

A 'Systematised-Review' and Meta-Analysis of The Anti-Cancer Therapeutic Potential of *Nigella sativa* (Black cumin) to Reduce Cancer Cell Proliferation and Induce Apoptosis within an In-vitro Framework.

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Systematic Review

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الشخص
الذي تم تجاهله
—
THE OVERLOOKED ONE

An Analytical Research Project

by

Zayan Hussein

زيان ابن خليفة

239005596

Submitted to

The School of Biological Sciences

جامعة لستر
—
The University of Leicester



١٤٤٧

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The Basmallah

(The Prince Ghazi Trust for Qur'anic Thought, n.d.).

سَمِعَ رَسُولَ اللَّهِ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ يَقُولُ
"فِي الْحَبَّةِ السَّوْدَاءِ شِفَاءٌ مِنْ كُلِّ دَاءٍ إِلَّا السَّامَ"

- ٥٦٨٨ رواه البخاري

I heard Allah's Messenger (ﷺ) saying, "There is healing in black cumin for all diseases except death."

- Bukhari 5688

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A 'Systematised-Review' and Meta-Analysis of The Anti-Cancer Therapeutic Potential of *Nigella sativa* (Black cumin) to Reduce Cancer Cell Proliferation and Induce Apoptosis within an *In-vitro* Framework.

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Abstract

Despite *Nigella sativa* (NS) being heralded nearly three decades ago for its exceptional antineoplastic action, it seems its expectation for clinical incorporation is still quite far from realisation. With current treatments taking a 'sledgehammer-to-crack-a-nut' approach many patients and researchers are looking for non-toxic alternatives that still retain efficacious anti-neoplastic results. Within this study we set out to analyse one potential option through a 'systematised-review' and meta-analysis of available pre-clinical research papers utilising NS to reduce cancer-proliferation. We set out to ascertain whether any significant effects were observed, and with which derived medium, along with if any effects observed were consistent across different cancer-types. Our results suggest NS is not merely another 'snake-oil' but rather generates consistently potent anti-neoplastic compounds, with promising therapeutic potential. All of the nine collated papers suggest NS's effect upon *in-vitro* cell-viability was of a concentration-dependent manner, across all common cancer-types, with the oil-medium being the statistically superior mode of administration with frequent low IC₅₀ values observed within MTT-assays. Additionally, the repeated selective-cytotoxicity displayed within the studies supports the notion of NS being utilised synergistically with current chemo-agents, to mitigate their substantial drawbacks upon a patient's quality-of-life. However, its journey from bench-to-bedside seems to be stagnant with present scarcity of *in-vivo* clinical-trials and sub-par knowledge of the underlying molecular-mechanisms that enable NS to exert its therapeutic effects. Thus, this project aims to encourage further research to develop novel alternative therapeutics, for the prevention and treatment of cancer, utilising bio-active constituents from NS. The overlooked one.

Keywords: *Nigella sativa*, 'Systematised-review', Meta-analysis, Anti-cancer, Oil, Extract, MTT, Cell-line, *In-vitro*

1. Introduction

1.1 *The Background of The Analytical Research Project*

N*igella sativa* (NS) has been recorded to have huge 'carcino-preventive' potential (2). If harnessed appropriately its constituents could create an avenue for future alternative-therapeutic research and incorporation in present clinical-care, through combination-therapy, preventing various cancer-types by enhancing cytotoxic-outcomes through synergy with established chemo-therapeutics (214) (317).

This conception is further supported by the low-occurrence of side-effects present once NS was administered, along with its high accessibility (275) (408). However, currently the available literature in regard to clinical-trials are scarce with those available being of subpar-quality (20) (168). A possible consequence stemming from the truncated funding and demand for additional research within the pharmaceutical-industry, for this subject area (155) (319).

1.2 *The Objectives for The Analytical Research Project*

This 'systematised-review' and meta-analysis aim to assess the significance of reported *in-vitro* cytotoxic effects of NS, across common cancer-types.

This method of comparative-analysis will elucidate this through the utilisation of results generated from the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as a measure of the anti-proliferative efficacy of NS-derived formulations, for each respective cancer-cell line (55).

