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# Biological Evaluation of *Nigella sativa* L. Seeds cultivated in Saudi Arabia

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**Abstract :** Evaluation of the soluble fractions of the alcoholic extract of *Nigella sativa* cultivated in Saudi Arabia for their cytotoxic, antibacterial, antifungal and antioxidant activities were done using the brine shrimp lethality assay and mouse lymphoma (L5178Y), rat brain tumour (PC12), human cervix cancer cells lines (HELA) and colon cancer colo-205 cell lines for the cytotoxic activity. The antibacterial activity was evaluated using the gram-positive bacteria *Bacillus subtilis* 168 and *Staphylococcus aureus* ATCC 25923, gram-negative bacteria *Escherichia coli* ATC 25922, *Salmonella* ATC 25921, *Klebsiella pneumoniae* and *Pseudomona aeruginosa* ATC 49189. Meanwhile, *Candida albicans*, *Saccharomyces cereviisae* and *Aspergillus flavus* were used for assessing the antifungal activity. Finally, evaluation of the antioxidant activity using the DPPH assay was also carried out.
**Key words :** *Nigella sativa*, cytotoxic activity, Brine shrimp assay, Antibacterial, Antifungal.

## Introduction

*Nigella sativa* L. (family Ranunculaceae), is developed in Middle Asia, Middle East, and North Africa. The capsule of its ripe fruit contains 3-7 joined follicles, each having oval black little seeds. Its seeds are synonymously known as 'black Seed' or 'black cumin' in English, 'Habba Al-Sauda' or 'Habba Al-Barakah' in Arabic, 'Kalonji' in Urdu, and 'SiyahDaneh' in Persian and 'Corek Out' in the Turkish language [1]. *N. sativa* has emerged as a miraculous herb with a wide spectrum of pharmacological activities. *N. sativa* seeds are most extensively studied, both phytochemically and pharmacologically. Both seeds and oil are known to possess various health properties like antitumor [2], antioxidant [3], anti-inflammatory [4], antibacterial activities [5], and a



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stimulatory effect on the immune system [6]. Furthermore, N. sativa seed extract have also proved to be potent antimicrobial agents against certain pathogenic Gram positive and Gram negative bacteria [7]. *N. sativa* have been shown to be rich in diverse phytoconstituents including volatile and fixed oil, monoterpenes, alkaloids, flavonoids, triterpenes, and saponins [8-12].

*N. sativa* has been used for the treatment of microbial diseases without any reported side effect. It is one of the important medicinal herb worldwide. It has considerable value of use in pharmaceutical, food and ornamental industries. Many literatures are available about the seeds of *N. sativa*, but the current study was planned to investigate the some of the biological activities of the plant cultivated in Saudi Arabia.

## Experimental

### Plant material

The seeds of *N. sativa* L. were obtained from the cultivated plant in Saudi Arabia purchased from local herbal market in Makkah.

### Extraction and Fractionation:

*N. sativa* seeds (100 g) were crushed into fine powder and were extracted with chloroform, then dried, and extracted with 75% MeOH (3 L × 1 L) at room temperature till exhaustion to get the polar extract. The combined methanol extract was filtered and then evaporated under reduced pressure to get a dark brown oily residue of the concentrated methanolic extract (17.2 g).Then, the extract was digested with 100 ml distilled water, transferred to a separating funnel and successively partitioned with n-hexane (3×300 ml), dichloromethane (3×300 ml), ethyl acetate (3×300 ml) and *n*-butanol (3×300 ml), the remaining aqueous portion considered as the aqueous fraction. Each fraction was individually concentrated under reduced pressure to give the corresponding n-hexane fraction (5.7 g), dichloromethane fraction (4.5 g), ethyl acetate fraction (2.9 g) and *n*-butanol fraction (1.3 g), respectively, and aqueous fraction (1.5 g).

### Solvent for extraction:

Solvents for extraction and fractionation were purchased from Riedel-Haan.

### Equipment:

### General Equipment:

Mettler 200 balance, freeze dryer (Sartorius RC210P, LYOVAC GT2, Pump TRIVAC D10E), Automatic pipette (Eppendorf), Magnetic stirrer (Variomag Multipoint HP), pH-meter (Inolab, Behrotest pH 10-Set), Rotary Evaporator (Büchi Rotavap RE111), Sonicator (Bandelin Sonorex RK 102), Vacuum desiccator(Savant Speed Vac SPD 111V, Savant Refrigerator Vapour Trap, RVT400, Pump Savant VLP80)

### Biological studies:

### Brine shrimp lethality assay

The test was performed using 5 and 10 ug of the obtained fractions as published by Mayer and Edrada et al. [13,14]. The test was done in triplicate.

