SYNTHESIS OF AMINO-DERIVATIVE FROM THYMOQUINONE EXTRACTED FROM NIGELLA SATIVA AND ITS SPECTRAL CHARACTERISATION

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Abstract

Thymoquinone (TQ) is a bioactive constituent of (*Nigella sativa*) black seed oil. TQ shows potential pharmacological properties against numerous diseases. It exhibits wonderful antioxidant, anti-inflammatory, anticancer, and other important biological activities. Thymoquinone (TQ), also known as 2-isopropyl-5-methyl-1,4-benzoquinone effectively extracted HPLC and compared to the standard. It is a solid bright yellow compound having scaly crystals with a melting point of 49–50 °C and gives a characteristic intense smell of pepper. It gives an in detail studied of spectrum and the synthesis of amino derivative form TQ . Its purification, characterization by FTMS, IR, ^{1} HNMR, ^{13}CNMR and synthesis of its amino derivative (ATQ) is reported along with characterization by LCMS, IR, ^{1} HNMR and ^{13}CNMR .Finally, the present status of adjuvant potency of TQ and its characterisations are summarized.

Keywords: Nigella sativa, Thymoquinone, antioxidant, anti-inflammatory, anticancer

Isolation of Thymoquinone :

Thymoquinone (TQ), also known as 2-isopropyl-5-methyl-1,4-benzoquinone, obtained from seeds of Nigella sativa [11-13] is an important constituent of oil. Through high performance liquid chromatography (HPLC), Ghosheh and co-workers developed the method for the analysis of the oil of Nigella sativa seeds. The constituents from the oil were separated by using C18 mini columns and quantification of these recovered constituents by HPLC was completed on a reversed-phase μBondapak C18 analytical column[4]. Isocratic mobile phase of water:methanol:2-propanol (50:45:5% v:v) at flow rate of 2 ml/min and 254 nm radiation was used for detection of TQ.

Isolation of TQ from seeds of Nigella sativa by subjecting 25 g of finely powdered seeds to Soxhlet extractor with hexane and solvent was separated under vacuum followed by stream of nitrogen[5]. The extract was encumbered on silica gel column and eluted with diethyl ether , hexane, 15% diethyl ether in hexane and methanol 500 mL each and analyzed on HPLC after evaporation and reconstitution in methanol. HPLC analysis showed 659 mg/g in 15% diethyl ether in hexane fraction, and 367 mg/g of thymoquinone in hexane fraction[6]. Supercritical fluid carbon dioxide extraction (SCFE-CO2) of Nigella sativa oil at 150 bar and 40^{o}C for 120 min produced 4.09 mg of thymoquinone per ml of CO2 extract as reported by Solati.

HPLC Extraction of TQ from Nigella sativa

Extraction of TQ from commercially available Nigella sativa oil was performed by sonication as this is reported by Velho-Pereira and colleagues. Nigella sativa oil was obtained for the

isolation of TQ[7]. 5 gm of oil sample was taken in a 30 ml volumetric flask with 10 ml of methanol and for 20 minutes it is sonicated. Methanol layer was separated from oil and evaporated. Concentrated viscous liquid was loaded on silica gel column (Mesh size 60-120) and eluted with petroleum ether (60°C-80°C). The fastest moving yellow colour spot (Fig.3) was concentrated after elution and found to be matching with standard TQ sample (Aldrich) on TLC plate in 10% chloroform in petroleum ether[8]. This sample was subjected to HPLC analysis and compared with standard sample of TQ obtained from Sigma Aldrich with methanol as the solvent. Retention time of 3.40 minutes shown by purified column fraction matched with that of standard TQ sample[9]

