

Open Access Article

## Potential Cytotoxic, Antifungal, and Antioxidant Activity of Dithymoquinone and Thymoquinone

Eman Ramadan Elsharkawy<sup>1,2</sup>, Emad M. Abdallah<sup>3</sup>, Ahmad Abo Markb<sup>4</sup>

<sup>1</sup>Department of Ecophysiology, Ecology and Range Management Division, Desert Research Center, Mathef El-Mataria, 15753, Egypt

<sup>2</sup>Department of Chemistry, Science Faculty, Northern Border University, Arar, North Region, Saudi Arabia

<sup>3</sup>Department of Laboratory Sciences, College of Sciences and Arts at Al-Rass, Qassim University, Saudi Arabia

<sup>4</sup>Department of Chemistry, Science Faculty, Assiut University, Assiut, Egypt

**Abstract:** *Nigella sativa* is a well-known plant with various applications in traditional medicine. The research goals of the current study were focused on discovering *Nigella sativa*'s active substances with antifungal, antioxidant, and anticancer properties. The major compound of the plant's essential oil was found to be thymoquinone (TQ), which was used to synthesize dithymoquinone (DTQ) by the simple photodimerization method. Formation of the compound was confirmed by high-performance liquid chromatography (HPLC), HNMR, IR, and ESI. Both compounds (TQ and DTQ) were assayed for antifungal, antioxidant, and cytotoxic properties, and the results showed higher activity of TQ compared to DTQ. In conclusion, thymoquinone is a promising antifungal, antioxidant, and anticancer agent and is recommended for further pharmaceutical evaluation. To the best of our knowledge, this is the first study to evaluate the bioactivity potential of DTQ synthesized from thymoquinone as it is present in low quantities in the essential oil of *Nigella sativa*.

**Keywords:** cytotoxicity, antioxidant, thymoquinone, dithymoquinone.

## 二百里醌和百里醌的潜在细胞毒性、抗真菌和抗氧化活性

**摘要：**黑种草是一种众所周知的植物，在传统医学中具有多种应用。当前研究的研究目标集中在发现具有抗真菌、抗氧化和抗癌特性的黑种草活性物质。发现该植物精油的主要化合物是百里醌（质量保证），它被用于通过简单的光二聚法合成二百里醌（DTQ）。通过高效液相色谱（高效液相色谱）、核磁共振、红外线和ESI确认了化合物的形成。测试了两种化合物（质量保证和DTQ）的抗真菌、抗氧化和细胞毒性特性，结果表明与DTQ相比，质量保证的活性更高。总之，百里醌是一种很有前途的抗真菌、抗氧化剂和抗癌剂，推荐用于进一步的药物评估。据我们所知，这是第一项评估由百里醌合成的DTQ生物活性潜力的研究，因为它在黑种草精油中的含量很低。

**关键词：**细胞毒性，抗氧化剂，百里醌，二百里醌。

Received: May 1, 2021 / Revised: June 6, 2021 / Accepted: August 3, 2021 / Published: September 30, 2021

About the authors: Eman Ramadan Elsharkawy, Department of Ecophysiology, Ecology and Range Management Division, Desert Research Center, Mathef El-Mataria, Egypt; Department of Chemistry, Science Faculty, Northern Border University, Arar, Saudi Arabia; Emad M. Abdallah, Department of Laboratory Sciences, College of Sciences and Arts at Al-Rass, Qassim University, Saudi Arabia; Ahmad Abo Markb, Department of Chemistry, Science Faculty, Assiut University, Assiut, Egypt

## 1. Introduction

Scientific interest in medicinal plants and their ingredients has increased because of the high efficiency of herb-derived drugs and increasing interest in natural products. The seeds of *Nigella sativa* (from the Ranunculaceae family) have been used for thousands of years as a condiment and food preservative. TQ, as the bioactive component of the fugacious oil of the plant's black seeds, has demonstrated potent medicinal effects in traditional medicine [1]. The sudden emergence of multidrug-resistant pathogens and the rapid spread of new diseases has prompted health institutions and pharmaceutical companies around the world to change their policies regarding avoidance of the production of antibiotics based on traditional ingredients to combat multidrug-resistant pathogens [2].

Antibiotic resistance in pathogenic fungi is a major international issue of great concern due to the various diseases that pathogenic fungi cause. Furthermore, mutant resistant fungi have been documented all over the world, both in the lab and in the clinic, that show resistance to a wide range of antibiotics other than those originally used for treatment. This problem is exacerbated by the insufficient number of antifungal medications available [3].

Accordingly, the quest for plant-based medications and dietary supplements has intensified in recent years. Microbiologists, botanists, natural products chemists, and even ethnopharmacologists are scouring the globe for phytochemicals and "leads" that could be formulated as new antimicrobial drugs with innovative mechanisms different from ineffective antibiotics [4].

In this study, the essential oil content of *N. sativa* L. was determined to be 0.4–0.5% (w/w). This extract was analyzed by gas chromatography-mass spectrometry, which revealed the presence of the following major components: 62.17% TQ, 16.84% carvacrol, 8.29% 2-methyl-5-Prop-2-enyldihydroquinone, 6.99% dihydrothymoquinone, 2.07% terpine-4-en-1-ol, and 3.11% monoterpene [5]. Ozer et al. [6] investigated the protective effects of TQ on survival, vascular reactivity, mesenteric artery blood flow, oxidative, and inflammatory responses in rats. TQ has been suggested as a potential agent for fighting chemotherapy-induced nephrotoxicity [7].

TQ has demonstrated histopathological protective effects on multiple organ injury [8]. It also exerts antioxidant effects via strengthening of the oxidant scavenging system. Thus, it also has antitoxic properties. In addition, TQ has shown potential anti-inflammatory effects [9, 10]. Hanieh [11] indicated protective features of TQ against inflammation and oxidative stress in renal disorders. Likewise, TQ has shown anti-inflammatory

and antioxidant properties in animal and in vitro models of several renal diseases caused by inflammation and oxidative stress. [11].

The main constituents of *Nigella sativa*'s black seeds are three benzoquinone compounds: (1) thymoquinone, (2) thymohydroquinone, and (3) dithymoquinone [12]. The first two compounds are available for purchase from Sigma-Aldrich, while the third compound is not available. Thus, the present study aimed to synthesize dithymoquinone by photodimerization of thymoquinone and investigate the cytotoxic, antioxidant, and antifungal activities of both compounds.

## 2. Materials and Methods

### 2.1. Raw Materials

Thymoquinone (acetone, ethanol [C<sub>2</sub>H<sub>5</sub>OH], and HPLC-grade methanol) was purchased from Merck. De-ionized water was used throughout this study. The characterization methods and typical conditions for dithymoquinone are listed in Table 1.

