOGICAL PROPERTIES OF THYMOQUINONE**

TQ is found in tautomeric forms such as enol, keto and combinations. The keto form accounted for approximately 90% of TQ's pharmacological effects. The pharmacological characteristics of TQ were investigated for their efficacy in pharmaceutical applications, prompting us to explore its numerous medical uses.[4]

#### **1] Antioxidant Activity**

Thymoquinone was studied for its anti-oxidant effect. TQ stimulated the generation of cytoprotective enzymes, which assisted in protecting cells against oxidative damage. TQ mediated mRNA upregulation induced cytoprotective enzymes including lipid peroxidation, H<sub>2</sub>O<sub>2</sub>, and glutathione peroxidase, which eliminates highly reactive oxygen.[5]

The interplay between TQ and oxidative stability establishes a synergistic relationship, resulting in a heightened resistance to autooxidation. *N. Sativa* seed oil can be correlated owing to the presence of antioxidant constituents. The combination of Thymoquinone and 4-terpineol possesses excellent anti-radical properties. The addition of 11.8%  $\gamma$ -terpinene has been observed to increase oxidative stability. Decreased oxidative damage might be one of the key routes that cause TQ's antioxidant activity, making it a potent antioxidant agent even at low concentrations.[6]

Thymoquinone exhibits potent phytochemical antioxidant properties due to its ability to scavenge towards several reactive oxygen compounds, which include anion of superoxide, hydroxyl radical, and singlet molecular oxygen, therefore it can counteract the detrimental effects of high Reactive Oxygen Species levels in several illnesses.[7]

#### **2] Immunomodulatory effect**

Thymoquinone based fascination of immunomodulatory effect was researched by IVIV studies (in vivo-in vitro studies) and Thymoquinone has been discovered to influence the proliferation and cellular response of numerous immune cells like B cells, neutrophils, T cells, NK cells, macrophages, and dendritic cells. Thymoquinone is a powerful antiviral drug that also activates T cells to increase adaptive immunity.[8]

Thymoquinone can decrease and reverse the pesticide-induced decrease in the level of leukocyte and immunoglobulin, as well as increase bacterial activity by stimulating macrophages. TQ is an immunomodulatory drug that can successfully stimulate innate immunity by activating many types of immune cells.[9]

#### **3] Chemotherapeutic activity**

Thymoquinone, a bioactive ingredient of black seed volatile oil, was shown to induce apoptosis in tumor cells, which is a fundamental action for successful chemotherapy treatments.

Thymoquinone based induction in several malignancies, such as colon, ovary, larynx, myeloblastic leukemia, lung, breast and osteosarcoma were explored in recent years, with reports of selective and considerable attenuation on these cells. Thymoquinone inhibited the multiplication of certain tumor cells as they advanced through various phases. TQ's potential as a chemotherapeutic agent was investigated.[10]

#### **4] Anti-microbial Action**

Thymoquinone has antibacterial action against a variety of bacterial species, such as *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *S. faecalis* and *B. subtilis*. Thymoquinone reduced the number of adhering bacteria while affecting the metabolic processes of cells trapped in biofilm. Thymoquinone effectively eliminated staphylococci in suspension and stopped biofilm development. Thymoquinone was reported to have significant growth inhibitory capability versus Gram-positive bacteria at low concentrations.[11]

TQ had shown antifungal efficacy towards *Candida* species like *C. albicans*, *C. tropicalis*, and *C. krusei*. TQ was employed as a medicinal drug to restore the dysregulated insulin production seen in HIV-1 positive individuals treated with very active antiretrovirals. These suggestions have elevated TQ to the status of a favored natural antimicrobial agent versus a wide range of diseases.[12]