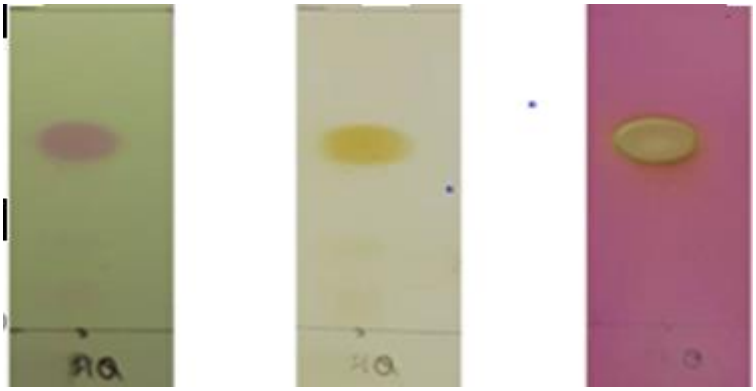


Fig.3 TLC plate in 10% chloroform in petroleum ether

(Figure 1 and 2). The weight of TQ obtained from column after drying was 1.13 g . Thus, the w/w percentage of TQ obtained from Nigella sativa oil sample was found to be 21.6% which is less than the reported value of 36.87% during GC-MS analysis in other report.

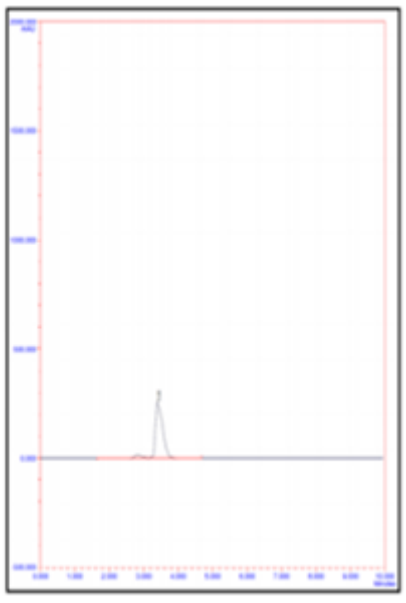


Fig.1. HPLC of TQ Fraction after Isolation and Purification

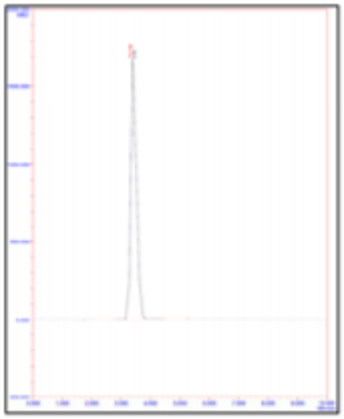
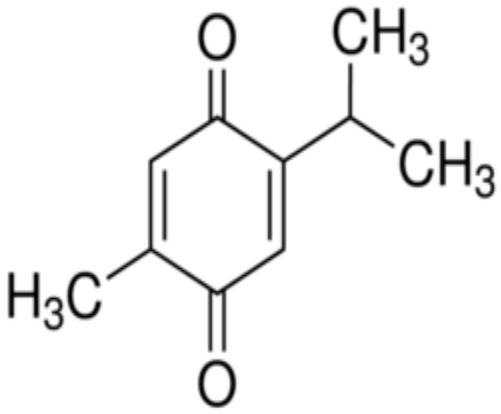


Fig.2 HPLC of Standard TQ Sample

Structural details of TQ:

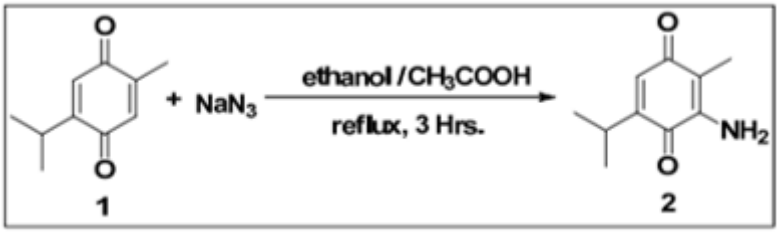


Structural chemistry of thymoquinone. TQ is a 10 carbon compound having the IUPAC name 2-isopropyl-5methyl-1,4-benzoquinone , bears the chemical formula $C_{10}H_{12}O_2$. It is a solid bright yellow compound having scaly crystals with a melting point of 49–50 °C and gives a characteristic intense smell of pepper. TQ is a 10 carbon compound having a basic quinone ring moiety of 6