Table 1 List of characterization methods for dithymoquinone

Analysis	Instruments
TLC	Silica TLC plates
FT-IR spectra	Brüker Tensor 27with ATR configuration.
<sup>1</sup> H NMR	BrükerAvance 400 NMR spectrometer
UV-Vis's spectra	Varian Cary® 50 Scan spectrometer in a 1.0 cm square cuvette.
ESIMS	LC-MS/(MS)

### 2.2. Synthesis of Dithymoquinone (DTQ)

TQ (0.50 g.) was dissolved in acetone (5.0 mL) in a 500 mL glass beaker. The bright yellow solution was gently rotated along the inner surface of the beaker until complete evaporation to a thin, crystalline layer. The resulting thin layer (solid state) of TQ was exposed to an ultraviolet lamp (345 λ max) in a fume hood at room temperature. The reaction was found to be > 99% complete after eight hours. The photodimerization reaction was monitored by TLC. Crude product was dissolved in a small amount of DCM, loaded on silica gel, and then purified by column chromatography using silica gel for the stationary phase and hexane and ethyl acetate (9:1) for the mobile phase.

Thymohydroquinone was dissolved in a minimal volume of ethyl acetate, transferred to a smaller Erlenmeyer flask, and then evaporated to dryness over gentle heat. Crystallization of thymohydroquinone into DTQ was performed using ethanol to render fine, pale yellow needle-like crystals. Ultra-pure water and cold 2-propanol were re-centrifuged and lyophilized overnight to dryness [13]; thymohydroquinone (110 mg, 22% yield, m.p. 200.5°C); UV<sub>max</sub> 250 nm and UV<sub>min</sub> 380 nm; IR

(solid state): 3060 (vinyl C = C-H stretch); 2969–2872 (C-H stretch of aliphatic groups);  $^1\text{H}$ NMR (600 MHz);  $\delta$  6.70 (s, 2H);  $\delta$  3.01 (s, 2H);  $\delta$  3.12–3.06 (septet,  $j = 6.6, 2$  H);  $\delta$  1.22 (s, 6H);  $\delta$  1.16–1.13 (2d,  $j = 7.2, 6.6, 12$  H); ESIMS: 329  $[\text{M} + 1]$ .

### 2.3. Microorganisms and Inoculum Preparation

The experiments were conducted at the University of Assiut Mycological Center, Egypt in November 2020. Six strains of yeast and fungi were tested: *Aspergillus flavus* AUMC 1276, *Candida albicans* AUMC 1299, *Fusarium oxysporum* AUMC 215, *Geotrichum candidum* AUMC 226, *Scopulariopsis brevicaulis* AUMC 1653, and *Trichophyton rubrum* AUMC 1804. Microorganisms were sub-cultured on Sabouraud Dextrose agar (SDA; Himedia, India), and, before conducting each antimicrobial test, a single colony from fresh microbial pure cultures was transferred to Sabouraud Dextrose broth (SDA; Himedia, India). The microbial cultures were then incubated at 37°C for 24 to 48 hours to ensure a fresh culture was used.

### 2.4. In Vitro Antimicrobial Bioassay

The well diffusion method was used for the antimicrobial assays as mentioned in Abdel Gawwad et al. [14] with minor modification. Sterile plates containing nutrient agar were prepared according to the manufacturer's instructions and allowed to solidify. The agar was then punched with a sterile cork borer (size 6 mm). Subsequently, the agar plates were seeded with fungal and yeasts strains adjusted to the McFarland solution using a sterile cotton swap, and a dose of 50  $\mu\text{L}$  test solution was loaded into wells. Standard antifungal drug (Clotrimazole 10 mg/ml) was served as positive control and was launched into a separate well on the agar plates. All the seeded plates were incubated for 24–48 h at 37 °C for yeasts or for 48–72 h at 30–35°C for fungi. Inhibition zones were measured using a transparent ruler in the nearest millimeters [14].

### 2.5. Minimum Inhibitory Concentration Test

The negative control consisted of a broth medium without inoculum and antimicrobial agent, and the positive growth control contained pure microbial culture without an antimicrobial agent. The microtiter plates were incubated aerobically at 35°C for 48 h. The lowest concentration of extracts that did not show visible growth was recorded as the MIC. A broth medium without inoculum or antimicrobial agent served as the negative control, while pure microbial culture served as the positive growth control. The microtiter plates were incubated aerobically for 48 h at 35°C in the Sabouraud Dextrose broth. The MIC was determined as the lowest

concentration of extracts that did not display visible growth [15].

### 2.6. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Capacity

The antioxidant potential was evaluated by the DPPH radical scavenging activity as described in [16]. Ascorbic acid was used as a positive control, and the radical scavenging activity was expressed as % DPPH inhibition, calculated from the graph of inhibition percentage plotted.

$$\text{DPPH radical scavenging activity} = \frac{(A_0 - A_1)}{A_0} \times 100,$$

where  $A_0$  is control absorption (DPPH), and  $A_1$  is the sample absorption.

Percentage radical activity was plotted against the concentration of the corresponding antioxidant substance to obtain the  $\text{IC}_{50}$  value.

### 2.7. Cytotoxicity

Cell viability was assessed with WST-1 assay using Abcam® kit (Proliferation Reagent). Aliquots of 50  $\mu\text{L}$  cell suspension ( $3 \times 10^3$  cells) were seeded in 96-well plates and incubated in complete media for 24 h. Cells were treated with another aliquot of 50  $\mu\text{L}$  media containing drugs at serial concentrations. After 48 h of drug exposure, cells were treated with 10  $\mu\text{L}$  WST-1 reagent, and the absorbance was measured after 1 h at 450 nm using a BMG LABTECH®- FLUOstar [17].

### 2.8. Statistical Analysis

Quantitative data were expressed as the mean  $\pm$  standard error of means. A paired sample t-test was employed to determine if there were any significant differences between each tested compound with the referenced antifungal drug. The program used was SPSS-Statistical Package, version 11.

## 3. Results and Discussion

Solid state photodimerization of 1 to 2 had been previously shown to proceed via a  $[2 + 2]$  cycloaddition reaction. Dithymoquinone (DTQ) was synthesized by a simple photodimerization reaction of compound 1 TQ (thymoquinone), which was adopted from Sigma-Aldrich. The synthetic pathway was outlined in Scheme 1. The prepared compound 2 DTQ was monitored by TLC [18] and confirmed by spectral analysis (UV, IR,  $^1\text{H}$ NMR, and ESIMS). HPLC detected the purity of the compound, which is further discussed in the experimental part.

