

Anti-Cancer Properties of *Nigella* spp. Essential Oils and their Major Constituents, Thymoquinone and β -Elemene

Amr E. Edris*

Aroma & Flavor Chemistry Department, National Research Center, Dokki, Cairo, Egypt

Abstract: Essential oils are the volatile fraction of aromatic and medicinal plants after extraction by steam or water distillation. They have been used for their pharmaceutical potential since early times, and even now are still subject to a great deal of attention, as is clear from the increasing number of publications each year on this subject. This review presents both fundamental and recent studies concerned with the role of *Nigella* species essential oils and their major constituents, thymoquinone and β -elemene, as potential chemotherapeutic and chemopreventive anti-cancer agents. The mechanism of action and the factors which determine the concentrations of these major constituents in the essential oil are also reviewed.

Key Words: Anti-cancer, essential oils, *Nigella sativa*, *Nigella damascene*, thymoquinone, β -elemene.

INTRODUCTION

Aromatic plants have been used since ancient times for their medicinal properties. These properties can be partially or wholly attributed to their volatile oil fractions (essential oils). The diverse therapeutic potentials of essential oils have attracted the attention of many researchers to investigate their anti-cancer activity, and it has been found that the mechanism of action of essential oils is dissimilar to that of most classic cytotoxic chemotherapeutic agents [1]. Early reports had indicated that essential oil components, especially the monoterpenes, have multiple pharmacological effects on the mevalonate pathway of metabolism which may account for the tumor suppressive activity exhibited by such terpenes [2].

Nowadays there are many review papers available which contain conclusive information about the anti-cancer activity of essential oils from various other aromatic plants [3-6], thus this review will focus only on essential oils from specific aromatic plants belonging to the genus *Nigella*.

The genus *Nigella* (family Ranunculaceae) includes at least 25 species. They are flowering, annual, herbaceous, dicotyledonous plants which are native to Southern Europe, North Africa and Southwest Asia. The shape and morphology of the flower are the most important features which enable the taxonomist to determine the exact species of *Nigella*. Among the different species, *Nigella sativa* is one of the most highly valued for its medicinal properties [7-10] and low toxicity [4, 11]. The seeds contain 30.0%-38.0% (by weight) of total oil [12], which is composed of approximately 97.5-99.9% fixed oil and about 2.5-0.1% volatile (essential) oil.

The anti-carcinogenic activities of *N. sativa* whole essential oil and purified components have been documented in earlier studies [13-15]. A recent study has indicated a high efficacy for the essential oil, when injected directly into the

tumor, in reducing tumor volume, inhibiting metastasis development and delaying mortality of P815 tumor-bearing mice [16]. These activities were attributed to the thymoquinone (TQ) component of the essential oil (Fig. 1a). Thymoquinone is generally the major constituent of *N. sativa* essential oils from most sources. TQ shows promising *in vitro* and *in vivo* antineoplastic growth inhibition against various tumor cell lines [13-15, 17, 18]. This activity may be attributed to its inhibitory effects on cancer cell growth and its capability for inducing apoptosis [19]. The growth inhibiting activity of TQ is associated with the induction of cell cycle arrest [17] and inhibition of DNA synthesis [13], and the apoptotic activity induced by TQ has been shown to be mediated *via* p53-independent and p53-dependent pathways [20, 21]. In addition, TQ has been reported to be active against many multidrug-resistant variants of different human cancer cell lines [13].

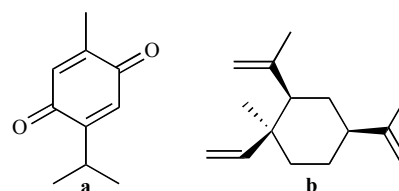


Fig. (1). Chemical structure of thymoquinone (a), and β -elemene (b).

The molecular events involved in the antineoplastic activities of TQ in prostate cancer cells have now been revealed: the compound effectively blocks G1-phase prostate cancer cells from entering the S phase, and thus may prove to be useful in treating prostate cancer, particularly in hormone refractory cases [22]. In the same investigation it was also observed that TQ possesses a high selectivity for inhibiting proliferation and viability of cancerous, but not non-cancerous, prostate epithelial cells. The selective anti-tumor activity of TQ compared with that of 5-fluorouracil suggests that this compound may be a safer alternative for the treatment of human colon cancer. Sustained delivery of TQ produced significant cellular destruction and interference of cellular metabolic functions of SW-626 human colon cancer

*Address correspondence to this author at the Aroma & Flavor Chemistry Department, National Research Center, Dokki, Cairo, Egypt; E-mail: amr_edris@hotmail.com

cells, which was comparable to the effect of 5-fluorouracil (the gold-standard for treatment of human colon cancer) [23]. TQ also triggers apoptotic cell death in human colorectal cancer cells, which correlates with the G1 phase arrest of the cell cycle [19].