#### **5] Anti-diabetic effect**

TQ contains anti-diabetic property in addition to numerous other beneficial pharmacological effects. TQ's glucose-lowering impact is not only connected to insulin impact; it reducing the formation of gluconeogenic. TQ increases glucose consumption and lowers hepatic glucose synthesis by altering carbohydrate metabolism. Thymoquinone showed an anti-glycaemic action, which alleviated diabetes complications caused by protein glycation. It reduced plasma cholesterol and triglyceride concentration in diabetic rats treated with Thymoquinone, plasma levels of cholesterol and triglycerides decreased considerably.[13]

## 6] Anti-respiratory activity

*N. Sativa* seeds provided medicinal benefits for respiratory disorders such as asthma and dyspnea. TQ might have mitigated the detrimental effects of some injurious substances. TQ inhibited apoptosis and decreased lung damage caused by long term toluene intake in rats. It also delayed the progression of pulmonary fibrosis induced by bleomycin in rats. Thymoquinone reduced cell inflammation, emphysema in air alveoli, and cell activation around bronchioles.[14]

TQ provided protection against cyclophosphamide-induced lung injury. Thymoquinone reduced the inflammation in the lung by suppressing the Th2-driven immune system reaction. Thymoquinone demonstrated its efficacy in respiratory problems and added to the traditional usage of black seeds for the treatment of respiratory issues such as bronchial asthma.[15]

## 7] Nephroprotective effect

Thymoquinone has been shown to protect kidneys against a number of pathogenic illnesses. Thymoquinone improved kidney lesions caused by numerous toxic substances because it had a defense impact, which included reducing oxidative damage and inflammation. Thymoquinone protected the rats against mercuric chloride (HgCl<sub>2</sub>) induced kidney injury.

TQ treatment improved the drop of antioxidant enzymes, proliferative response, elevation in serum creatinine, and histological damage caused by HgCl<sub>2</sub>. TQ treatment has prevented the kidneys from the oxidative damage induced by pyelonephritis. TQ might be useful as a preventive measure for nephropathies.[16]

## 8] Anti-cardiovascular effect

Thymoquinone is a treatment option for heart issues. Doxorubicin has a broad range of antitumor action, with cardiotoxicity being a prominent adverse effect. TQ pretreatment prevented against DOX-induced cardiotoxicity while maintaining antitumor efficacy.[17]

TQ reduced high creatinine levels, raised GSH levels, and prevented superoxide radical formation in enzymatic processes.[18]

### Extraction of Thymoquinone [19-20]

The pharmacological activity and yield of extract from *N. Sativa* L. seed oils were influenced by the extraction procedures used. Thymoquinone was generated from essential oil derived from seeds using a variety of processes.

**Supercritical Fluid Extraction:** Food-grade liquid carbon dioxide was used in high pressure cylinder to perform supercritical fluid extraction. Carbon dioxide was injected into the Supercritical Fluid Extraction system until the desired pressure was reached. The supercritical carbon dioxide travels from the extractor to the separator vessel. The extractor samples were collected, and the carbon dioxide flow rate was monitored using a flow meter. The entire extraction was performed in two separate conditions by varying the pressure and temperature.

**Soxhlation:** At first, the air-dried plant sample was mixed with a lab mill. In the Soxhlet device, 5g of dry powder onto a paper cartridge was extracted for 6 hrs. The resulting solution was then filtered with anhydrous sodium sulfate and re-extracted three times with methanol in a separator funnel. Re-extracted methanol portions were collected and placed in a round bottom flask, evaporated with a vacuum-operated rotary evaporator, and 10 mL of the internal standard solution was added.

**Hydro-distillation (HD):** Supercritical fluid extraction with solid carbon dioxide extract was utilized to perform hydro distillation, which (HD) for 6 hrs using the Clevenger equipment. The yellow-coloured pure essential oil (HD-SFE) with a nice odour was produced. The extract was dried with anhydrous sodium sulfate.







**Maceration:** 1 gm of black seed was extracted by using 20mL of each hexane and methanol use as solvent taken in different conical flasks and macerated at room temperature for 4 hrs. After extraction the resultant solution was centrifuged at 4000 rpm and 4°C for 10 minutes.





