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Analytical profile of anti-diabetic constituents - Thymoquinone from seed of Nigella Sativa

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Abstract : Nigella sativa Linn. (Ranunculaceae) (N. sativa), commonly known as black seed or black cumin, is an herbaceous plant, mainly grows in the Middle East, Central Europe and Western Asia. It is widely used in indigenous system of medicine for treatment of numerous disorders for over2 000 years. Its seed oil had been widely used in antidiabetic, due presence of high content of thymoquinone. Apart from the antidiabetic used it has other uses like seed oil Arabtraditional of medicine for the treatment of arthritis, lung diseases and hypercholesterolemia. Some of the reported pharmacological properties of N. sativa include hypotensive, anti-nociceptive, uricosuric, choleretic, antifertility, anti-histaminic, anti-oxidant, anti-inflammatory, anti-microbial, anti-tumor and immune modulatory effects. Most pharmacological properties of the whole seeds or their extracts of N. sativa are mainly attributed to the volatile oil of which thymoquinone, about 27%-57%, is the most abundant component. In this article for Intial profiling study physical properties, reported λ_{max}in solvent water –ethanol it is 254 nm, solubility in different solvent, melting point in DSC and FTIR data.

Keywords : $\lambda_{max,}$ Solubility, Anti-dibetic, DSC and FTIR.

Introduction:

Diabetes mellitus is a growing public health problem in both developed and developing countries. According to the report of World Health Organization (August, 2011), 346 million people have diabetes worldwide. It is also estimated that 3.4 million patients died from diabetes-related complications in 2004. Without urgent action, this number is likely to double by 2030.Nigella sativa Linn. (Ranunculaceae) (N. sativa), known with common name as black seed or black cumin, is an herbaceous plant, it is abundantly found in region in the Middle East, Central Europe and Western Asia. It is widely used in indigenous system of medicine for treatment of numerous disorders for over2000 years.Most pharmacological properties of the whole seeds or their extracts of *N. Sativa* are mainly attributed to the it's volatile oil, of which thymoquinone, about 27%-57%, is the most abundant component.

The hidden capacity of this plant is variable and it is expande over the time. Known to be as one of the predictive medicines and the emerging demands in utilizing natural products to replace the synthetic drugs, *N. sativa* has become amongst the top candidates selected by researchers as a natural product in medical field (Paarakh, 2009).An important study showed that 2 g/day dose managed to reduce insulin resistance and at the same time seems to increase β-cell function.

A study based on humans reported that significant decrease in blood glucose level after 1 week of oral ingestion of NS powder at a dose of 2 g/day. An important study based on streptozotocin induced diabetic rats

reported that treatment of TQ caused a sharp decrease in the elevated serum glucose, and an increase in the lowered serum insulin concentrations and other study based on normal rats showed that oral treatment with aqueous extract of NS with dose of 2 g/kg daily for 6 weeks improved glucose tolerance as metformin (300 mg/kg daily) and also reduced body weight.

Administration of black seed oil to diabetic rats has shown that significant decrease the blood glucose, triglycerides, cholesterol, low density lipoprotin-cholesterol ALT, AST and uric acid compared to untreated diabetic rats.

A thorough literature survey revealed that, several analytical techniques reported for the determination of thymoquinone. These methods include high-performance thin layer chromatography (HPTLC) method in N. sativa extracts. holographic and high performance liquid chromatography with UV detection in black seed oil, and ultra-performance liquid chromatography with UV detection in bulk drug. Before performing the advance analytical technique complete the initial profiling by studying like physical properties ,solubility study , DSC, IR and UV –Spectroscopy.

Experimental:

Materials and Reagents:

All chemicals and solvents used were of analytical grade and obtained from sigma Aldrich (New Delhi). Standard solution prepared in aqueous medium and methanol phosphate buffer of different pH is prepared and also a 0.1 N Hcl, solubility in other organic solvent likes DMSO and dimethyl foramamide (DMF).

Preparation of standard solution and sample:

For solubility check prepare a solution of 549-669 µg/mL in aqueous medium. Other solution in solvent 0.1N Hcl, Phoshphate buffer pH- 5 pH- 7.4 pH -9 and organic solvent DMSO and dimethyl foramamide. Analysis in UV-spectroscopy prepares solution in water- methanol in concentration range 1 µg/ml- 16 µg/ml. For IR-analysis KBr pellet technique.