### Cytotoxic activity

Cytotoxicity was evaluated by the [$^3$H] thymidine assay [15], against mouse lymphoma (L5178Y), rat brain tumour (PC12) , human cervix cancer cells lines (HELA) and colon cancer colo-205 [15,16]. All cells were mycoplasma-free and cultures were propagated under standardized condition as decribed by K. G. Steube [17]. All tests were carried out in triplicates and repeated three times. As controls, media with 0.1% EGMME or DMSO were included in the experiment. Results are listed in table 2.

**Antioxidant activity**

The antioxidant activity was evaluated using 2,20-diphenylpi-crylhydrazyl (DPPH) assay as previously outlined by Stefan and Joyeux [18,19]. Standard propyl gallate (synthetic antioxidant) set as 100% antioxidant activity. The % free radical scavenging activity was calculated (Table 3), using the following formula:

$$\text{antioxidant activity} = 100 \times \left[1 - \frac{\text{absorbance with the sample}}{\text{absorbance of the blank}}\right]$$

**Antibacterial activity:**

The obtained soluble fractions were tested for activity against the following standard strains: gram-positive bacteria *Bacillus subtilis* 168 and *Staphylococcus aureus* ATCC 25923, gram-negative bacteria *Escherichia coli* ATC 25922, *Salmonella* ATC 25921 and *Pseudomona aeruginosa* ATC 49189. The agar diffusion assay was performed according to the Bauer-Kirby-Test [20].

For screening agar diffusion assay was applied [21], aliquots of the test solution were applied to sterile filter-paper discs to give a final disc loading concentration of 20 and 50 µg for the soluble fractions. The impregnated discs were placed on agar plates previously seeded with the selected test organisms, along with discs containing solvent blanks. The plates were incubated at 37$^{o}$C for 24 h then antibacterial activity was represented by the diameter in mm of the clear zone of inhibition in millimeter surrounding the tested substance disc. Results are listed in table 3.

**Antifungal activity:**

Soluble fractions were tested for activity against *Aspergillus flavus*, *Saccharomyces cereviisae* and the human pathogenic standard fungal strain *Candida albicans*. Culture preparations were done according to Mackay and Mackarthy [20]. The tested fractions were added using the agar diffusion method described before, the plates were incubated for 20 hours then the diameter of zones of inhibition were measured (mm). Results are listed in table 4.

**Statistical analysis**

All data were expressed as a mean ± standard error of the mean. Normal distribution of data was analyzed using the Student's *t*-test. The statistical significance was evaluated by one-way analysis of variance. The values were considered to be statistically significant different when $P < 0.05$.

## Results and discussions

**Brine shrimp assay.**

The brine shrimp cytotoxicity assay was considered as a convenient rapid simple bioassay for preliminary assessment of lethality, which in most cases correlates reasonably well with cytotoxic and anti-tumor properties [22]. Since the aimed biological response is attributed to a mixture of mixture of bioactive components rather than a single component. The brine shrimp lethality assay has been proved to be a convenient assay for monitoring biological activities of natural products [23].

**Table 1: Brine shrimp lethality assay of the total extract and its fractions of *N. sativa*.**

| Sample | % Mortality (24 hrs) | | % Mortality (48 hrs) | |
|---|---|---|---|---|
| | 5o µg\ml | 100 µg\ml | 50 µg\ml | 100 µg\ml |
| Total extract | 60 | 85 | 75 | 90 |
| n-Hexane | 20 | 55 | 30 | 65 |
| Ethyl acetate | 35 | 60 | 45 | 72 |
| n-Butanol | 65 | 80 | 75 | 90 |
| Aqueous | 55 | 70 | 65 | 80 |

**Cytotoxicity.**

The methanolic extract and its fractions were all cytotoxic to colon cancer (COLO-205), the total extract and the aqueous fraction were the most active and the dichloromethane fraction has the least activity, while the ethyl acetate and the n-butanol fractions have moderate cytotoxicities. The aqueous fraction, total extract and n-butanol, respectively, were the most active against mouse lymphoma (L5178Y) cell line, while the ethyl acetate has moderate cytotoxicity. The total extract, aqueous, ethyl acetate and n-butanol fractions, respectively, have moderate cytotoxicity against human cervix cancer (Hela) cell line; meanwhile the n-hexane fraction was inactive. Only the total extract and aqueous fraction were cytotoxic to mouse brain tumor (PC12) cell line. The dichloromethane fraction was inactive against L5178Y, Hela and PC12 cell lines and the n-hexane fraction was inactive against all the tested cell lines (Table 2).