**Percolation:** 1 gram of black seed was extracted with 20 mL of solvent. In this hexane and methanol are used as solvent taking in different conical flasks and then heated in a water bath at 40°C for 4 hrs. The resulting mixture was centrifuged at 4000 rpm and 4°C for 10 minutes.

Table 1: Phytochemical properties of Thymoquinone

PROPERTY	CHARACTERISTIC
Appearance	white to tan crystalline powder
Molecular Formula	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
Chemical Formula	2-isopropyl-5-methyl benzo-1,4-quinone
Molecular Weight	164.20 g/mol
Melting Point	45-47 °C
Boiling Point	230-232 °C
Solubility	Soluble in organic solvent Ethanol DMSO Dimethyl formamide

Table 2: Source of Thymoquinone

Family	Species & genera	Part of the Plant Used	Photos
Ranunculaceae	<i>Nigella sativa</i> (Black Cumin)	Seed	
Lamiaceae	Agastache (Hummingbird Mint)	Aerial part	
	<i>Coridothymus</i> (Spanish oregano)	Aerial part	
	Monarda (Scarlet Beebalm)	Leaf, stem	
	Mosla (Miniature Beefsteakplant)	Aerial part	
	Origanum (wild marjoram)	Aerial part	

	Satureja (pink savory)	Aerial part	
	Thymbra (Mediterranean thyme)	Aerial part	
Cupressaceae	Tetraclinis (Barbary thuja)	Twig	
	Juniperus (haubera)	Twig	

### ANALYTICAL TECHNIQUES FOR THYMOQUINONE

The Analytical techniques used for the estimation of Thymoquinone are described in Table 3.

Table 3: Analytical Techniques for Thymoquinone

Sr. No.	Research Object	Method	Description	Ref. No.
1.	Thymoquinone + its nanoformulation	HPTLC	<b>Stationary Phase:</b> Silica gel aluminum plate 60 F254 (10 × 20 cm, 250 μm) <b>Mobile Phase:</b> n-hexane: Methanol: ethyl acetate (7:1:2 v/v/v) <b>Detection:</b> 254 nm <b>Conc. Range:</b> 75-500 ng/band <b>Correlation Coefficient:</b> 0.9970 <b>R<sub>f</sub>:</b> 0.77 <b>LOD:</b> 18 ng/band <b>LOQ:</b> 54 ng/band	[21]
2.	Thymoquinone in Black Seed	HPTLC	<b>Stationary Phase:</b> Silica gel aluminum plate 60 F254 (10 × 20 cm, 250 μm) <b>Mobile Phase:</b> dichloromethane: hexane (1:1 v/v) <b>Detection:</b> 254 nm <b>Conc. Range:</b> 2-12 ng/band <b>R<sub>f</sub>:</b> 0.30	[22]
3.	Guesstimate of thymoquinone	HPTLC	<b>Stationary Phase:</b> Silica gel aluminum plate 60 F254 (10 × 20 cm, 200 μm) <b>Mobile Phase:</b> ethyl acetate: n-hexane (2:8 v/v) <b>Detection:</b> 254 nm <b>Correlation Coefficient:</b> 0.9925 <b>R<sub>f</sub>:</b> 0.77 <b>LOD:</b> 0.77 ng/spot <b>LOQ:</b> 2.34 ng/spot	[23]