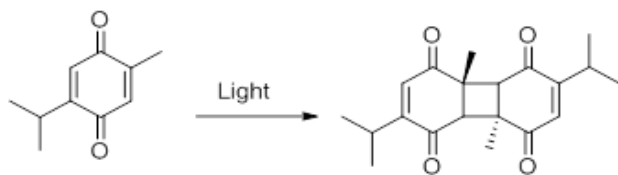


Fig. 1 Scheme for the synthesis of compound 2 (DTQ) from compound 1(TQ)

### 3.1. FTIR of Thymoquinone and Dimer

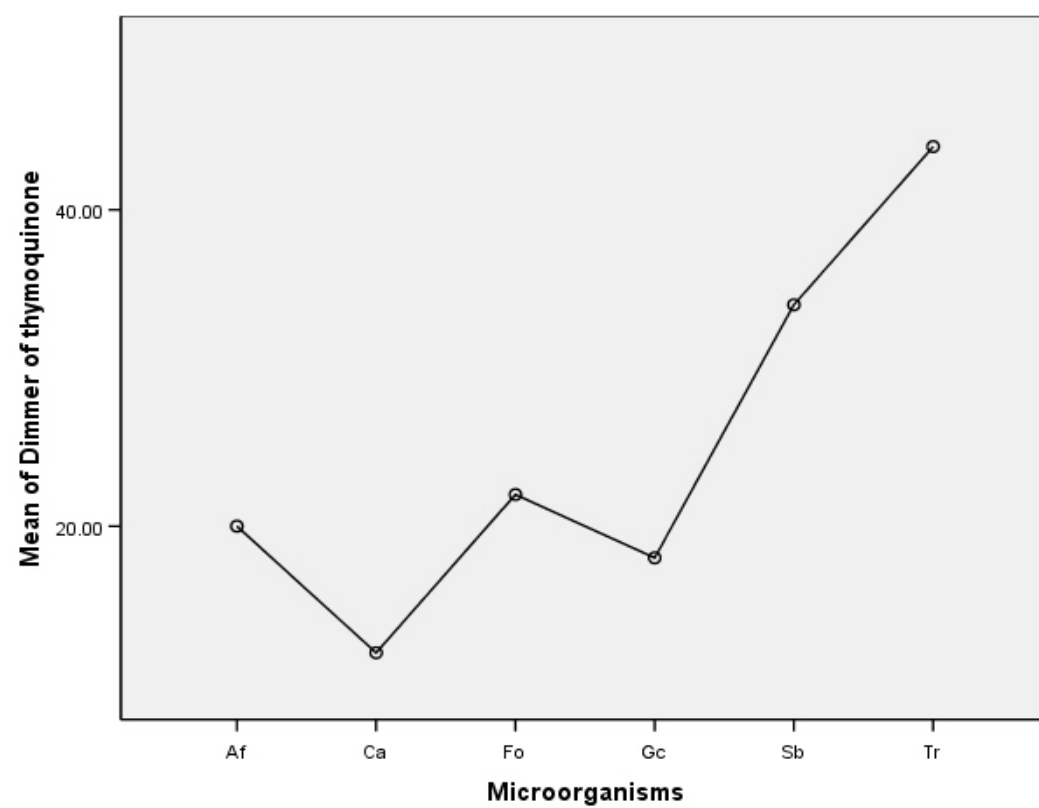
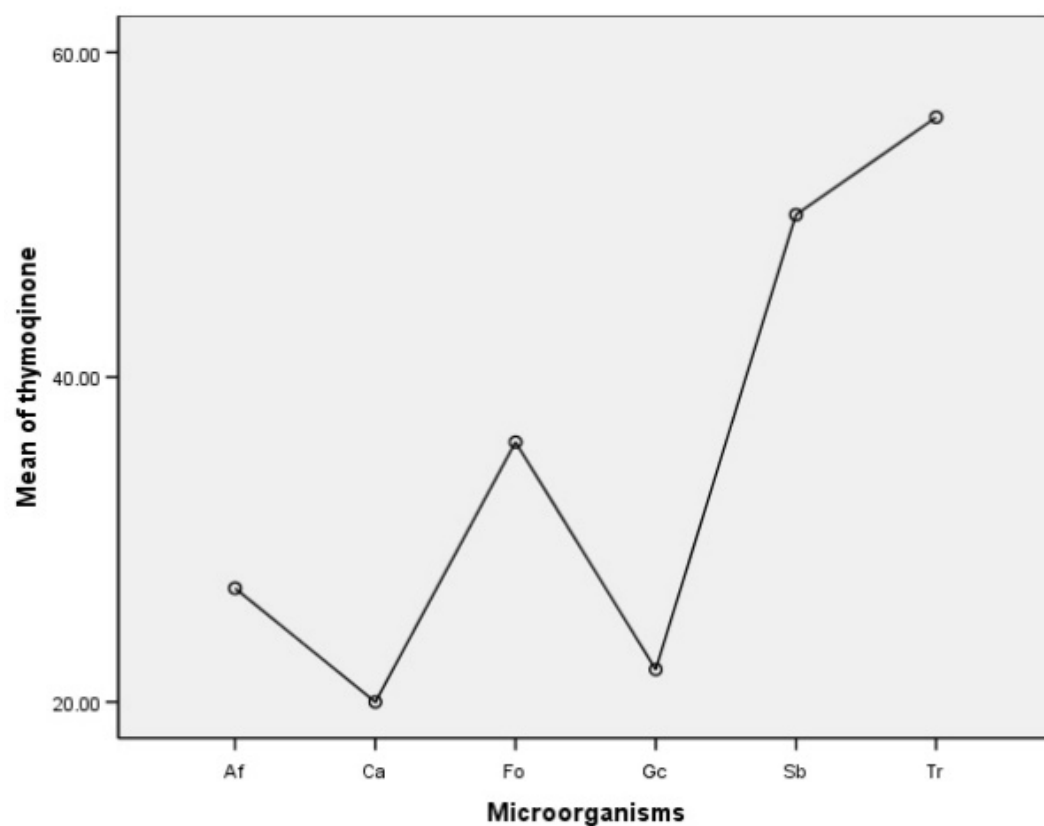
In the experimental spectra of both thymoquinone and dimer, medium bands were detected at a range of 2974–2875  $\text{cm}^{-1}$ , which can be associated with the alkane molecular stretching ( $\nu\text{CH}_2$ ) vibration. The strong band appeared at 1646  $\text{cm}^{-1}$  due to stretching vibrations of  $\nu(\text{C}=\text{O})$  of conjugated ketone. At a wavenumber of  $\approx 1606 \text{ cm}^{-1}$ , the strong band attributed to stretching vibrations of  $\nu(\text{C}=\text{C})$  was of  $\alpha, \beta$ -unsaturated ketone. The obtained FTIR spectra of the bending  $\delta(\text{C-H})$  vibration resulted in the absorption band [19]. The strong band appeared at 1231  $\text{cm}^{-1}$  due to stretching vibrations of  $\nu(\text{C-O})$  of aromatic ether. The two medium absorption bands at 1124  $\text{cm}^{-1}$  and 900  $\text{cm}^{-1}$  attributed to bending vibration of  $\delta(\text{C}=\text{C})$  of  $\alpha, \beta$ -unsaturated alkene.