Synthesis of 3-amino-5-isopropyl-2-methylcyclohexa-2,5-diene-1,4-dione

3-amino Thymoquinone (2) was synthesized with the method described by Moore and co-worker in molar ratio of reactants and acid catalyst with modifications. A mixture of TQ (1 mmol, 0.164 g) and sodium azide (1.3 mmol 0.084 g) in ethanol was refluxed for 3 Hrs in the presence of 3 ml of glacial acetic acid. Reaction was followed by TLC in $CHCl_3$. Reaction was worked up by neutralization of acetic acid with $NaHCO_3$ and extracted with chloroform (20 ml × 2). $CHCl_3$ was evaporated under vacuum and residue was taken up for purification by column chromatography starting with petroleum ether and gradual increase of polarity up to 10% ethyl acetate in petroleum ether. Eluted compound 2 was obtained by evaporation of solvent as

viscous oily red liquid[10]. It was dissolved in HPLC grade methanol and solution is kept for slow evaporation at room temperature which lead to red crystals of compound 2 in 45% yield.



Structural Characterization Thymoquinone (TQ):

2-isopropyl-5-methylcyclohexa-2,5-diene-1,4-dione Yield 20.6%. FTMS Peak for mass number 187.22, which is sodiated TQ adduct with 100% intensity in accordance with M.F. C10H12O2 + sodium peak. IR (cm-1): 2967 (CH), 1637 (C=O), 1610 (C=C), 1 H NMR (500 MHz, DMSO-D6): ppm = 1.06 (d, J=7Hz, 6H), 1.9 (s, 3H), 2.86 (m, J=7Hz, 1H), 6.5 (s, 1H), 6.7 (s, 1H); 13C NMR (125 MHz DMSO): ppm = 14.77, 21.03, 26.01, 130.1, 133.36, 144.92, 153.95, 187.08 [i1], 188.09 [i1]. (i stand for interchangeable). (Figure 3a-3d)

3-amino thymoquinone (ATQ): 3-amino-5-isopropyl-2-methylcyclohexa-2,5-diene1,4-dione Yield 45%. LCMS : RT = 1.94 min. M+ peak 179.09 (M=179.1 in accordance with MF C10H13NO2). IR (cm-1): 3462-3332 (-NH2), 1645 (C=O), 1 H NMR (500 MHz, DMSOD6): ppm = 1.06 (d, J=7Hz, 6H), 1.71 (s, 3H), 2.85 (m, J=7Hz, 1H), 6.26 (s, 1H), 6.42 (s, 2H); 13C NMR (125 MHz DMSO): ppm = 8.5, 21.0, 25.8, 106.6, 131.9, 145.1, 148.9, 183.6 [i1], 184.7[i1]; (Figure 4a-4e)

Result and Discussion

LCMS shows chromatogram of ATQ (Fig.4a) as intense peak at 1.94 minutes and mass spectrum (Fig.4b) shows m/z peak of 179.09. ^{1}HNMR spectrum of ATQ (Fig.4d) shows first peak for six hydrogen atoms as a doublet at 1.06 ppm, which belongs to two methyl groups of iso-propyl group present at quinone ring at fifth position, with 7Hz. One hydrogen of the same moiety appears as multiplet due to neighbouring methyl groups at 2.85 ppm and 7 Hz splitting constant[13]. Methyl group at second position of quinone ring appears as a singlet at 1.71 ppm which appears slightly upfield as compared to TQ because of electron donating –NH2 group on the adjacent carbon at third position. Hydrogen which is at sixth position is a singlet at 6.26 ppm and the position is somewhat shielded as comparative to the parent TQ.

Hydrogen atoms of – NH2 group appear as singlet at 6.42 ppm and integration shows that the peak is for two hydrogen atoms. In ^{13}C NMR of ATQ, (Fig. 4e) methyl group which is at second position of quinone ring seems at 25.87 ppm. The iso-propyl moiety of methyl groups appears at 8.4 ppm with –CH at 21.09 ppm[14].

Doubly bonded carbon atoms of quinone ring appear in de shielded region with carbon bearing methyl group appearing at 106.6 ppm and its neighbouring carbon bearing –NH2 at 148.9 ppm which matches with reported value of similar alkylamino derivatives of benzoquinone [11].

Carbon bearing iso-propyl group appears at 145.1 ppm and its neighbouring -CH at 131.9 ppm. Carbonyl carbon at first position appears 183.6 ppm and carbonyl carbon at fourth position appears at 184.7 ppm and these values may be interchangeable [i1].