4.	Thymoquinone marketed formulations +	HPTLC	<b>Stationary Phase:</b> Silica gel aluminum plate 60 F254 (20 × 10 cm, 250 μm) <b>Mobile Phase:</b> ethyl acetate: n-hexane (2:8 v/v) <b>Detection:</b> 254 nm <b>Correlation Coefficient:</b> 0.9989 <b>Rf:</b> 0.48 <b>LOD:</b> 8.67 ng/spot <b>LOQ:</b> 17.43 ng/spot	[24]
5.	<i>Nigella Sativa</i> L.	HPTLC	<b>Stationary Phase:</b> Silica gel aluminum plate 60 F254 (20 × 10 cm, 250 μm) <b>Mobile Phase:</b> n-Butanol: water: acetic acid (3:1:1 v/v/v) <b>Detection:</b> 254 nm <b>Conc. Range:</b> 75-500 ng/ band <b>Correlation Coefficient:</b> 0.9970 <b>Rf:</b> 0.5	[25]
6.	Thymoquinone Formulations +	HPTLC	<b>Stationary Phase:</b> Silica gel aluminum plate 60 F254 (20 × 10 cm, 200 μm) <b>Mobile Phase:</b> cyclohexane: toluene (2: 8 v/v) <b>Detection:</b> 254 nm <b>Correlation Coefficient:</b> 0.9921 <b>Rf:</b> 0.28 <b>LOD:</b> 50 ng/spot <b>LOQ:</b> 150 ng/spot	[26]
7.	Thymoquinone in Rat Plasma	RP-HPLC	<b>Stationary Phase:</b> Symmetry C18 column (3.9 x 150 mm, 5 μm) <b>Mobile Phase:</b> methanol: potassium dihydrogen phosphate buffer acetonitrile: (50:30:20 v/v/v) <b>Detection:</b> 254 nm <b>Correlation Coefficient:</b> 0.9900 <b>Rt :</b> 2.2 min <b>LOD:</b> 0.119 μg/mL <b>LOQ:</b> 0.361 μg/mL	[27]
8.	Thymoquinone	HPLC	<b>Stationary Phase:</b> Eclipse XDB-C18 reversed phase column (4.6 x 150 mm, 5 μm) <b>Mobile Phase:</b> 2-propanol: methanol: water (20:30:50 v/v/v) <b>Detection:</b> 254 nm <b>Flow rate:</b> 0.9 mL/min <b>Injection volume:</b> 20 μL <b>Rt :</b> 15 min	[28]
9.	Thymoquinone	RP-HPLC	<b>Stationary Phase:</b> C18 RP- column (3.9 x 150 mm, 5 μm) <b>Mobile Phase:</b> water: 2-propanol: methanol: water (45:5:50 v/v/v) <b>Detection:</b> 254 nm <b>Flow rate:</b> 1 mL/min <b>Injection volume:</b> 20 μL <b>Rt :</b> 7.7 min	[29]
10.	Thymoquinone + Antibacterial Activity	HPLC	<b>Stationary Phase:</b> C18 column (4.6 x 150 mm, 5 μm) <b>Mobile Phase:</b> water: methanol (40: 60 v/v) <b>Detection:</b> 254 nm <b>Flow rate:</b> 1.5 mL/min <b>Injection volume:</b> 10 μL <b>Rt :</b> 6.1 min	[30]
11.	Thymoquinone+ PLGA-nanoparticles	UHPLC	<b>Stationary Phase:</b> Pinnacle DB Cyanom column (2.1 x 30 mm, 1.9 μm) <b>Mobile Phase:</b> Methanol: Water (80:20 v/v) <b>Detection:</b> 254 nm <b>Flow rate:</b> 0.300 mL/min <b>Injection volume:</b> 5 μL <b>Rt :</b> 0.4 min	[31]

## CONCLUSION

The present review covered extraction, detection, and estimation techniques of Thymoquinone. For the estimation of Thymoquinone, various analytical techniques have been used like HPLC, RP-HPLC, UPLC, LC-MS, HPTLC, and the X-ray diffraction method. The HPTLC technique is a reliable method for measuring its

active components within a sample at concentrations ranging from nanograms to micrograms. It could be concluded that all the techniques were found to be precise, accurate, less costly, and less time consuming.