### 3.2. $^1\text{H}$ NMR Spectra

NMR spectra are consistent with forming a single isomer of photodimerization reaction, with only one set of peaks in  $^1\text{H}$  NMR spectra. Furthermore, the olefinic methyl ( $\delta$  2.03 ppm, s) and proton of the same double bond ( $\delta$  6.18 ppm, s) of thymoquinone not shown, disappear upon irradiation and are accompanied by the appearance in the aliphatic region of the spectrum two new singlets (1.21 and 3.29). This result identifies the double bond involved in dimerization. ESI of the compound proved and confirmed the formation of dimer. There is a peak at  $m/z$ , 351 [ $\text{M}^+ \text{Na}$ ], corresponding to the molecular weight of Compound 2 DTQ. The absence of a peak at  $m/z$  165 [ $\text{M}^+ \text{H}$ ] relayed to TQ confirmed the complete reaction; purity was followed by HPLC.

Results of the antifungal potential of the dimer of thymoquinone and thymoquinone are shown in Table 2. The results were very interesting; both compounds showed noticeable antifungal activity. The dimer of thymoquinone isolated from the black seeds showed moderate to high antifungal activity. The zones of inhibitions of the most susceptible microorganism were

*Trichophyton rubrum* (44.0 mm), *Scopulariopsis brevicaulis* (34.0 mm), *Fusarium oxysporum* (22.0 mm), *Aspergillus flavus* (20.0 mm), *Geotrichum candidum* (18.0 mm), and *Candida albicans* (12.0 mm). On the other side, the thymoquinone isolated from black seeds showed higher antifungal activity than the dimer of thymoquinone. The highest inhibition zones made by tested microorganisms were *Trichophyton rubrum* (56.0 mm), *Scopulariopsis brevicaulis* (50.0 mm), *Fusarium oxysporum* (36.0 mm), *Aspergillus flavus* (27.0 mm), *Geotrichum candidum* (22.0 mm), and *Candida albicans* (20.0 mm). However, the thymoquinone recorded significant activity when we statistically compared each isolated compound with the referenced antifungal drug (Clotrimazole). In contrast, the dimer of thymoquinone revealed non-significant effects at  $P < 0.05$  (Fig. 2). This amazing result means that thymoquinone isolated from black seeds has a comparable antifungal effect and could be used as an effective antifungal drug. To the best of our knowledge, this is the first study recommending this compound as a novel antifungal drug. The MIC results support this claim, with the results ranging between 12.5 to 0.78 mg/ml for the dimer of thymoquinone, and between 12.5 to 1.56 mg/ml for thymoquinone isolated from black seeds. Previous studies mentioned that the essential oil and various extracts of black seeds, particularly thymoquinone, have high antifungal activity against three pathogenic dermatophyte strains, namely *Trichophyton mentagrophytes*, *Microsporum canis*, and *Microsporum gypseum*, and the study suggests the use of thymoquinone as an anti-dermatophyte drug [20]. Fixed oil of black seeds (*Nigella sativa*) showed good antifungal efficacy against *Candida parapsilosis* ATCC 22019 (13.33 mm) and *Candida glabrata* ATCC 90030 (12 mm) with minimal MIC values [21]. The essential oils of the black seeds exhibited potent antifungal activity against eight seed borne fungi, namely *Fusarium oxysporum*, *F. moniliforme*, *F. nivale*, *F. semitectum*, *Aspergillus niger*, *A. flavus*, *Alternaria alternata*, and *Drechslera hawaiiensis* [22]. Another in vivo study showed that the *Nigella sativa* seeds have a remarkable inhibitory effect against candidiasis induced in experimental mice [23]. Therefore, we highly recommend the development of an antifungal drug based on thymoquinone from black seeds.



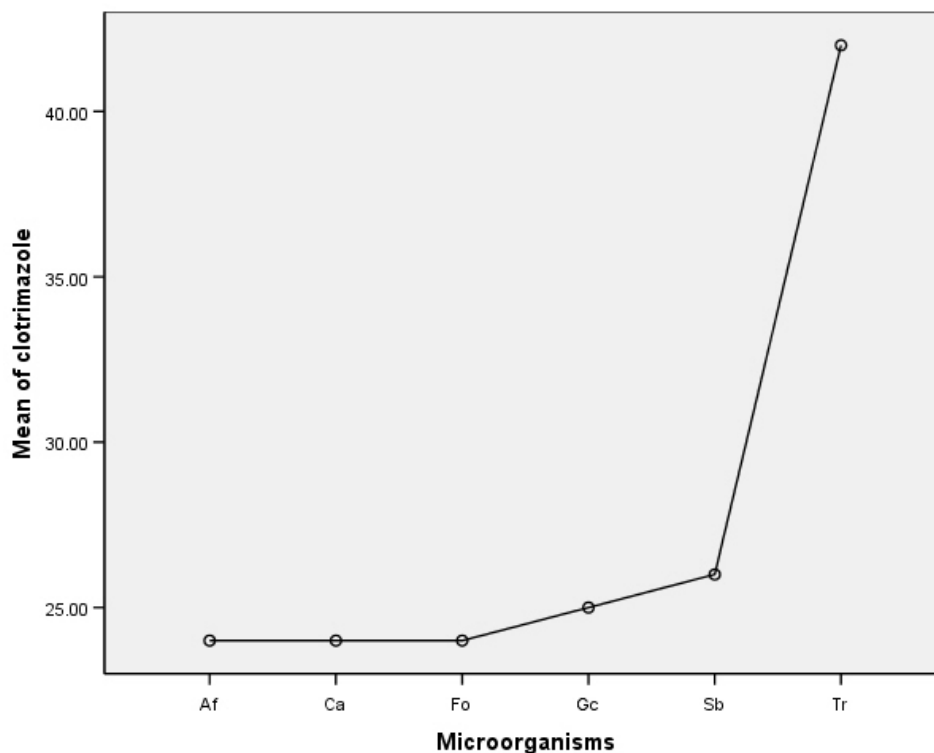


Fig. 2 Mean plots of the antifungal potential of the isolated compounds compared to the referenced antifungal drug