TQ exhibited antiproliferative effects and induced apoptosis in myeloblastic leukemia HL-60 cells [21]. The same study also indicated that TQ could be used as a dietary supplement to enhance the effects of other anti-cancer drugs. A combination of TQ with anti-cancer drugs used clinically may decrease side effects and enhance the performance of these drugs. For example, the combination of TQ with doxorubicin (a widely-used anti-cancer drug) was able to decrease the cardiotoxicity of the latter in rats, which is caused by the generation of the superoxide free-radical [24]. The mechanism of action of TQ is based on its free radical scavenging as well as its anti-lipid peroxidation activity [24, 25].

In the same way, a combination of TQ with ifosfamide leads to improvements in the therapeutic index and prevents non-tumor tissues from sustaining chemotherapy-induced damage [26]. TQ also ameliorates the nephrotoxicity of cisplatin and enhanced its antitumor properties [27].

In addition to its chemotherapeutic potentials, TQ has also shown chemopreventive activity against various chemical carcinogens including benzo(a)pyrene, methylcholanthrene and carbon tetrachloride. TQ reduced the frequencies of chromosomal aberrations induced by benzo(a)pyrene in mouse bone marrow cells [28] and administration of TQ in drinking water resulted in a powerful inhibition of methylcholanthrene-induced fibrosarcoma tumorigenesis and benzo(a)pyrene-induced stomach tumors [29, 30]. TQ also inhibited carbon tetrachloride-induced hepatotoxicity in mice [31-33] and Mansour *et al.* [31] determined that the dose of TQ that ameliorated hepatotoxicity of carbontetrachloride was of the order of 12.5 mg/kg, administered intraperitoneally. They also concluded that the other major components of *N. sativa* essential oil, for example *p*-cymene and α -pinene, did not show significant hepatoprotective activity, emphasizing the important role of TQ.

Beside its protective activity for the internal organs, TQ has also been reported to be a potential skin chemopreventive agent, particularly at the early stages of skin tumorigenesis [34]. However the irritation and the potent allergenic effect of TQ should be taken also into consideration when testing on the skin [35].

The whole essential oil of *N. sativa* has also shown chemopreventive effects against carcinogenesis: oral administration of the oil was found to have the ability to inhibit colon carcinogenesis in the post-initiation stage in rats, with no evidence of adverse side effects [36].

The possible mechanism of the chemopreventive actions of TQ was suggested to be the result of its antioxidant and anti-inflammatory activities, coupled with an enhancement of detoxification processes [30]. In fact dihydrothymoquinone, which is an *in-vivo* metabolite of thymoquinone *via* reductase enzymes [37], also participated in the overall protection of organs against oxidative damage [38, 39]. Recently, the possible mechanism of the antioxidant activity of

TQ was investigated [40]. The study suggested that reduced TQ, or its conjugate forms after *in vivo* reaction with glutathione (GSH), could play a significant role in the antioxidant activities reported for *in vivo* treatments with TQ or the parent essential oil of *Nigella sativa*.

After reviewing the anti-cancer potential of TQ it is worth mentioning that there are certain environmental factors which influence the quantity of that compound. These factors include both agricultural practice (e.g. type of fertilization, irrigation, etc.) and climatic conditions (e.g. temperature, latitude, altitude, etc.). Moreover, there are two additional major factors which will be discussed in more detail in this review: the method of essential oil extraction and the species of *Nigella* seeds.

Extracting the oil directly from the crushed seeds, using steam or water distillation (method A) leads to a dramatic decrease in the amount of TQ in the resulting essential oil [41]. On the other hand, hydraulic expression (or hexane extraction) of the crushed seeds to obtain the total oil (fixed and volatile) followed by steam or water distillation of that oil (method B), produces a significant increase in the amount of TQ in the essential oil [41]. To confirm these claims quantitatively, we investigated both methods in our lab and found that, using GC-FID analysis, an essential oil containing 30.2% TQ can be isolated from *N. sativa* seeds using method B. This value was reduced to only 2.5% (using the same seeds and the same conditions of distillation) when applying method A (Fig. 2, previously unpublished data). The mechanisms by which the different extraction methods produce such a dramatic effect on the concentration of TQ have not yet been elucidated. However, it could be correlated with the

