EXTRACTION OF BLACK CUMIN BY HYDRODISTILLATION PROCESS AND SPECTRAL DETERMINATION BY MASS, IR AND ^{1}H AND ^{13}C NMR

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Abstract

Nigella Sativa can produce essential oil that is important in medicine and also important in developing our agriculture sector nowadays. Nigella Sativa essential oil, is extracted by researchers nowadays by hydro distillation method. The objective of the study is to obtain essential oil from Nigella Sativa plant source using modern hydro distillation technique because of by investigating and understanding hydro distillation extraction process the essential oils present can be studied better. In this research, the rotary evaporator is used and the parameters expected are dominated in producing maximum yield of Nigella Sativa oil was the time and the surface area of the Nigella Sativa seeds exposed to the extraction process. The extraction temperature for the process is maintained at 100 ^{o} C and 1 atm pressure. It is expected that the optimum operating time is established from the experimental result. Thymoquinone is the main compound of the essential oil, which is found to be around 60% from overall compounds. Therefore, the presence of this compound should take as the characteristic for the essential oil and is analysed using High Performance Liquid Chromatography (HPLC).

Keywords: Nigella Sativa, hydro distillation, Thymoquinone, HPLC

Characteristic of Nigella Sativa

Nigella Sativa is a twelve-monthly flowering plant, native to southwest Asia. It grows linear (but not thread-like) to 25-30 cm tall, with finely divided leaves. The flowers are subtle, and usually pale blue and white coloured with 5-10 petals[1]. The fruit is a huge and inflated casing composed of 3-8 united follicles, each containing numerous seeds. Nigella sativa seeds are commonly used as an enriched spice [12]. Figure 1, Figure 2 below show the flower and bud of Nigella Sativa.

Fig.1 Flower of Nigella Sativa

Fig.2 Bud of Nigella Sativa

Nigella seeds Figure.3 are small, black matte grains with a coarse surface and an oily white interior. They are unevenly triangulate, 1 1/2 - 3 mm (1/16 to 1/8 in) long. They are comparable to onion seeds[2]. The seeds have little bouquet, though when they are rubbed, they give off an aroma redolent of oregano. It is also slightly peppery and bitter with a crusty texture[3].

Fig.3 Seeds of Nigella Sative

Main constituents of Nigella Sativa

The seeds contain bulk of esters which are structurally unsaturated fatty acids with terpene alcohols (7%); besides, traces of alkaloids are found which are isochinoline alkaloids are represented by nigellimin N-oxide and nigellimin , and pyrazol alkaloids comprise nigellicin

and nigellidin [14]. In the essential oil (avg. 0.5%, max. 1.5%), thymoquinone was identified as the major component (up to 70%) besides α-pinene (up to 12%), p-cymene (40%), thymoquinone and thymohydroquinone. Other terpene derivatives which were found in traces were: carvone, Carvacrol, citronellol, limonene and 4-terpineol. Furthermore, the essential oil contains 10% significant quantity of fatty acid ethyl esters[4]. On storing, thymoquinone yields higher oligo condensation products i.e., nigellone and thymoquinone .

The seeds also have unsaturated fatty acids which are mainly rich with dihomolinoleic acid (10%) , linoleic acid (50 –55%), eicodadienoic acid (5%) and oleic acid (20%) which is characteristic for the genus[5]. Saturated fatty acids such as palmitic and stearic acid was found about ± 30% . Commercial nigella oil ("Black Seed Oil", "Black Cumin Oil") also contain thymoquinone as the essential oil, which it processes an aromatic flavour [6].

Essential Oil Extraction Process

Many methods are used to extract the essential oils; majority of essential oils are produced by distillation[7]. There are different distillation processes such as steam distillation, hydro distillation, vapor-cracking, turbo-extractor, cold pressing and various other solvent extractors. Hydro Distillation method is used in this research because of its efficiency and easy to process.

Hydro distillation

Hydro distillation is generally used in the extraction and manufacturing of essential oils. The botanical material is engrossed in water then boiled with water[8]. The aromatic molecules from the plant material will be released into the hot water, since the hot water forces to disrupt the pouches in which the oils are kept in the plant materials. The volatile oils then escape from the plant material and evaporate as steam[9].

During this process the temperature needs to be carefully controlled - just enough to force the plant material to release the essential oil, yet not too hot as to burn the plant material or the essential oil[10]. The steam which then holds the essential oil is passed over a cooling system to condense which converts as a liquid from which the essential oil and water is then separated.

During distillation, only minuscule molecules can evaporateas shown in Figure.4 . These extremely small molecules convert an essential oil[11]. Volatile oils of tiny molecules, are noted as 'top notes' in the perfumery world; those containing the weightiest and less

volatile of the tiny molecules are noted as ‘base notes. Those in between are known as middle notes [12].

Fig.4 Instrument of Modern Hydro Distillation system

Results & Discussions:

FTMS of Thimoquinone shows two peaks of m/z 187.22 and 187.07 with relative abundance at 100% appears as a peak of sodiated adduct of TQ [TQ-Na] + where the m/z expected peak of 164.20 appears with added 23Na at 187.22 (Figure 3a & 3b).