Table 2 Antifungal activity of tested compounds (inhibition zone and MIC) \*

Tested compounds	Af	Ca	Fo	Gc	Sb	Tr
	ZI(MIC)	ZI(MIC)	ZI(MIC)	ZI(MIC)	ZI(MIC)	ZI(MIC)
Dimmer of Thymoquinone	20(12.5)	12(1.56)	22(6.25)	18(0.78)	34(6.25)	44(3.125)
Thymoquinone	27(12.5)**	20(6.25)**	36(3.125)**	22(1.56)**	50(1.56)**	56(1.56)**
Clotrimazole	27(0.039)	24(0.156)	24(0.039)	25(0.0039)	26(0.312)	42(0.019)

\* Inhibition zone in mm, MIC in mg/ml, Af - *Aspergillus flavus*, Ca - *Candida albicans*, Fo - *Fusarium oxysporum*, Gc - *Geotrichum candidum*, Sb - *Scopulariopsis brevicaulis*, Tr - *Trichophyton rubrum*, \*\* - Significant at  $P < 0.05$

### 3.3. Antioxidant Activity

*Nigella sativa* is a natural source of many active compounds used to overcome the effects of oxidative stress underlying pathogenic disease. The DPPH radical scavenging activity of two major compound of plant *N. sativa*, TQ and DTQ, were assayed by DPPH radical scavenging methods [19], with the results illustrated in Table 3. The results demonstrate the variation in antioxidant activity of the compounds, with TQ showing high antioxidant activity with IC<sub>50</sub> ( $78.38 \pm 0.01$  mg\ml) compared to the activity of Ascorbic acid, while DTQ shows the lowest DPPH antioxidant activity with IC<sub>50</sub> ( $95.95 \pm 0.01$  mg/ml).

Table 3 DPPH scavenging activity of compounds (thymoquinone and dithymoquinone)

Test	TQ	DTQ	AC
IC <sub>50</sub> (mg/ml)	$78.38 \pm 0.01$	$95.95 \pm 0.01$	$66.33 \pm 0.001$

Note: Ac - Ascorbic acid, TQ - thymoquinone, DTQ - dithymoquinone

TQ is a major compound of *N. sativa* that, in a previous study, exhibited high antioxidant activity, and is recommended as a substitute for synthetic drugs to handle oxidative damage caused to DNA, lipids, and proteins [24]. Antioxidant activity of TQ varies depending on extraction procedure [25]. These previous studies agree with the current study which also recommends the use of TQ as an antioxidant agent.

The scavenging activity increases as concentration increases. Similar studies in petroleum ether, distilled water, and methanol extract of *N. sativa* revealed a dose dependent increase in scavenging activity [26].

### 3.4. Cytotoxicity of Compounds Isolated from *Nigella Sativa*

Cell viability of compounds TQ and DTQ was assayed by the WAST-1 method [17] against three cell lines (MCF-7, MDA-MB-231 breast cancer cell line, and A549 lung cancer cell line), with the results indicating a higher activity of thymoquinone compound against MCF-7 and MDA-MB-231 with IC<sub>50</sub> (7.9 ug/ml and 28.1mg/ml) than dimmer of thymoquinone (DTQ), while

DTQ shows good cytotoxic activity with IC<sub>50</sub> (35.5 ug/ml) against the lung cancer cell line (Table 4).

Table 4 Cytotoxicity of TQ and DTQ on different cell line

Tested compounds	MCF-7 ug IC <sub>50</sub> ug/ml	MDA-MB 231 IC <sub>50</sub> ug/ml	A549 IC <sub>50</sub> ug/ml
Dimmer of Thymoquinone	36.7	42	35.5
Thymoquinone	7.9	28.1	39.8
Doxorubicin	26.12	32.22	28.32

Thymoquinone (TQ) is a naturally occurring compound drawing great attention as an anti-cancer agent and chemo modulator for chemotherapies.

In the current study WST-1 assay was used to assess the viability of TQ and DTQ against three different cell lines over concentration range 0.01- 100 ug. Treatment with TQ and DTQ induced a significant dose-dependent cytotoxic effect on MCF-7, MDA-MB 231 breast cancer, and A549 (Fig. 2). Thymoquinone isolated from *N. sativa* exerted a closely dependent antiproliferative effect on the hella cancer cell line. Many previous studies confirmed the antiproliferative effect against different cell lines [27, 28, 29], but there are no previous studies concerned with the cytotoxicity of DTQ.

## 4. Conclusion

*N. sativa* seeds (Ranunculaceae family) have been used for thousands of years as a condiment and food preservative. The revival of traditional medicine using modern technologies could lead to noticeable drug development against some serious ailments. TQ and DTQ as the bioactive component of fugacious oil of the black seed has indicated potent medicinal effects in traditional medicine. DTQ was synthesized by photodimerization. Two compounds were studied for antifungal, antioxidant, and cytotoxic activity. TQ showed higher cytotoxic activity against MCF-7 and MDA-MB 231 cell lines with IC<sub>50</sub> (7.9 and 28.1) than dimmer of thymoquinone (DTQ); both compounds showed noticeable antifungal activity. The results recommended the use of thymoquinone as an anticancer drug against breast cancer cell lines. To the best of our knowledge, this is the first study on TQ and DTQ together, and the DTQ represented remarkable bioactivities. However, this study has some potential limitations: the promising antifungal, antioxidant, and anticancer activities are based on the *in vitro* assays. Accordingly, we recommend further *in vivo* studies in parallel with other pharmacological evaluations that could lead to the development of interesting drugs from this plant product.

