Journal of Medicinal and Organic Chemistry

Extended Abstract Open Access

Liquid-liquid extraction method developed for thymoquinone from seed powder of *Nigella sativa*, characterized it by UV-spectrophotometer

Mohamad Taleuzzaman

Glocal University, India

Keywords: Liquid-liqud extraction, Thymoquinone, methanolic extracts, Spectrometry, and Rotary evaporator

Nigella Sativa Linn. (Ranunculaceae), commonly known as black seed or black cumin, is an herbaceous plant. Liquidliquid extraction method developed, the seed powder *Nigella sativa* was packed in a muslin cloth and placed in a beaker containing sufficient quantity of methanol for 72 hrs. Thereafter the methanolic extracts were filtered through Whatman filter paper no. 42 and the resultant filtrates were concentrated under reduced pressure using rotary evaporator characterize by UVspectrometer. The λmax of thymoquinone is found 254 nm and it confirms pure compound of thymoquinone. The accuracy of recovery studies by the standard addition technique was carried out by adding 50, 100 and 150% of the thymoquinone concentration in the sample. The percentage recoveries of the three concentrations were found to be (99.95-101.68) and % RSD (1.63-1.02). The precision method was assessed by analyzing thymoquinone in three different concentrations as 10, 25 and 40 µg mL-1 of thymoquinone. Repeatability (intra-day) was assessed by analyzing thymoquinone in three different concentrations (10, 25 and 40 µg mL-1) three times a day the %RSD (1.83-0.91). Intermediate precision (inter-day) was established by analyzing three different concentrations (10, 25 and 40 µg mL-1) of thymoquinone for three different days, %RSD (1.85-0.93). The low values of % RSD for repeatability and intermediate precision suggested an excellent precision of the developed UV spectrophotometric method. The optical, linear regression and validation data of UV spectrophotometry for the quantification of thymoquinone in methanol-Optical characteristics E1%, 1cm (201.82±2.43). Regression analysis-slope=0.0204±0.0002, intercept=0.0062±0.0012, regression coefficient (R2)=0.9984±0.0003, validation-range=5-50 µg mL-1), detection limit=0.99 µg mL-1) and quantitation limit=2.89 µg mL-1).

LLE is the classic method used to isolate herbicides, particularly from water and biological samples of fluids. Among the preferred extraction solvents for phenylureas, triazoles, amides, carbamates, benzimidazoles, and chlorotriazines are ethyl acetate, dichloromethane and their mixtures. LLE, also known as the extraction and partitioning of solvents, is derived from the Luke method but is not used in multiresidue. This process is commonly used for sample cleanup. This approach is used to distinguish compounds in two distinct immiscible liquids, typically water and an organic solvent, based on their relative solubility. LLE applies to the grapes and by-products. The key drawback of the LLE approach is that this process is time-consuming, repetitive, laborious and involves vast quantities of toxic solvents that pose a possible threat to human health, the environment, the issue of emulsion formation and, where there are several target compounds with substantial variations in sample polarity. LLE is one of the oldest extraction techniques and is widely used.

The LLE theory requires the transition of an analyte from an aqueous matrix to an extraction solvent which can be analyzed by GC-MS. Sometimes, LLE is applied to a number of matrices including blood, plasma, urine, and gastrics. In the aqueous matrix, the extraction solvent used for LLE should be immiscible, so that the two liquids can be separated easily. Analytes should be soluble in the solvent for extraction, and should ideally show high partition coefficients in the solvent. Using a handheld pipette, extraction solvents which are less dense than water can be removed whereas more dense solvents can be evacuated from the bottom of a separate funnel. It may take two or even three extractions to remove the majority of the analyte from the matrix. Some protocols allow the analyte to be extracted back into a small amount of acidified methanol or other polar solvent to remove many of the neutral compounds that may interfere with GC-MS analysis. Liquid-liquid extraction, also known as partitioning, is a separation process which consists of transferring a solvent from one solvent to another, the two solvents being either immiscible or partially miscible. One of the solvents is often water, or an aqueous mixture, and the other is a non-polar organic liquid.

Extraction of liquid-liquid is an important method of separation in the research and chemical analysis. It is commonly used as a industrial tool in the chemical and mining industries and for the downstream recovery of fermentation products (antibiotics, amino acids, steroids). Its food applications are limited to isolated cases, such as the conversion of carotenoid pigments from organic solvents to edible oils or the production of "terpeneless" basic citrus oil by extracting the basic oil's oxygenated compounds from aqueous ethanol. This cheap and easy to deploy method is still widely used. Nonetheless, it has the inconvenience of being time-consuming, hard to automate, often consuming toxic solvents and less efficient for highly polar compounds. The choice of solvent is dependent upon its polarity; polar solvents typically remove polar analytes more efficiently than apolar analytes do. In recent times, *Nigella sativa* has drawn healers to ancient civilizations and researchers. Traditionally, many diseases including asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza have been treated in different forms.

The chief bioactive constituent of black seed oil (*Nigella sativa*) is Thymoquinone (TQ). TQ possesses promising pharmacological properties against various diseases. It displays remarkable antioxidant, anti-inflammatory, anticancer and other important biological activities. TQ effectively transforms the signaling pathways for cancer progression. It not only increases the efficacy of chemotherapeutic drugs against cancer but also mitigates their side effects. Taking into account TQ's exceptional behavior, this chapter describes the root of TQ and its pharmacological characteristics. There has been discussion of recent advances in the form of chemical modifications and no formulations for designing TQ analogues. Oral distribution of TQ is limited as it is insoluble in water due to its poor bioavailability. TQ-loaded NLCs were successfully prepared and assessed against breast cancer and cervical cancer cell lines for their physicochemical characteristics, stability, and *in vitro* cytotoxicity. In several mechanisms, cell death may occur, including apoptosis, necrosis (see subsection, Anti-inflammatory activity), and autophagy. Autophagy is a catabolic process which retains cellular homeostasis in response to various factors of cellular stress. TQ-treatment also improves autophagosome accumulation. A xenograft model of the *in vivo* BALB / c nude mouse showed that TQ administered by oral gavage inhibits tumor growth by induced autophagy and apoptosis. Bafilomycin-A1, an inhibitor of autophagy, increases cytotoxicity of TQ, but does not facilitate apoptosis. Cell viability in autophagous-defective cells is eradicated.