**Table 2: $ED_{50}$ of the total methanolic extract and the obtained fractions of *N. sativa* against L5178Y, Hela, PC12 and COLO-205 cell lines.**

| Compound | ED50 (µg/mL) | | | |
|---|---|---|---|---|
| | **L5178Y** | **Hela** | **PC12** | **COLO-205** |
| Total extract | 23.6 | 27.7 | 31.6 | 22.4 |
| *n*-Hexane | - | - | - | - |
| Dichloromethane | - | - | - | 43.6 |
| Ethyl acetate | 32.2 | 28.4 | - | 29.3 |
| *n*-Butanol | 26.5 | 30.3 | - | 25.9 |
| Aqueous | 25.2 | 28.6 | 34.6 | 21.4 |

**Antioxidant Activity**

The assay revealed high antioxidant activity of the n-butanol and aqueous fractions, followed by the ethyl acetate fraction and the total extract (Table 3). The synergistic effect of flavonoid and phenolic derivatives may be responsible for the high antioxidant activity of the extract and the active fractions. The n-hexane fraction has no activity; while the dichloromethane fraction has weak antioxidant activity.

**Table 3: Antioxidant activity of the total extract and the obtained fractions of *N. sativa*.**

| Sample | Antioxidant % | |
|---|---|---|
| | **0.5 ug** | **1 ug** |
| DPPH (blank) | - | - |
| Propyl gallate (PG) (reference) | 100 | 100 |
| Total extract | 54.8 | 66.4 |
| *n*-Hexane fr. | - | - |
| Dichloromethane fr. | 27.2 | 36.7 |
| Ethyl acetate fr. | 67.4 | 76.9 |
| *n*-Butanol fr. | 79.3 | 85.5 |
| Aqueous fr. | 73.0 | 81.0 |

**Antibacterial Activity**

The n-butanol and the aqueous fractions showed pronounced activity in a dose dependent manner, the ethyl acetate fraction was the least active. Meanwhile, the n-hexane and dichloromethane fractions were inactive against all the test organisms as shown in table 4. None of the tested fractions was active against Salmonella.

**Table 4: Antibacterial activity of the soluble fractions of *N. sativa* soluble fractions.**

| Group | Conc. $\mu$g | Zone of inhibition | | | | | |
|---|---|---|---|---|---|---|---|
| | | B. subtilis | Stph | E. coli | Salm. | P. aeruginosa | K. pneumonia |
| Ethyl acetate | 50 | 1.1 | 0.8 | 0.9 | 0 | 0.7 | 0 |
| | 20 | 0.8 | 0.6 | 0.4 | 0 | 0.5 | 0 |
| n- Butanol | 50 | 1.7 | 1 | 1.1 | 0 | 0.8 | 0.7 |
| | 20 | 1.1 | 0.7 | 0.6 | 0 | 0.6 | 0.4 |
| Aqueous | 50 | 2.1 | 1.3 | 1.4 | 0 | 1.0 | 0.6 |
| | 20 | 1.0 | 0.7 | 0.6 | 0 | 0.6 | 0.4 |
| Streptomycin | 10 | 3.5 | 2.7 | 3 | 2.5 | 2 | 2.3 |
| Gentamycin | 10 | 3 | 2.5 | 2.1 | 1.9 | 1.8 | 2 |
| Ampicillin | 10 | 4.1 | 3 | 2.1 | 2 | 1 | 1.4 |

**Antifungal Activity**

The n-butanol and aqueous fractions showed antifungal activity in a dose dependent manner with the highest activity against *S. cereviisae* and *A. flavus*, and weak activity against *C. albicans*. Meanwhile, the ethyl acetate fraction showed weak activity against *S. cereviisae* and *A. flavus* at the higher concentration (50 $\mu$g) only, with no effect on *C. albicans* (Table 5).

**Table 5 : Antifungal activity of the soluble fractions of *N. sativa* extract.**

| **Sample** | **Conc. $\mu$g** | **Zone of inhibition** | | |
|---|---|---|---|---|
| | | ***C. albicans*** | ***S. cereviisae*** | ***A. Flavus*** |
| Ethyl acetate | 50 | 0 | 0.3 | 0.4 |
| | 20 | 0 | 0 | 0 |
| n- Butanol | 50 | 0.6 | 1.5 | 1.3 |
| | 20 | 0.4 | 1 | 0.8 |
| Aqueous | 50 | 0.7 | 1.3 | 1.5 |
| | 20 | 0.5 | 0.9 | 1 |
| Nystatin | 10 | 2.5 | 2.7 | 3 |

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