## References

- [1] GHOLAMNEZHAD Z., KEYHANMANESH R., and BOSKABADY M. Anti-inflammatory, antioxidant, and immunomodulatory aspects of *Nigella sativa* for its preventive and bronchodilatory effects on obstructive respiratory diseases: A review of basic and clinical evidence. *Journal of Functional Foods*, 2015, 17: 910-927. <https://doi.org/10.1016/j.jff.2015.06.032>
- [2] ABDALLAH E.M. Plants: An alternative source for antimicrobials. *Journal of Applied Pharmaceutical Science*, 2011, 1(6): 16-20. [https://www.japsonline.com/admin/php/uploads/118\\_pdf.pdf](https://www.japsonline.com/admin/php/uploads/118_pdf.pdf)
- [3] GULSHAN K., & MOYE-ROWLEY W. S. Multidrug Resistance in Fungi. *Eukaryotic Cell*, 2020, 6(11), 1933-1942. <https://doi.org/10.1128/EC.00254-07>
- [4] COWAN M. M. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 1999, 12(4): 564-582. <https://doi.org/10.1128/CMR.12.4.564>
- [5] HASSANIEN M. F., ASSIRI A. M., ALZOHAIY A. M., and ORABY H. F. Health-promoting value and food applications of black cumin essential oil: an overview. *Journal of Food Science and Technology*, 2015, 52(10): 6136-6142. <https://doi.org/10.1007/s13197-015-1785-4>
- [6] CASCELLA M., PALMA G., BARBIERI A., BIMONTE S., AMRUTHRAJ N. J., and MUZIO M. R. Role of *Nigella sativa* and its constituent thymoquinone on chemotherapy-induced nephrotoxicity: evidence from experimental animal studies. *Nutrients*, 2017, 9(6): 625. <https://doi.org/10.3390/nu9060625>
- [7] OZER E. K., GOKTAS M. T., TOKER A., PEHLIVAN S., BARISKANER H., and UGURLUOGLU C. Thymoquinone protects against the sepsis induced mortality, mesenteric hypoperfusion, aortic dys-function and multiple organ damage in rats. *Pharmacological Reports*, 2017, 69(32): 683–690. <https://doi.org/10.1016/j.pharep.2017.02.021>
- [8] FAISAL R., SHINWARI L., and JEHANGIR T. Comparison of the therapeutic effects of thymoquinone and methotrexate on renal injury in pristane induced arthritis in rats. *Journal of the College of Physicians and Surgeons Pakistan*, 2015, 25(8): 597-601. [https://applications.emro.who.int/imemrf/J\\_Coll\\_Physicians\\_Surg\\_Pak/J\\_Coll\\_Physicians Surg Pak 2015 25 8 597 601.pdf](https://applications.emro.who.int/imemrf/J_Coll_Physicians_Surg_Pak/J_Coll_Physicians Surg Pak 2015 25 8 597 601.pdf)
- [9] GALI-MUHTASIB H., ROESSNER A., and SCHNEIDER-STOCK R. Thymoquinone: A promising anti-cancer drug from natural sources. *The International Journal of Biochemistry & Cell Biology*, 2006, 38(8): 1249-1253. <https://doi.org/10.1016/j.biocel.2005.10.009>
- [10] RAMADAN M. F. Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): an overview. *International Journal of Food Science & Technology*, 2007, 42(10): 1208–1218. <https://doi.org/10.1111/j.1365-2621.2006.01417.x>
- [11] SHATERZADEH-YAZDI H., NOORBAKSH M.-F., SAMARGHANDIAN S., and FARKHONDEH T. An Overview on Renoprotective Effects of Thymoquinone. *Kidney Diseases*, 2018, 4(2): 74-82. <https://doi.org/10.1159/000486829>
- [12] GHOSHEH O. A., HOUDI A. A., and CROOKS P. A. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in



- the oil of the black seed (*Nigella sativa* L.). *Journal of Pharmaceutical and Biomedical Analysis*, 1999, 19(5): 757–762. [https://doi.org/10.1016/S0731-7085\(98\)00300-8](https://doi.org/10.1016/S0731-7085(98)00300-8)
- [13] PATHAN S. A., JAIN G. K., ZAIDI S. M. A., AKHTER S., VOHORA D., CHANDER P., KOLE P. L., AHMAD F. J., and KHAR R. K. Stability-indicating ultra-performance liquid chromatography method for the estimation of thymoquinone and its application in biopharmaceutical studies. *Biomedical Chromatography*, 2011, 25(5): 613–620. <https://doi.org/10.1002/bmc.1492>
- [14] ABDEL GAWWAD M. R., MAHMOOD A., AL FARRAJ D. A., EL-ABEDELIN A. I. Z., MAHMOUD A. H., and BUKHARI S. M. In-vitro antimicrobial activities of *Solanum villosum* (L.) lam; crude extract solvent comparison. *Journal of King Saud University – Science*, 2020, 32: 2129–2133. <https://doi.org/10.1016/j.jksus.2020.01.035>
- [15] WONG J. X., & RAMLI S. Antimicrobial activity of different types of *Centella asiatica* extracts against foodborne pathogens and food spoilage microorganisms. *LWT*, 2021, 142: 111026. <https://doi.org/10.1016/j.lwt.2021.111026>
- [16] ELSHARKAWY E. R., & SHIBOUB M. Antioxidant Activity of Phenolic and Alkaloid Fractions Accumulated in *Artemisia judaica* and *Artemisia herba alba*. *Journal of Natural Remedies*, 2017, 17(4): 2320-3358. <https://doi.org/10.18311/jnr/2017/18731>