^{1} H NMR spectrum of TQ (Fig. 3c) shows first peak as a doublet at 1.06ppm which belongs to two methyl groups of iso-propyl moiety present at second position of quinone ring, with splitting constant of 7Hz. Methine hydrogen of the same moiety appears as multiplet due to neighbouring methyl groups at 2.86 ppm and 7 Hz splitting constant. At fifth position the methyl group in quinone ring appears as a singlet at 1.9 ppm. At third position hydrogen appears at 6.5 ppm and hydrogen at sixth position hydrogen appears slightly downfield with shift of 6.7 ppm as singlet appears to be in arrangement with the reported value of 1 HNMR by earlier group, which used advance NMR techniques like Two-Dimensional Heteronuclear Single Quantum Coherence Transfer Spectra (2D HSQCT) on Bruker Avance AQS 500 MHz.

^{13}C NMR of TQ(Fig.3d) showed aliphatic carbon atoms at shielded positions. Methyl group at fifth position of quinone ring appears at 26.01 ppm, methyl groups of isopropyl moieties appear at 14.77 ppm with –CH appearing at 21.03 ppm[12]. carbon atoms which are doubly bonded in quinone ring appear in shielded region with carbon bearing -CH_3 group appears at

144.92 ppm (145.1 ppm reported 21) and the neighbouring group –CH appears at 133.36 ppm. At 153.95 ppm (156.4 ppm reported 21) Carbon bearing iso-propyl group appears and its neighbouring -CH at 130.1 ppm. At fourth position carbonyl carbon position appears at 188.09 ppm [i1] (188.3 ppm reported 21) and at first position carbonyl carbon appears at 187.08 ppm [13] (187.3 ppm reported 21). The chemical shifts of two carbon pair cannot be attributed with certainty and they are mentioned as interchangeable values with [i] and reported 13C chemical shifts of TQ in parenthesis. IR spectrum shows C=O peak at 1637 cm-1 with a shoulder and C-H stretching of methyl groups at 2967 cm-1.