Result and Discussion:

Physical Properties:

Thymoquinone (THQ) procured from sigma Aldrich (New Delhi) was characterized for various physical properties like colour, nature, melting point and loss on drying (Table-I).

Table-I

S. No	Parameters	Inferences
1	Nature	Crystalline powder
2	Colour	Yellow
3	Odour	Odourless
4	Melting point	45.00 to 47.00 °C. @ 760.00 mm Hg
5	Loss on drying	3.36 %

The Solubility Study:

The solubility parameter takes as very characteristic property of a chemical substance. After performing literature survey it is found a deficiency of data about TQ solubility in commonly used solvent. A signified solubility data in different solvent helpful for the analytical study of drugs for characterisation as well as in formulation study. Here showing solubility data of TQ ranging from concentration 549-669 µg/mL in aqueous medium starting from 24 hrs. -72 hrs. in different pH. From this study it is observed probably due to chemical and physical instability of the drug in these solvents that may interfere with soluble drug fraction. These data helpful for the degradation study of the drug. (Table-II)

Summary of the solubility study results, the values represent the concentration of TQ ± SD in µg/mL, H (hours), P.B (phosphate buffer).

It is reported TQ is soluble in organic solvent like ethanol, DMSO and dimethyl foramamide (DMF) which should be purged with an inert gas. It is found solubility in ethanol and DMF is approximately 16 µg/ml and in DMSO 14 µg/ml.

Table-II

Time (H)	Water	0.1 N HCl	P.B pH 5	P.B pH 7.4	P.B pH 9
24 h	670.17 ± 5.2	652.62 ± 1.8	617 ± 7.2	564.43± 0.25	548.19± .44
48 h	696.16 ± 1.6	636.4 ± 2.73	718.1 ± 1.47	548.12 ±1.60	725.57 4.60
72 h	738.63 ± 5.2	476.48 ± 0.92	736.20 ± 10	608.88 ± 3.5	663.66 ±16.5

Table-III

S.No.	Concentration	λ (nm)	Absorbance
1	1 µg/ml	252	0.02
2	4µg/ml,	252	0.7
3	6 µg/ml,	252	1.3
4	10µg/ml,	254	1.9
5	14 µg/ml	254	2.4
6	16 µg/ml	254	2.8

U.V. Spectroscopy reading:

Prepare a powder from seed part of the *Nigella Sativa*take 1 g of powder and dissolve in aqueous – methanol solvent of 60:40 ratio in 250 ml. From this stock solution make a dilution of 1 µg/ml, 2µg/ml, 4 µg/ml, 6 µg/ml,10µg/ml, , 14µg/ml, and 16 µg/ml. (Table-III), Calibration curve –Figure-6

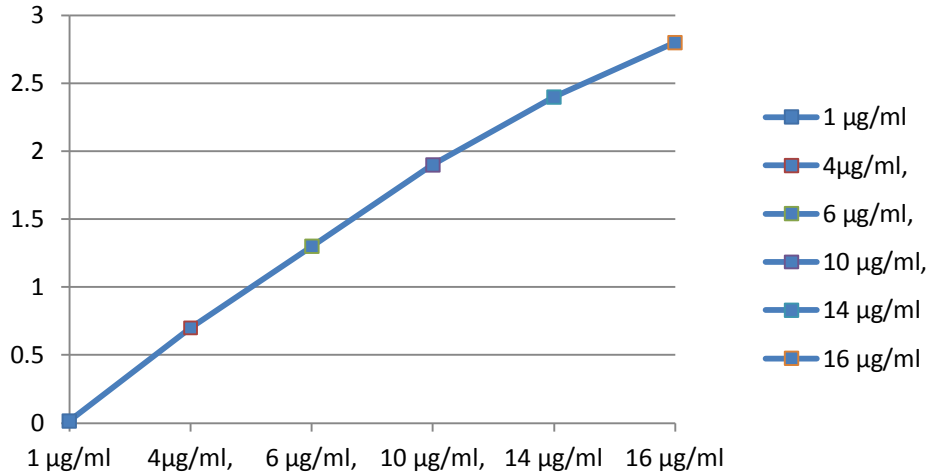


Figure-6: Calibration Curve in UV-Spectroscopy

U.V.-Spectrum:

The wavelengths of absorption peaks can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups present within a molecule. Thymoquinone is characterized by the presence of one prominent peak (λmax) at 254–257 nm as shown in Figure-1-4, and in Figure –5 comparison of Thymoquinone test and reference The spectra have been recorded using concentration of range 1 µg/ml- 16 µg/mL. By having such a prominent peak at this concentration, the use of this wavelength for the detection of TQ in HPLC method would be applied for futher study in HPLC and other instrument

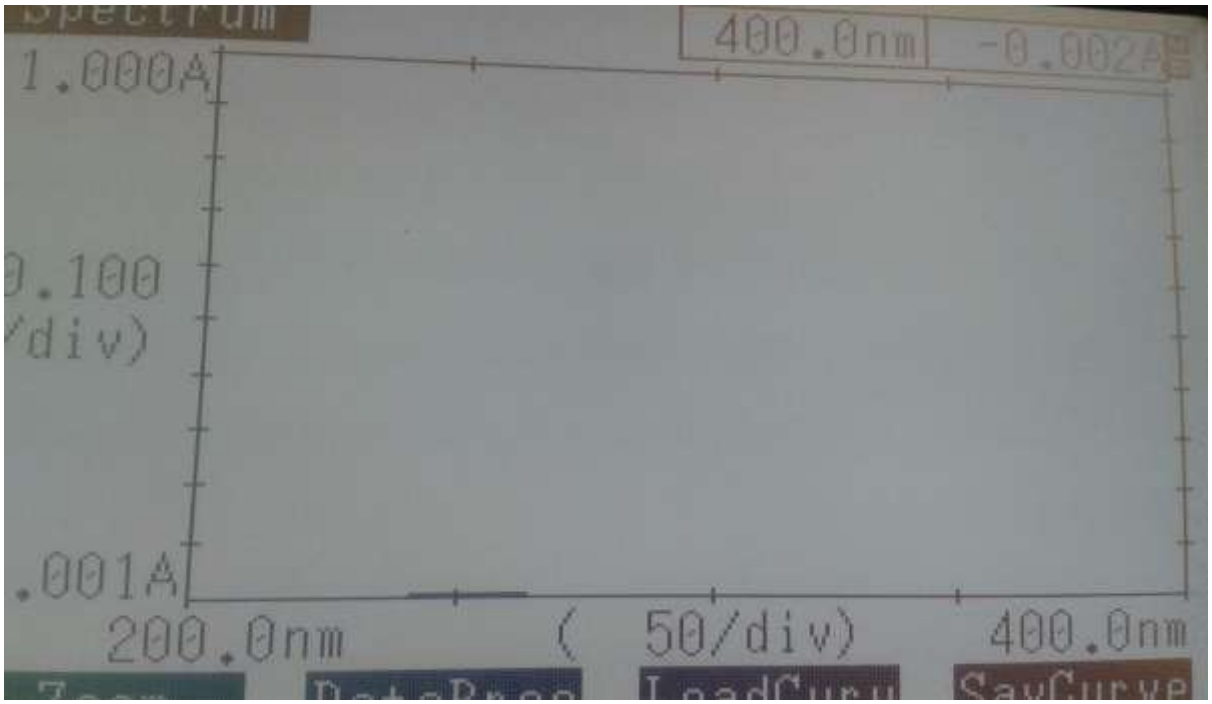


Fig-1: Dilution- 2µg/ml

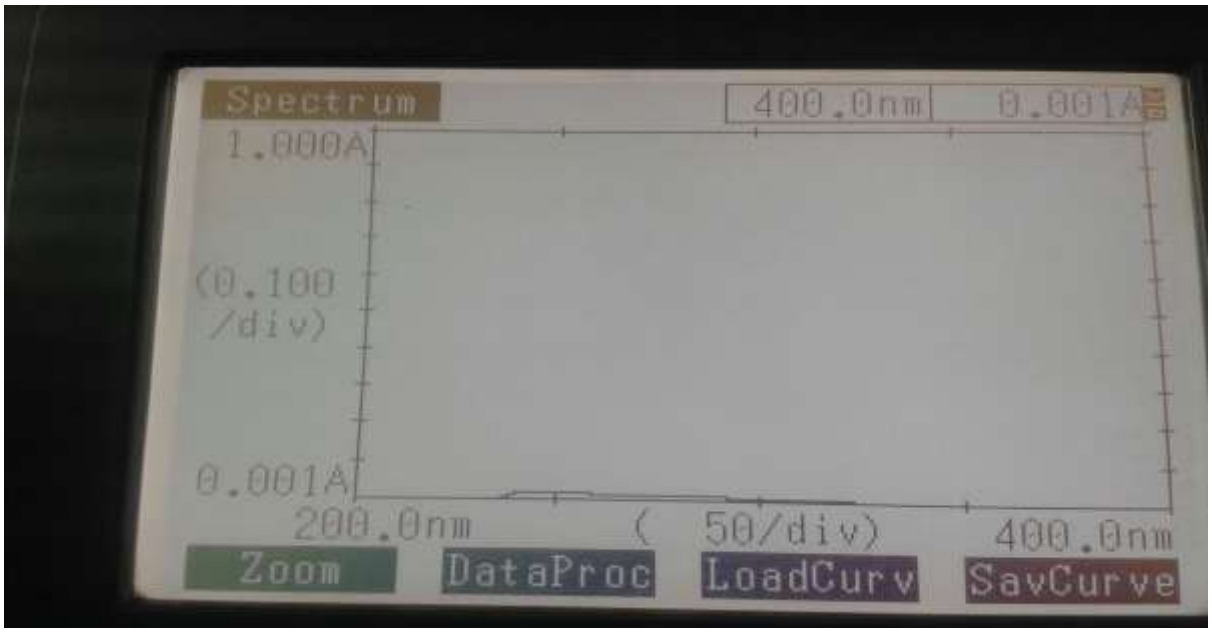


Fig-2: Dilution- 4µg/ml

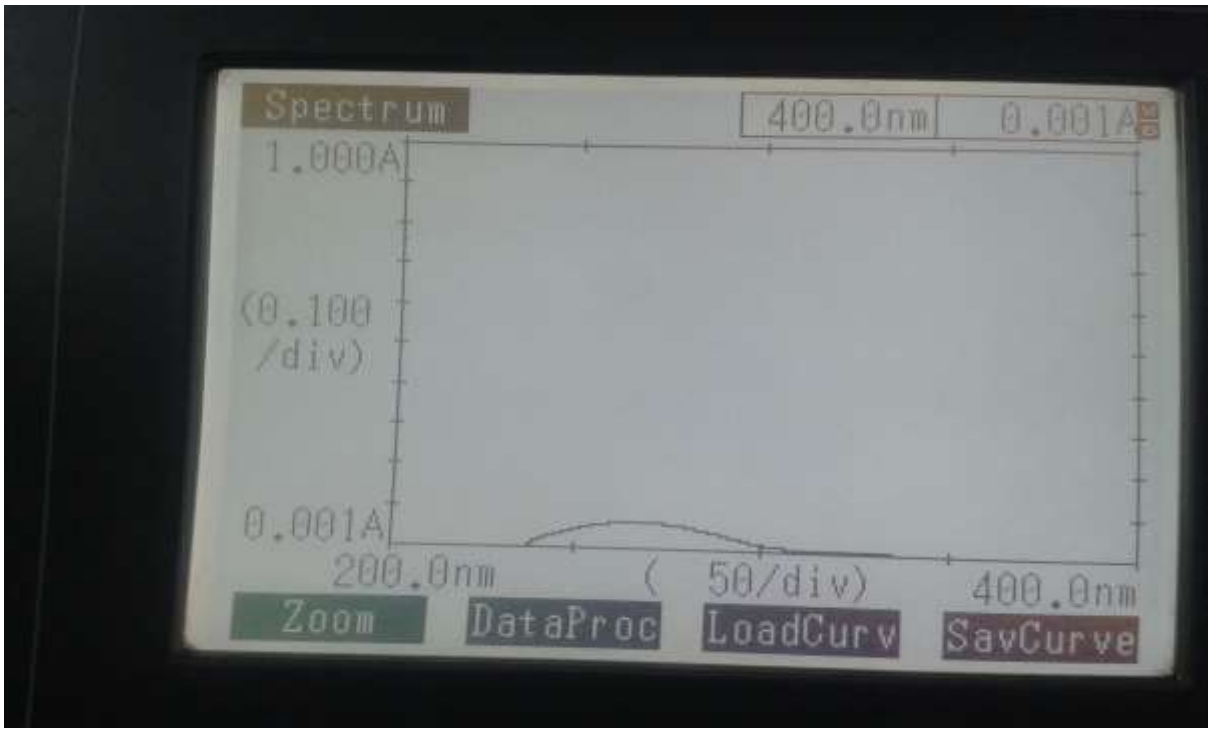


Fig-3: Dilution 8 µg/ml