- [17] ALAUF O. M., NOORWALI A., ZAHARAN F., AL-ABD A. M., and AL-ATTAS S. Cytotoxicity of thymoquinone alone or in combination with cisplatin (CDDP) against oral squamous cell carcinoma in vitro. *Scientific Reports*, 2017, 7(1): 13131. <https://doi.org/10.1038/s41598-017-13357-5>
- [18] BASHA L. I. A., RASHED M. S., and ABOULENEIN H. Y. TLC Assay of Thymoquinone in Black Seed Oil (*Nigella sativa* Linn) and Identification of Dithymoquinone and Thymol. *Journal of Liquid Chromatography & Related Technologies*, 1995, 18(1): 105–115. <https://doi.org/10.1080/10826079508009224>
- [19] YUSUFI M., BANERJEE S., MOHAMMAD M., KHATAL S., VENKATESWARA S. K., KHAN E. M., ABOUKAMEEL A., SARKAR F. H., and PADHYE S. Synthesis, characterization and anti-tumor activity of novel thymoquinone analogs against pancreatic cancer. *Bioorganic & Medicinal Chemistry Letters*, 2013, 23(10): 3101-3104. <https://doi.org/10.1016/j.bmcl.2013.03.003>
- [20] MAHMOUDV H., SEPAHVAND A., JAHANBAKHSH S., EZATPOUR B., and MOUSAVI S. A. A. Evaluation of antifungal activities of the essential oil and various extracts of *Nigella sativa* and its main component, thymoquinone against pathogenic dermatophyte strains. *Journal de Mycologie Medicale*, 2014, 24(4): 155-161. <https://doi.org/10.1016/j.mycmed.2014.06.048>
- [21] SITARA U., NIAZ I., NASEEM J., and SULTANA N. Antifungal Effect of Essential Oils on In Vitro Growth of Pathogenic Fungi. *Pakistan Journal of Botany*, 2008, 40(1): 409-414. [https://www.pakbs.org/pjbot/PDFs/40\(1\)/45.pdf](https://www.pakbs.org/pjbot/PDFs/40(1)/45.pdf)
- [22] HARZALLAH H., NOUMI E., KARIMA B., BAKHROUF A., and MAHJOUB T. Chemical composition, antibacterial and antifungal properties of Tunisian *Nigella sativa* fixed oil. *African Journal of Microbiology Research*, 2012, 6(22): 4675-4679. <https://doi.org/10.5897/AJMR11.1073>
- [23] KHAN M. A. U., ASHFAQ M. K., ZUBERI H. S., MAHMOOD M. S., and GILANI A. H. The in vivo antifungal activity of the aqueous extract from *Nigella sativa* seeds. *Phytotherapy Research*, 2003, 17(2): 183-186. <https://doi.org/10.1002/ptr.1146>
- [24] TOMA C. C., OLAH N. K., VLASE L., MOGOȘAN C., and MOCAN A. Comparative studies on polyphenolic composition, antioxidant, and diuretic effects of *nigella sativa* L. (black cumin) and *nigella damascena* L. (Lady-in-aMist) seeds. *Molecules*, 2015, 20(6): 9560-9574. <https://doi.org/10.3390/molecules20069560>
- [25] REDDY S. H., AL-KALBANI A. S., and AL-RAWAHI A. S. Studies on Phytochemical Screening - Gc-MS Characterization, Antimicrobial and Antioxidant Assay of Black Cumin Seeds (*Nigella sativa*) and Senna Alexandria (*Cassia angustifolia*) Solvent Extracts. *International Journal of Pharmaceutical Sciences and Research*, 2018, 9(2): 490-497. [https://doi.org/10.13040/IJPSR.0975-8232.9\(2\).490-97](https://doi.org/10.13040/IJPSR.0975-8232.9(2).490-97)
- [26] ISHTIAQ S., ASHRAF M., HAYAT M. Q., and ASRAR M. Phytochemical analysis of *nigella sativa* and its antibacterial activity against clinical isolates identified by ribotyping. *International Journal of Agriculture and Biology*, 2013, 15(6): 1151-1156. [https://www.fspublishers.org/published\\_papers/7026..pdf](https://www.fspublishers.org/published_papers/7026..pdf)
- [27] YUSUFI M., BANERJEE S., MOHAMMAD M., KHATAL S., VENKATESWARA S. K., KHAN E. M., ABOUKAMEEL A., SARKAR F. H., and PADHYE S. Synthesis, characterization and anti-tumor activity of novel thymoquinone analogs against pancreatic cancer. *Bioorganic & Medicinal Chemistry Letters*, 2013, 23(10): 3101-3104. <https://doi.org/10.1016/j.bmcl.2013.03.003>
- [28] BUTT A. S., NISAR N., GHANI N., ALTAF I., and MUGHAL T. A. Isolation of thymoquinone from *Nigella sativa* L. and *Thymus vulgaris* L., and its anti-proliferative effect on HeLa cancer cell lines. *Tropical Journal of Pharmaceutical Research*, 2019, 18(1): 37-42. <https://doi.org/10.4314/tjpr.v18i1.6>
- [29] BAÑUELOS A., REYES E., OCADIZ R., ALVAREZ E., MORENO M., MONROY A., and GARIGLIO P. Neocarzinostatin induces an effective P53-dependent response in human papillomavirus-positive cervical cancer cells. *Journal of Pharmacology and Experimental Therapeutics*, 2003, 306(2): 671-680. <https://doi.org/10.1124/jpet.103.051557>