## REFERENCES

1. Darakhshan S, Pour AB, Colagar AH, Sisakhtnezhad S. (2015). Thymoquinone and its therapeutic potentials. *Pharmacological Research*, 95(4):138-58.
2. Cited on: 10th Feb. 2024. Available From: [Thymoquinone | C10H12O2 | CID 10281 - PubChem \(nih.gov\)](#)
3. Taborsky J, Kunt M, Kloucek P, Lachman J, Zeleny V, Kokoska L. (2012). Identification of potential sources of thymoquinone and related compounds in Asteraceae, Cupressaceae, Lamiaceae, and Ranunculaceae families. *Central European Journal of Chemistry*, 10(6):1899-1906.
4. M.L. Salem. (2005). Immunomodulatory and therapeutic properties of the *Nigella Sativa* L. seed. *Int. Immunopharmacology*, 5 (13-14): 1749-70.
5. Badary OA, Taha RA, Gamal El-Din AM, Abdel-Wahab MH. (2003). Thymoquinone is a potent superoxide anion scavenger. *Drug and Chemical Toxicology*, 26(2):87-98.
6. Mokhtari-Zaer A, Norouzi F, Askari VR, Khazdair MR, Roshan NM, Boskabady M, Hosseini M, Boskabady MH. (2020). The protective effect of *Nigella Sativa* extract on lung inflammation and oxidative stress induced by lipopolysaccharide in rats. *Journal of Ethnopharmacology*, 253:1-28.
7. M. Kanter. (2011). Thymoquinone attenuates lung injury induced by chronic toluene exposure in rats. *Toxicology and Industrial Health*, 27(5): 387-95.
8. Salem ML, Alenzi FQ, Attia WY. (2011). Thymoquinone, the active ingredient of *Nigella Sativa* seeds, enhances survival and activity of antigen-specific CD8-positive T cells in vitro. *British Journal of Biomedical Science*, 68(3):131-37.
9. Mohany M, El-Feki M, Refaat I, Garraud O, Badr G. (2012). Thymoquinone ameliorates the immunological and histological changes induced by exposure to imidacloprid insecticide. *The Journal of Toxicological Sciences*, 37(1):1-11.
10. A.M. Shoieb, M. Elgayyar, P.S. Dudrick, J.L. Bell, P.K. Tithof. (2003). In vitro inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. *Int. J. Oncol*, 22 (1): 107-13.
11. Kokoska, J. Havlik, I. Valterova, H. Sovova, M. Sajfirtova, I. Jankovska. (2008). Comparison of chemical composition and antibacterial activity of *Nigella Sativa* seed essential oils obtained by different extraction methods. *J. Food Prot.*, 71(12): 2475-80.
12. S.H. Aljabre, M.A. Randhawa, N. Akhtar, O.M. Alakloby, A.M. Alqurashi, A. Aldossary. (2005). Antidermatophyte activity of ether extract of *Nigella Sativa* and its active principle, thymoquinone. *J. Ethnopharmacology*, 101 (1-3): 116-19.
13. S. Anwar, M.A. Khan, A. Sadaf, H. Younus. (2014). A structural study on the protection of glycation of superoxide dismutase by thymoquinone. *Int. J. Biol. Macromol.*, 69(4): 476-81.
14. D. El-Khouly, W.M. El-Bakly, A.S. Awad, H.O. El-Mesallamy, E. El-Demerdash. (2012). Thymoquinone blocks lung injury and fibrosis by attenuating bleomycin-induced oxidative stress and activation of nuclear factor Kappa-B in rats. *Toxicology*, 302 (2-3): 106-13.
15. A.F. Isik, I. Kati, I. Bayram, H. Ozbek. (2005). A new agent for treatment of acute respiratory distress syndrome: thymoquinone. An experimental study in a rat model. *Eur. J. Cardiothoracic. Surg.*, 28 (2): 301-305.
16. A.M. Fouda, M.H. Daba, G.M. Dahab, O.A. Sharaf El-Din. (2008). Thymoquinone ameliorates renal oxidative damage and proliferative response induced by mercuric chloride in rats. *Basic Clin. Pharmacol. Toxicol.*, 103 (2): 109-18.
17. M.N. Nagi, M.A. Mansour. (2000). Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection. *Pharmacol. Res.*, 41(3): 283-89.
18. M.M. Khattab, M.N. Nagi. (2007). Thymoquinone supplementation attenuates hypertension and renal damage in nitric oxide deficient hypertensive rats. *Phytother. Res.*, 21(5): 410-14.
19. Chung KX, Wei PL, Akowuah GA. (2007). Different extraction methods for Thymoquinone from *Nigella Sativa* L. seeds and antioxidant activity. *NIScPR Online Periodicals Repository*, 21(1): 75-80.
20. Goyal SN, Prajapati CP, Gore PR, Patil CR, Mahajan UB, Sharma C, Talla SP, Ojha SK. (2007). Therapeutic potential and pharmaceutical development of thymoquinone: a multitargeted molecule of natural origin. *Frontiers in Pharmacology*, 8(4): 1-19.
21. Taleuzzaman M, Imam SS, Gilani SJ. (2017). Quantitative Determination of thymoquinone in *Nigella Sativa* and its nanoformulation using validated stability indicating HPTLC densitometric method. *International Current Pharmaceutical Journal*, 6(10): 53-60.
22. Belete Y, Dagne E. (2014). HPTLC assay of thymoquinone in black seed and black seed oil (*Nigella Sativa* Linn) and identification of thymoquinone conversion with Uv-Vis. *Journal of Drug Delivery and Therapeutics*, 4(4): 5-9.
23. Ravi Y, Vethamani IP, Saxena SN, Velmurugan S, Santanakrishnan VP, Raveendran M, Bariya H, Harsh M. (2023). Guesstimate of thymoquinone diversity in *Nigella Sativa* L. genotypes and elite varieties collected from Indian states using HPTLC technique. *Open Life Sciences*, 18(1): 1-10.
24. Alam P, Yusufoglu H, Alam A. (2013). HPTLC densitometric method for analysis of thymoquinone in *Nigella Sativa* extracts and marketed formulations. *Asian Pacific Journal of Tropical Disease*, 3(6): 467-71.
25. Mazhar M, Agrawal SS. (2021). Standardization of *Berberis aristata* DC and *Nigella Sativa* L. using HPTLC and GCMS and their antineoplasia activity in 7, 12-dimethylbenz [a] anthracene-induced mouse models. *Frontiers in Pharmacology*, 12(4):1-13.