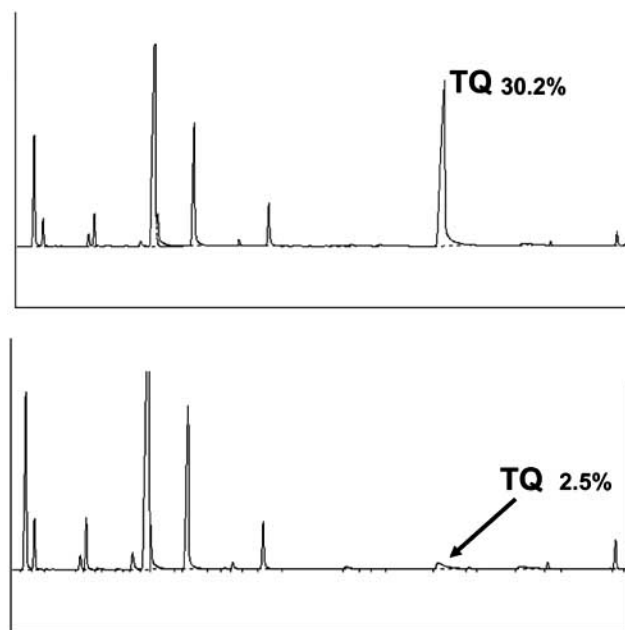


Fig. (2). Effect of extraction method on the amount of TQ in essential oil of *N. sativa*. Upper chromatogram: extraction of the essential oil indirectly from the total oil via water distillation (*method B*). Lower chromatogram: extraction of the essential oil directly from the crushed seeds via water distillation (*method A*).

extent of binding of TQ to the tissue of the oil gland in the seeds of *N. sativa*.

The second major factor affecting the concentration of TQ in the essential oil is the species of *Nigella* seed used. Generally, *N. sativa* produces the highest amount of TQ (27.0%-57.0%) [18], although a rare case has been reported in which *N. sativa* essential oil contained only 0.6% TQ [42]. There are also species of *Nigella* which contain no TQ at all, for example *N. orientalis*, *N. damascene* and *N. arvensis* [43-46]. Despite the absence of TQ in these species, their essential oils still possess anti-tumor activity, and this is mainly due to the presence of a sesquiterpene hydrocarbon called β -elemene (Fig. 1b). The concentration of β -elemene can reach up to 69.0%-73.0% in the essential oils of *N. orientalis* and *N. damascene* respectively [44, 45].

β -Elemene was recently investigated as a novel anti-cancer plant-based drug. Combinations of β -elemene with clinically used anti-cancer drugs like paclitaxel or docetaxel can lead to synergistic interactions [47]. The authors of this study concluded that β -elemene induced an alteration of cell membrane permeability which may enhance cellular uptake of the drug. β -Elemene was found to inhibit the growth of laryngeal cancer cells both *in-vitro* and *in-vivo*, probably by enhancing caspase-3 activity, thus inhibiting protein expression of eIFs (4E, 4G), bFGF, and VEGF [48]. β -Elemene also had differential inhibitory effects on cell growth between non-small-cell lung cancer cell lines and lung fibroblast and bronchial epithelial cell lines. The study indicated that the effect of β -elemene on lung cancer cell death may be through the cytochrome c-mediated apoptotic pathway [49].

β -Elemene was also able to inhibit the proliferation of cisplatin-resistant human ovarian cancer cells and their parental cells, but had only a marginal effect on normal ovarian cells [50]. This compound could also partially reverse drug resistance to adriamycin in the human breast cancer cell line MCF-7/ADM, which was related to the increased accumulation of intracellular adriamycin and the decreased expression of bcl-2 [51]. β -Elemene exhibited a marked antiproliferative effect on rat glioma C6 cells and to a lesser extent on human glioma SHG-44 cells at the same concentration [52].

Unfortunately, there are no conclusive studies on the toxicity of β -Elemene so far. On the other hand the acute oral toxicity of TQ was investigated in male Swiss albino mice [53] and the reported LD₅₀ value was 2.4 g/kg b.wt. In addition the oral LD₅₀ of the whole oil of *N. sativa* tested on mice was 28.8ml/kg b.wt. [11]. These data indicate low toxicity and a wide margin of safety for both TQ and *N. sativa's* total oil.

CONCLUSIONS

To date, the chemotherapeutic potential of TQ has not been tested clinically [54]. Thus, for future research, it is recommended that both TQ and β -elemene, either as a pure isolates or as part of the whole essential oils of *Nigella Spp.*, be assessed clinically against human cancers. If the whole essential oil is used, the chemical composition should be characterized first in order to confirm the presence of TQ or β -elemene, both qualitatively and quantitatively. It would also be of interest to test these plant extracts after encapsula-

tion in nano- targeted delivery systems, for instance liposomes [55-59], solid lipid nano-particles [60] and/or structured fluids like micelles and microemulsions [61,62]. Such systems can enhance the therapeutic index of these biologically active compounds by the specifically targeting their delivery to malignant cells.

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