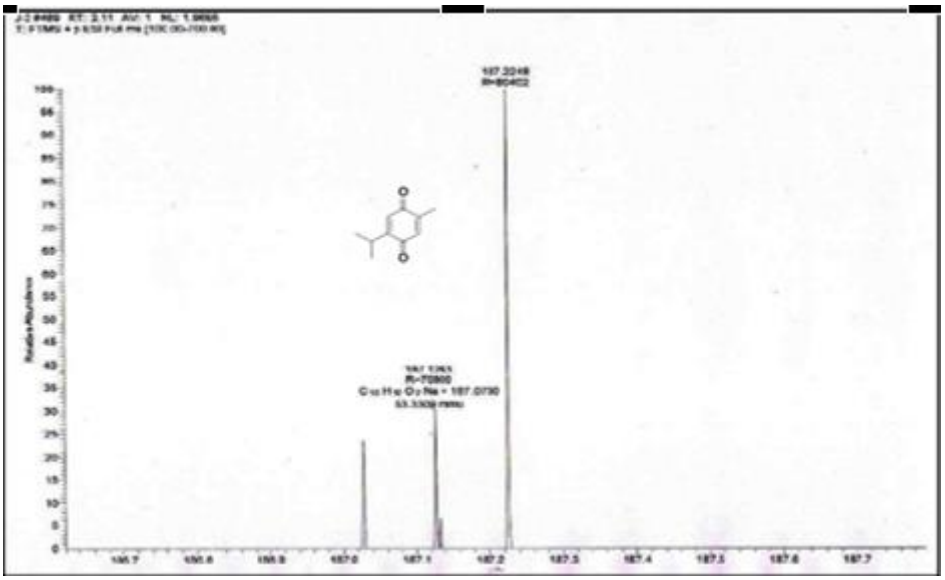

Fig 3a. A mass spectrum of isolated Thimoquinone

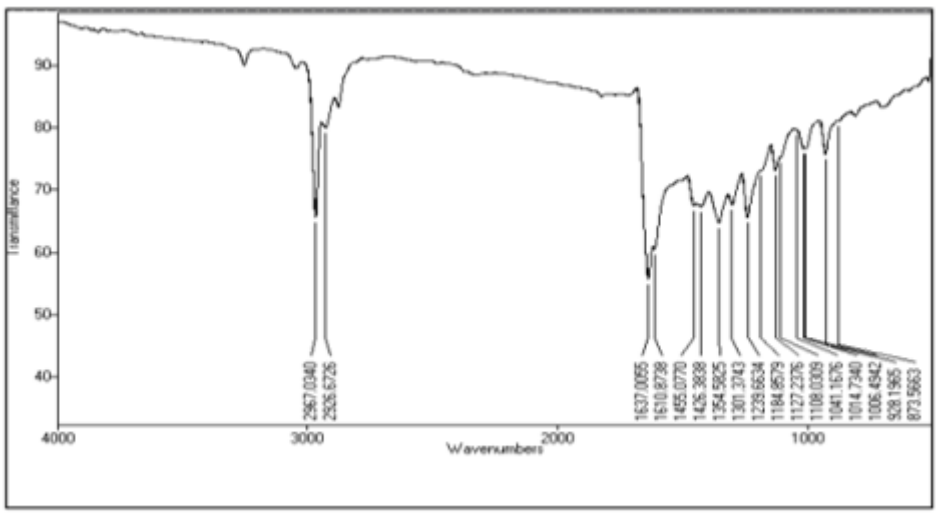

Fig 3b. **An infrared Spectrum of Thimoquinone**

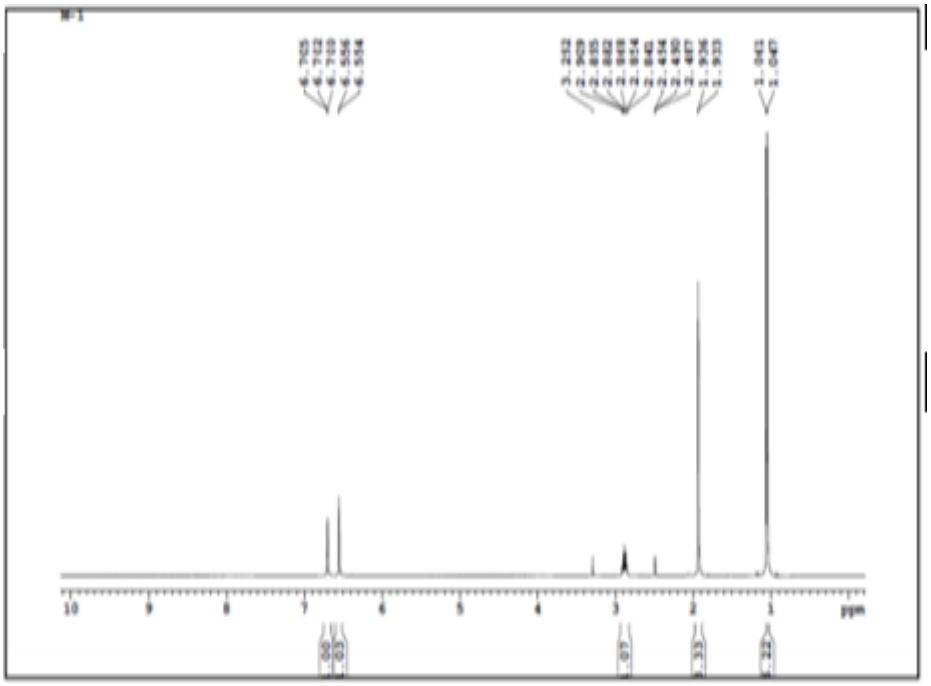

Fig.**3c Proton (^{1}H) NMR Spectrum of Thimoquinone**

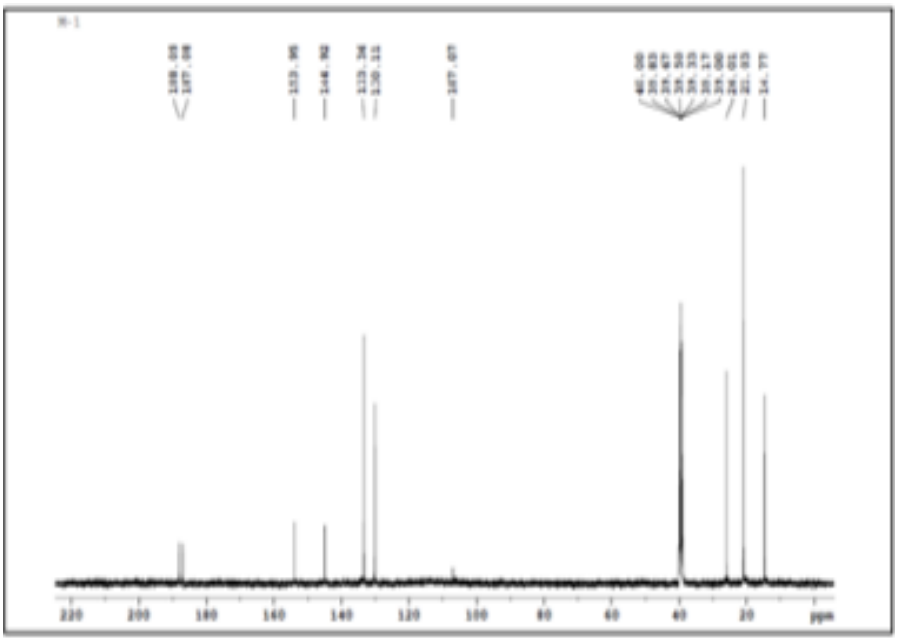

Fig. 3d.A Spectrum of ^{13}C NMR Thimoquinone

Summary and Conclusion

In this chapter isolation of thymoquinone from the sample of Nigella sativa oil, its purification, characterization by FTMS, IR, ^{1} HNMR, ^{13}CNMR) is reported along with characterization by LCMS, IR, ^{1} H NMR and ^{13}C NMR[14] .

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