26. Velho-Pereira RM, Barhate CR, Kulkarni SR, Jagtap AG. (2011). Validated high-performance thin-layer chromatographic method for the quantification of thymoquinone in *Nigella Sativa* extracts and formulations. *Phytochemical Analysis.*, 22(4): 367-73.
27. Ahmad A, Khan RM, Alkharfy KM. (2015). Development and validation of RP-HPLC method for simultaneous estimation of glibenclamide and thymoquinone in rat plasma and its application to pharmacokinetics. *Acta Chromatographica.*, 27(3): 435-48.
28. Usman AG, Işik S, Abba SI. (2021). Hybrid data-intelligence algorithms for the simulation of thymoquinone in HPLC method development. *Journal of the Iranian Chemical Society.*, 18(4): 1537-49.
29. Gilani SJ, Imam SS, Ahmed A, Chauhan S, Mirza MA, Taleuzzaman M. (2021). Formulation and evaluation of thymoquinone niosomes: Application of developed and validated RP-HPLC method in the delivery system. *Drug Development and Industrial Pharmacy.*, 45(11): 1799-806.
30. Habib N, Choudhry S. (2021). HPLC quantification of thymoquinone extracted from *Nigella Sativa* L.(Ranunculaceae) seeds and antibacterial activity of its extracts against *Bacillus* species. *Evidence-Based Complementary and Alternative Medicine.*, 202(1): 1-11.
31. Ahmad N, Ahmad R, Al Qatifi S, Alessa M, Al Hajji H, Sarafroz M. (2020). A bioanalytical UHPLC-based method used for the quantification of Thymoquinone-loaded-PLGA-nanoparticles in the treatment of epilepsy. *BMC Chemistry.*, 14(4): 1-5.

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