Infra red spectrum of ATQ (Fig.4c) showed some prominent peaks like 3405 cm-1 and 3465 cm-1 for –NH2 group [15]. Observed carbonyl stretching peaks are in typical range at 1643 cm-1 and 1667 cm-1 with some shoulder, which appear to be in agreement with reported values by Raschi et al. The band at 1446 cm-1 appears for -CH3 antisymmetric bending modes and 1397 cm-1 band for symmetric mode appears to be in agreement with reported values.

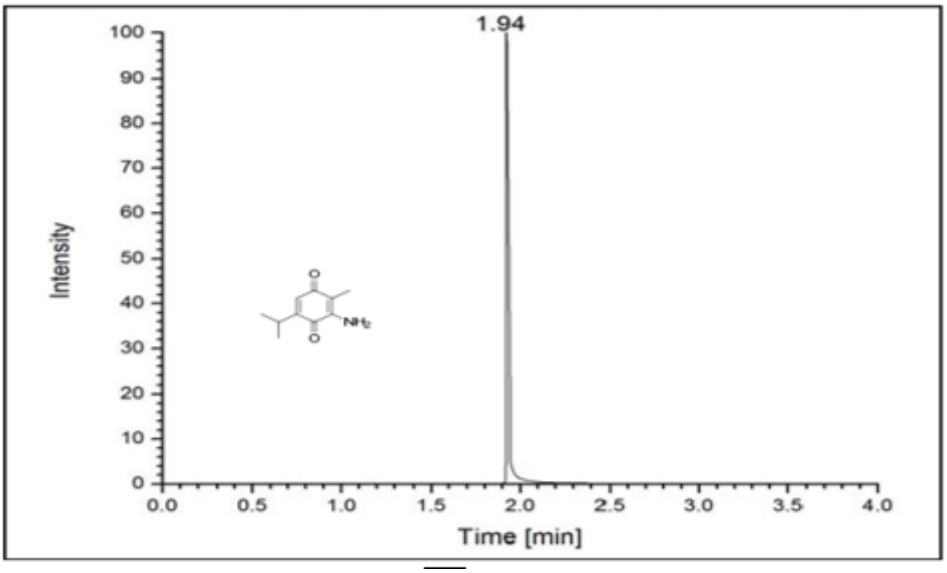


Figure 4a: Chromatogram of ATQ

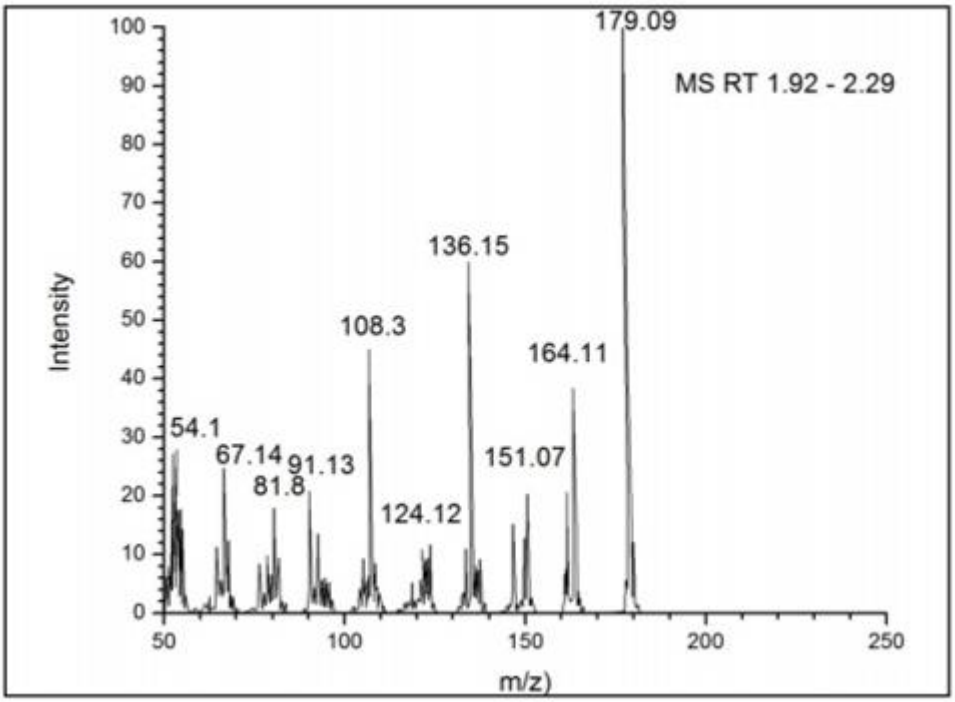


Fig.4b: Mass Spectrum of ATQ

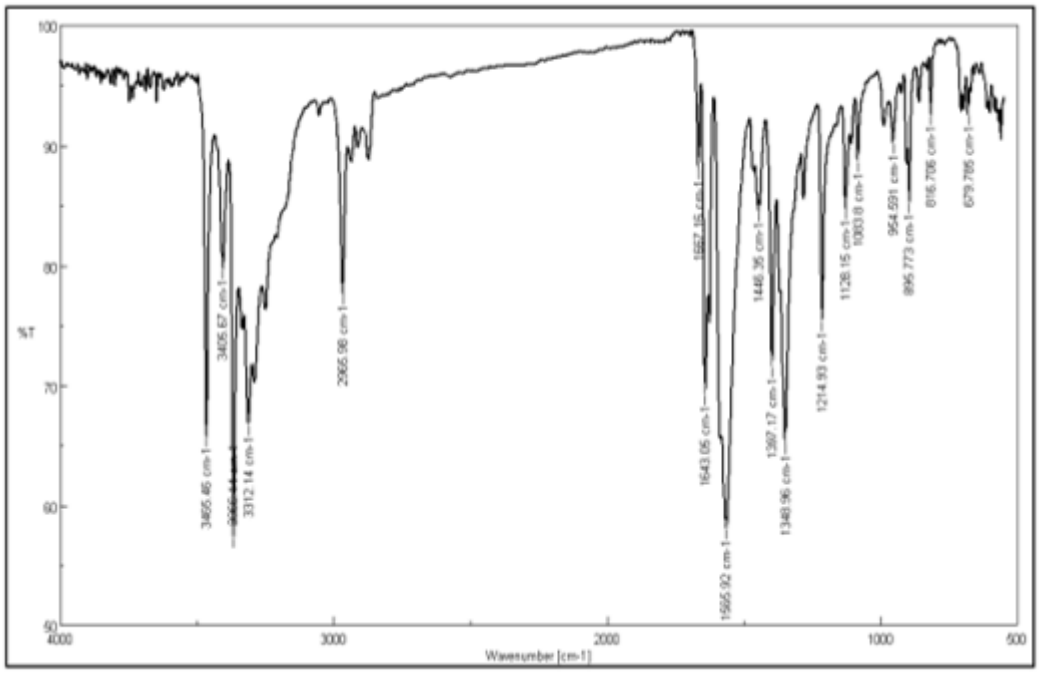


Fig.4c Infra Red spectrum of ATQ

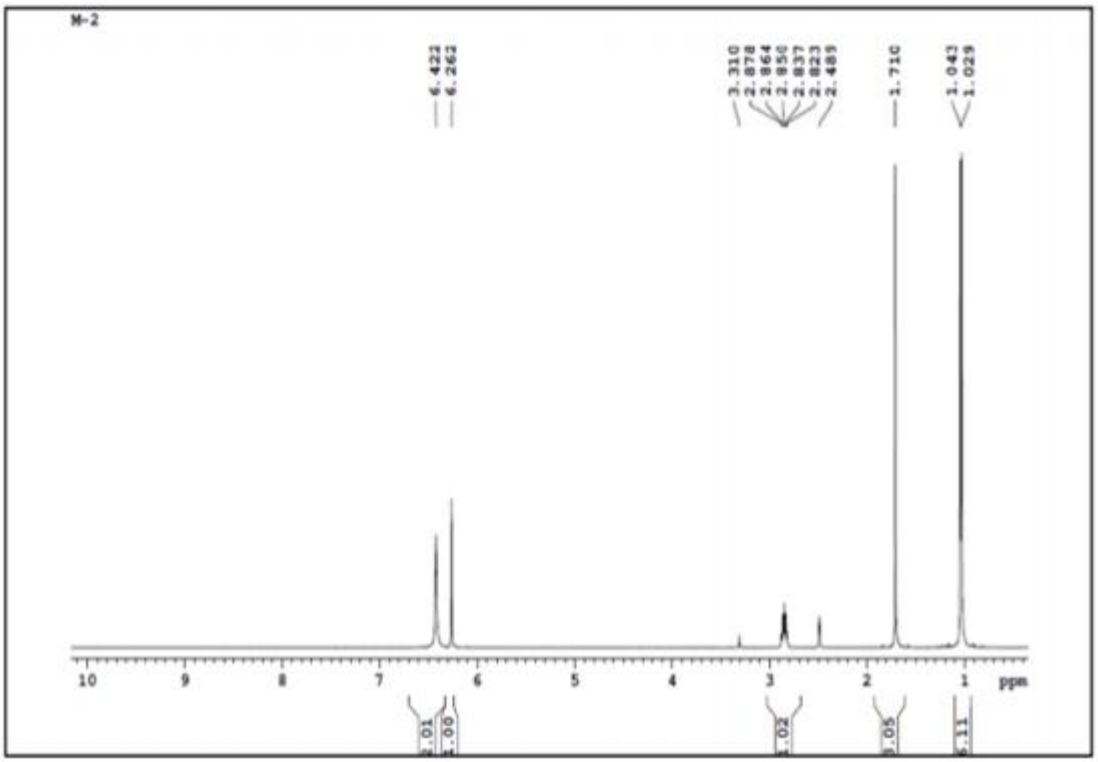