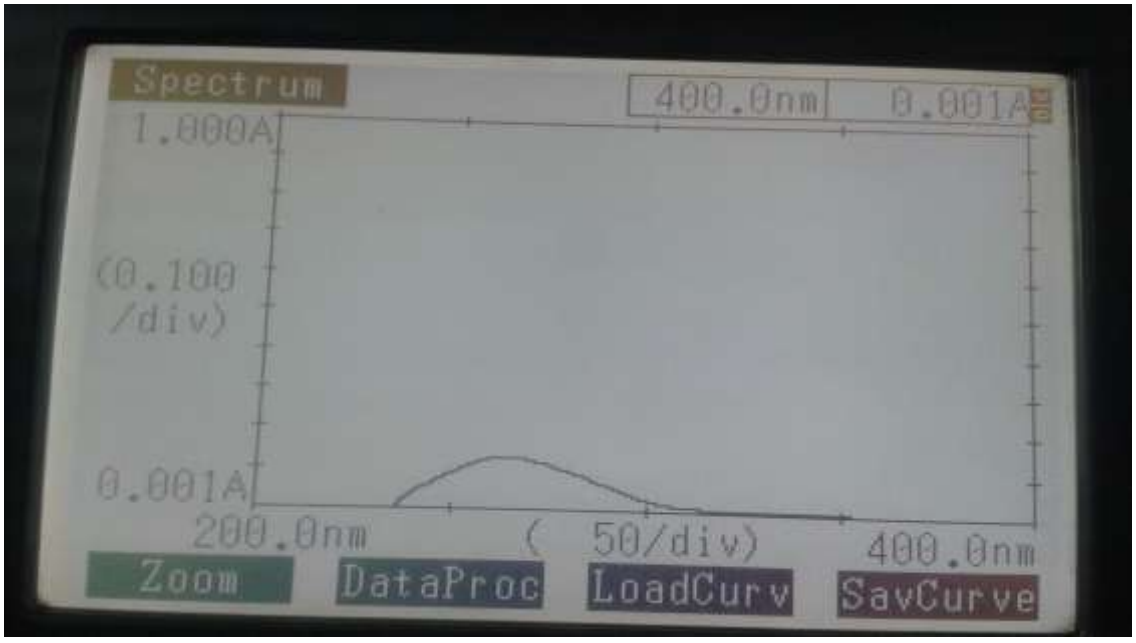


Fig-4: Dilution 16 µg/ml

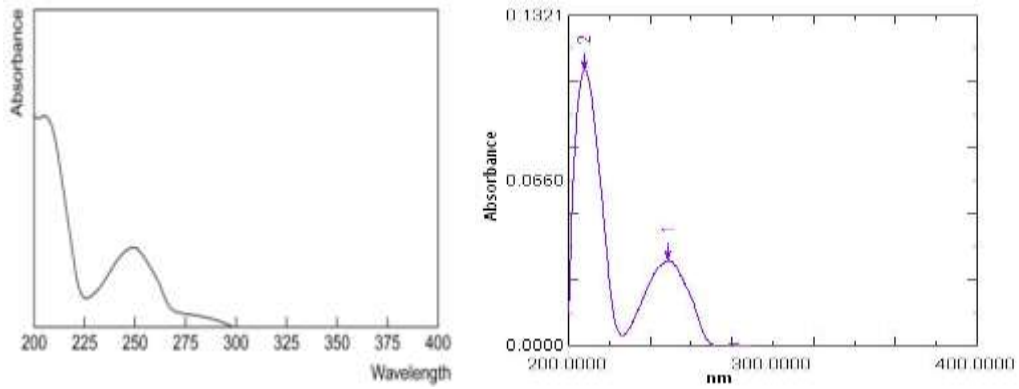


Figure-5: Comparison of UV-spectra of Thymoquinonein methanol (λ_{max} – 254 nm) with reference

Inference: The λ_{max} of Thymoquinone in methanol was 254 nm and was found to be identical with reference spectrum.

Differential scanning calorimetry (DSC):