#### 参考文献:

- [1] GHOLAMNEZHAD Z., KEYHANMANESH R. 和 BOSKABADY M. 黑种草的抗炎、抗氧化和免疫调节方面对阻塞性呼吸道疾病的预防和支气管扩张作用：基础和临床证据综述。功能食品杂志，2015，17：910-927. <https://doi.org/10.1016/j.jff.2015.06.032>
- [2] ABDALLAH E.M. 植物：抗菌素的替代来源。应用药理学杂志，2011，1(6)：16-20. [https://www.japsonline.com/admin/php/uploads/118\\_pdf.pdf](https://www.japsonline.com/admin/php/uploads/118_pdf.pdf)



- [3] GULSHAN K., & MOYE-ROWLEY W. S. 真菌中的多重耐药性。真核细胞, 2020, 6(11), 1933-1942. <https://doi.org/10.1128/EC.00254-07>
- [4] COWAN M. M. 植物产品作为抗菌剂。临床微生物学评论, 1999, 12(4): 564-582. <https://doi.org/10.1128/CMR.12.4.564>
- [5] HASSANIEN M. F., ASSIRI A. M., ALZOHAIY A. M. 和 ORABY H. F. 黑孜然精油的健康促进价值和食品应用: 概述。食品科学与技术学报, 2015, 52(10): 6136-6142. <https://doi.org/10.1007/s13197-015-1785-4>
- [6] CASCELLA M., PALMA G., BARBIERI A., BIMONTE S., AMRUTHRAJ N. J. 和 MUZIO M. R. 黑种草及其成分百里醌对化疗引起的肾毒性的作用: 来自实验动物研究的证据。营养素, 2017, 9(6): 625. <https://doi.org/10.3390/nu9060625>
- [7] OZER E. K., GOKTAS M. T., TOKER A., PEHLIVAN S., BARISKANER H. 和 UGURLUOGLU C. 百里醌可防止大鼠败血症引起的死亡率、肠系膜灌注不足、主动脉功能障碍和多器官损伤。药理学报告, 2017, 69(32): 683-690. <https://doi.org/10.1016/j.pharep.2017.02.021>
- [8] FAISAL R., SHINWARI L., 和 JEHangIR T. 胸腺醌和甲氨蝶呤对原始烷诱发的大鼠关节炎肾损伤的治疗效果比较。巴基斯坦内科和外科医生学院杂志, 2015, 25(8): 597-601. [https://applications.emro.who.int/imemrf/J\\_Coll\\_Physicians\\_Surg\\_Pak/J\\_Coll\\_Physicians\\_Surg\\_Pak\\_2015\\_25\\_8\\_597\\_601.pdf](https://applications.emro.who.int/imemrf/J_Coll_Physicians_Surg_Pak/J_Coll_Physicians_Surg_Pak_2015_25_8_597_601.pdf)
- [9] GALI-MUHTASIB H., ROESSNER A. 和 SCHNEIDER-STOCK R. 百里醌: 一种来自天然来源的有前途的抗癌药物。国际生物化学与细胞生物学杂志, 2006, 38(8): 1249-1253. <https://doi.org/10.1016/j.biocel.2005.10.009>
- [10] RAMADAN M. F. 黑孜然(黑种草升.)的营养价值、功能特性和营养保健应用: 概述。国际食品科学与技术杂志, 2007, 42(10): 1208-1218. <https://doi.org/10.1111/j.1365-2621.2006.01417.x>
- [11] SHATERZADEH-YAZDI H., NOORBAKHSH M. F., SAMARGHANDIAN S. 和 FARKHONDEH T. 百里醌对肾脏保护作用概述。肾脏疾病, 2018, 4(2): 74-82. <https://doi.org/10.1159/000486829>
- [12] GHOSHEH O. A., HOUDI A. A. 和 CROOKS P. A. 对黑色种子(黑种草升.)油中具有药理活性的醌类和相关化合物的高效液相色谱分析。药物与生物医学分析杂志, 1999, 19(5): 757-762. [https://doi.org/10.1016/S0731-7085\(98\)00300-8](https://doi.org/10.1016/S0731-7085(98)00300-8)
- [13] PATHAN S. A., JAIN G. K., ZAIDI S. M. A., AKHTER S., VOHORA D., CHANDER P., KOLE P. L., AHMAD F. J., 和 KHAR R. K. 稳定性指示超高效液相色谱法估计百里醌及其应用在生物制药研究中。生物医学色谱, 2011, 25(5): 613-620. <https://doi.org/10.1002/bmc.1492>
- [14] ABDEL GAWWAD M. R., MAHMOOD A., AL FARRAJ D. A., EL-ABEDELIN A. I. Z., MAHMOUD A. H. 和 BUKHARI S. M. 茄属植物(升.)我是的体外抗菌活性; 粗提物溶剂比较。沙特国王大学学报 - 科学, 2020, 32: 2129-2133. <https://doi.org/10.1016/j.jksus.2020.01.035>
- [15] WONG J. X., & RAMLI S. 不同类型积雪草提取物对食源性病原体和食品腐败微生物的抗菌活性。轻量级, 2021, 142: 111026. <https://doi.org/10.1016/j.lwt.2021.111026>
- [16] ELSHARKAWY E. R., & SHIBOOB M. 积累在蒿和阿尔巴蒿中的酚类和生物碱成分的抗氧化活性。自然疗法杂志, 2017, 17(4): 2320-3358. <https://doi.org/10.18311/jnr/2017/18731>
- [17] ALAUFI O. M., NOORWALI A., ZAHARAN F., AL-ABD A. M. 和 AL-ATTAS S. 百里醌单独或与顺铂(开发计划署)联合使用对体外口腔鳞状细胞癌的细胞毒性。科学报告, 2017, 7(1): 13131. <https://doi.org/10.1038/s41598-017-13357-5>
- [18] BASHA L. I. A., RASHED M. S. 和 ABOUL-ENEIN H. Y. 薄层色谱黑籽油(黑种草)中百里醌的薄层色谱分析以及二百里醌和百里酚的鉴定。液相色谱及相关技术杂志, 1995, 18(1): 105-115. <https://doi.org/10.1080/10826079508009224>
- [19] YUSUFI M., BANERJEE S., MOHAMMAD M., KHATAL S., VENKATESWARA S. K., KHAN E. M., ABOUKAMEEL A., SARKAR F. H., 和 PADHYE S. 新型百里醌类似物对胰腺的合成、表征和抗肿瘤活性癌症。生物有机与药物化学快报, 2013, 23(10): 3101-3104. <https://doi.org/10.1016/j.bmcl.2013.03.003>
- [20] MAHMOUDV H., SEPAHVAND A., JAHANBAKHSH S., EZATPOUR B. 和 MOUSAVI S. A. A. 评估黑种草及其主要成分百里香醌的精油和各种提取物对致病性皮肤癣菌菌株的抗真菌活性。真菌学医学杂志, 2014, 24(4): 155-161. <https://doi.org/10.1016/j.mycmed.2014.06.048>
- [21] SITARA U., NIAZ I., NASEEM J. 和 SULTANA N. 精油对病原真菌体外生长的抗真菌作用。巴基斯坦植物学杂志, 2008, 40(1): 409-414. [https://www.pakbs.org/pjbot/PDFs/40\(1\)/45.pdf](https://www.pakbs.org/pjbot/PDFs/40(1)/45.pdf)
- [22] HARZALLAH H., NOUMI E., KARIMA B., BAKHROUF A. 和 MAHJOUB T. 突尼斯黑种草固定油的化学成分、抗菌和抗真菌特性。非洲微生物学研究杂志, 2012, 6(22): 4675-4679. <https://doi.org/10.5897/AJMR11.1073>
- [23] KHAN M. A. U., ASHFAQ M. K., ZUBBERI H. S., MAHMOOD M. S. 和 GILANI A. H. 黑种草种子水提取物的体内抗真菌活性。植物疗法研究, 2003, 17(2): 183-186. <https://doi.org/10.1002/ptr.1146>
- [24] TOMA C. C., OLAH N. K., VLASE L., MOGOŞAN C. 和 MOCAN A.

黑种草. (黑孜然) 和大马士革黑种草升. (小姐) 的多酚成分、抗氧化和利尿作用的比较研究-雾) 种子。分子, 2015, 20(6): 9560-9574。

<https://doi.org/10.3390/molecules20069560>

[25] REDDY S. H.、AL-KALBANI A. S. 和 AL-RAWAHI A. S. 植物化学筛选研究 - 黑孜然种子 (黑种草) 和番泻叶 (狭叶决明子) 溶剂提取物的气相色谱仪表征、抗菌和抗氧化测定。国际药物科学研究杂志, 2018, 9(2): 490-497。 [https://doi.org/10.13040/IJPSR.0975-8232.9\(2\).490-97](https://doi.org/10.13040/IJPSR.0975-8232.9(2).490-97)

[26] ISHTIAQ S.、ASHRAF M.、HAYAT M. Q. 和 ASRAR M.

黑种草的植物化学分析及其对通过核糖分型鉴定的临床分离株的抗菌活性。国际农业与生物学杂志, 2013, 15(6): 1151-1156。 [https://www.fspublishers.org/published\\_papers/7026\\_..pdf](https://www.fspublishers.org/published_papers/7026_..pdf)

[27] YUSUFI M.、BANERJEE S.、MOHAMMAD M.、KHATAL S.、VENKATESWARA S. K.、KHAN E. M.、ABOUKAMEEL A.、SARKAR F. H. 和 PADHYE S. 新型百里醌类似物对胰腺的合成、表征和抗肿瘤活性癌症。生物有机与药物化学快报, 2013, 23(10): 3101-3104。 <https://doi.org/10.1016/j.bmcl.2013.03.003>

[28] BUTT A. S.、NISAR N.、GHANI N.、ALTAF I. 和 MUGHAL T. A. 从黑种草. 和百里香. 中分离百里醌, 及其对海拉癌细胞系的抗增殖作用。热带药物研究杂志, 2019, 18(1): 37-42。 <https://doi.org/10.4314/tjpr.v18i1.6>

[29] BAÑUELOS A.、REYES E.、OCADIZ R.、ALVAREZ E.、MORENO M.、MONROY A. 和 GARIGLIO P. 新制癌素在人乳头瘤病毒阳性宫颈癌细胞中诱导有效的磷53依赖性反应。药理学和实验治疗学杂志, 2003, 306 (2) : 671-680。 <https://doi.org/10.1124/jpet.103.051557>