Fig 4d: [1]HNMR Spectrum of ATQ

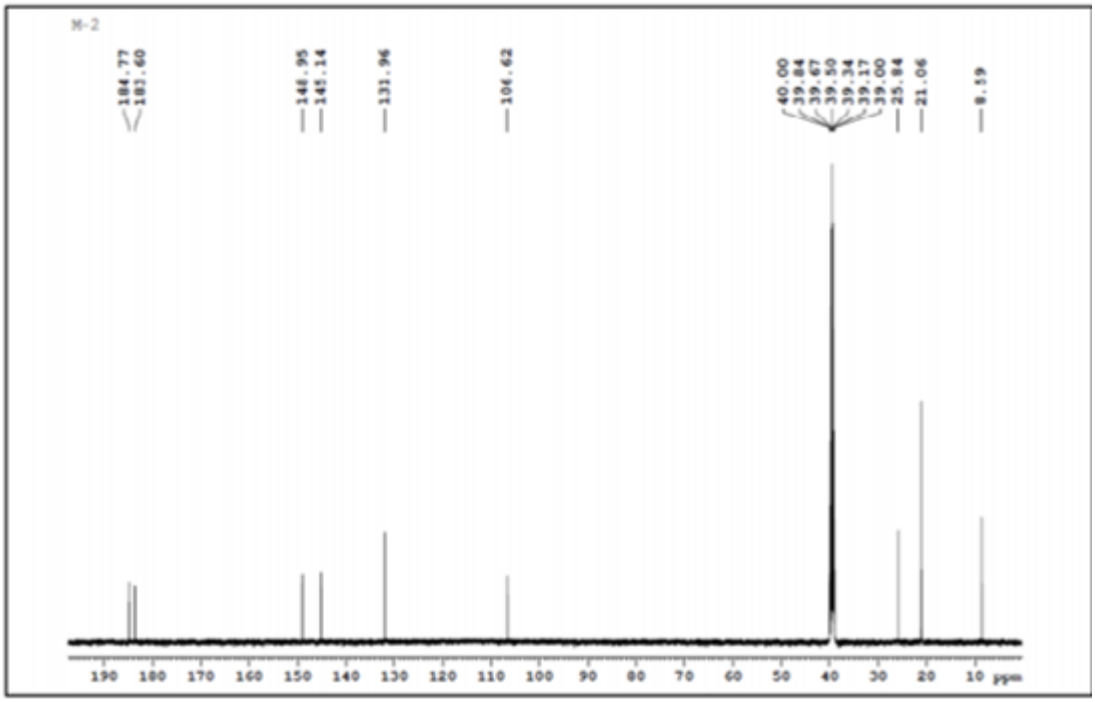


Fig. 4e. [13]CNMR Spectrum of ATQ

Summary and Conclusion

In this chapter isolation of synthetic thymoquinone amino derivative (ATQ) is reported along with characterization by LCMS, IR, ^{1}H NMR and ^{13}C NMR .

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