The melting point of the thymoquinone (THQ) sample was determined by capillary method which was 45° C. The reported value was 45-47□ C. This was also confirmed by the DSC thermogram, showing endotherm peak at 47° C with enthalpy of 110.6 J/gl (Figure -7). It proves the sample was authentic. (Pagola et al, 2004).

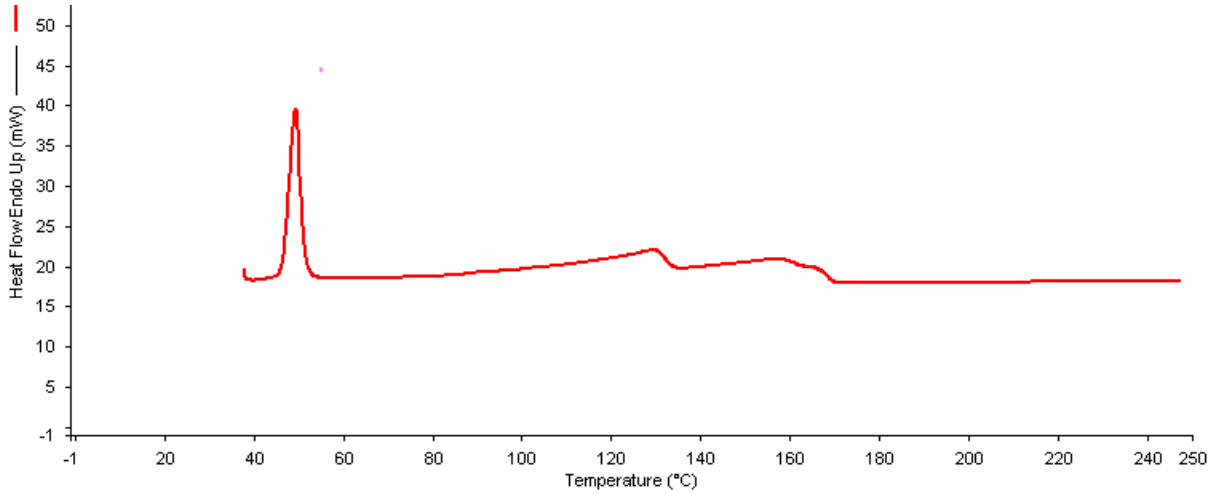


Figure -7: Differential scanning calorimetric (DSC) of Thymoquinone sample.

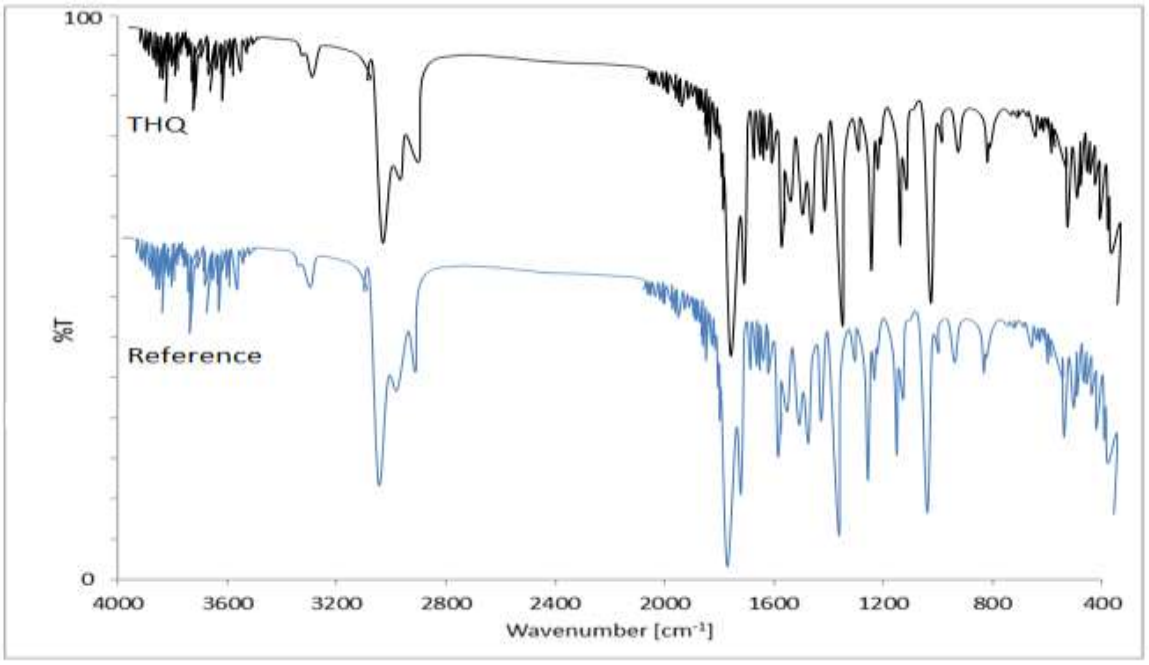
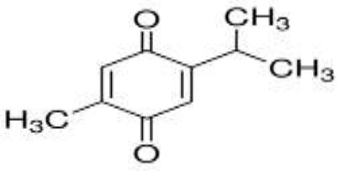


Figure -8: FT-IR spectra of Thymoquinone showing characteristics band and stretching.



Molecular Formula: $C_{10}H_{12}O_2$
Molecular Weight: 164.204g/mole.
Figure -9

Fourier transforms infrared absorptions spectrum (FTIR).

The FT-IR absorption spectra of Thymoquinone (THQ) was obtained using KBr pellet technique and exhibited the characteristics (IR)strong stretching band of the carbonyl group of a cyclohexadiene is observed at the wavenumber 1650cm^{-1}, which is supported by the values reported for thymoquinone (1.648 cm^{-1}) and 1,4 benzoquinone (1661cm^{-1}). The intense band present at 2967 cm^{-1} corresponds to the C-H stretching of aliphatic groups, and the value previously reported was 2969 cm^{-1}. The weaker band observed at a higher wavenumber (~3040 cm^{-1}) was assigned to the stretching observed in the vinylic C-H in the C = C-H groups, which had previously been reported at wave number 3041 cm^{-1}. This feature can be seen more clearly as an isolated band in the spectrum of 1, 4-benzoquinone (with-out aliphatic C-H stretchings) at 3058 cm^{-1}. The C = C stretching (1640-1675 cm^{-1}) yielded an isolated and moderately strong band at 1640 cm^{-1} in 1, 4-cyclohexadiene. The C = C stretching band cannot be unambiguously identified because the strong carboxylic stretching band in thymoquinone is present in this frequency range. In addition, the intensity of the C = C band is expected to be lower than both the carboxylic band and the C = C stretching in 1, 4-cyclohexadiene, which contains no methyl and isopropyl substituents; however, it must be noted that there is a transition at 1673 cm^{-1} slightly separated from the carbonylic band (the same feature is present at 1678 cm^{-1} in 1, 4-benzoquinone) that could be tentatively ascribed to the C = C stretching band. The spectrum was found to exhibit peaks similar to those reported in the literature. The chemical structure of THQ.(Figure-8-9).

Conclusion:

An Initial qualitative and quantitative test performed for the seed extract of N. sativa and it is characterised in DSC, FTIR and UV-spectroscopy. The given result gives the exact molecular structure of TQ and this data is helpful for further analytical study in instrument like HPLC,LC-MS/MS.

Conflict of interest statement: We declare that we have no conflict of interest.

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