The analysis aims to scrutinise the concluded results and elucidate if any findings of anti-proliferative character hold any significance across the included studies.

Lastly, this analytical research project aims to be a resource to encourage further *in-vivo* research to develop novel alternative anti-cancer therapeutics, specifically utilising bio-active constituents from the overlooked NS plant.

1.3. *Current Cancer Statistics and The Limitations of Conventional Therapies*

Cancer. A persistent global-issue, with an estimated number of 20 million new-cases and 9.7 million deaths, during 2022. Current statistical-estimates illustrate the dire need for effective preventative-measures with estimated global increases, of over 30 million new cases in 2050 (111)(445); additionally, local current estimates place a patient's survival-rate at 49.8% (post-treatment) across all known cancer types.

Contemporary-treatments utilised include surgery, chemotherapy, and radiation-therapy; with each respective method having undergone significant advancements over the past few years (308)(459). Despite this, the afore-mentioned treatments have substantial drawbacks, resulting from the invasive side-effects produced, with such by-products causing severe impairment to a patient's quality-of-life (developmental-resistance), elevation of tumour-

recurrence-risk (low-selectivity) and economical-constraints (post-recovery complications) (31) (273) (400).

1.4. Why Practitioners, and Patients Alike, May Consider Nutraceutical-therapy?

One area that provides a promising avenue for a non-toxic approach to preventative anti-cancer therapy, is the use of nutraceuticals which have garnered interest over recent-years, from the scientific research-community (312). Simply, the utilisation of commercially available plants provides an opportunity for developing novel approaches towards mitigating by-product production from current-treatments (467). This developmental approach may help manufacture ingredients to decrease treatment-periods, through amplifying chemotherapeutic-efficacy whilst reducing harmful side-effects, that often lead to secondary-malignancy from prolonged exposure (148).

These characteristics are indispensable in addressing the World Health Organisation's (WHO) global integration strategy of incorporating indigenous medicinal-practices, within a clinical-setting; agreeing with previous resolutions determined by The World Health Assembly (WHA) (protocol: 1976 WHA 30.49) (18). Hence, this paper also hopes to inform researchers of a possible way towards increasing accessibility for preventative cancer-treatment, within 'low/middle-income' countries, in addition to communicating a possible viable-way of reducing the financial-burden and time deducted from a patient's life (76) (176) (451).

1.5. The Nigella sativa Landscape

NS. A multifaceted source for disease treatment and prevention since antiquity. From its place in the pantry of pharaohs, to the luggage of old Hittite-travellers (figure 1; figure 2) (184) (369). Therefore, its utilisation in contemporary-applications may be the solution, from the past, that aids in mitigating issues of the future - not just reserved for culinary-use. NS's diverse chemical-composition (figure 3) may be the paramount asset needed towards advancing preventative oncological-care.

1.6. Ascertaining Nigella sativa

NS is part of the genera *Ranunculus* of the Ranunculaceae family, native to North-Africa and the Mediterranean, having widespread agricultural-use within Asia (figure 2).

For many staple foods, NS remains the sought-after addition in which its absentia would incomplete a magnitude of global dishes. The plant consists of delicately-thin and linearly arranged leaves upon a well-established stem-frame, growing to a height of 25cm. Placed upon its elongated-structure is a crown of spear-pointed white petals detailed with pale-blue accent-tones; springing from the angulate-centre originate yellow-stamens in tandem with triplet-groupage of green pistil-structures which homogenise into distinct capsuled-fruits (159).

The 'triquetrous-shaped' seeds within the fruit-pods have an epidermal-mixture of black-brown hues, typically 1mm in length (figure 4). Once disturbed the tiny-structures emit a

poignant aroma, semblance to nutmeg. In terms of flavour, this characteristic tends to be time-bound with unripe produce presenting a rancid taste compared to a fruity and 'smoky' flavour, with 'peppery' after-tones, once mature (161) (250). These seeds are identified by many names (table 1) across different ages, regions, and civilisations (11)(12).

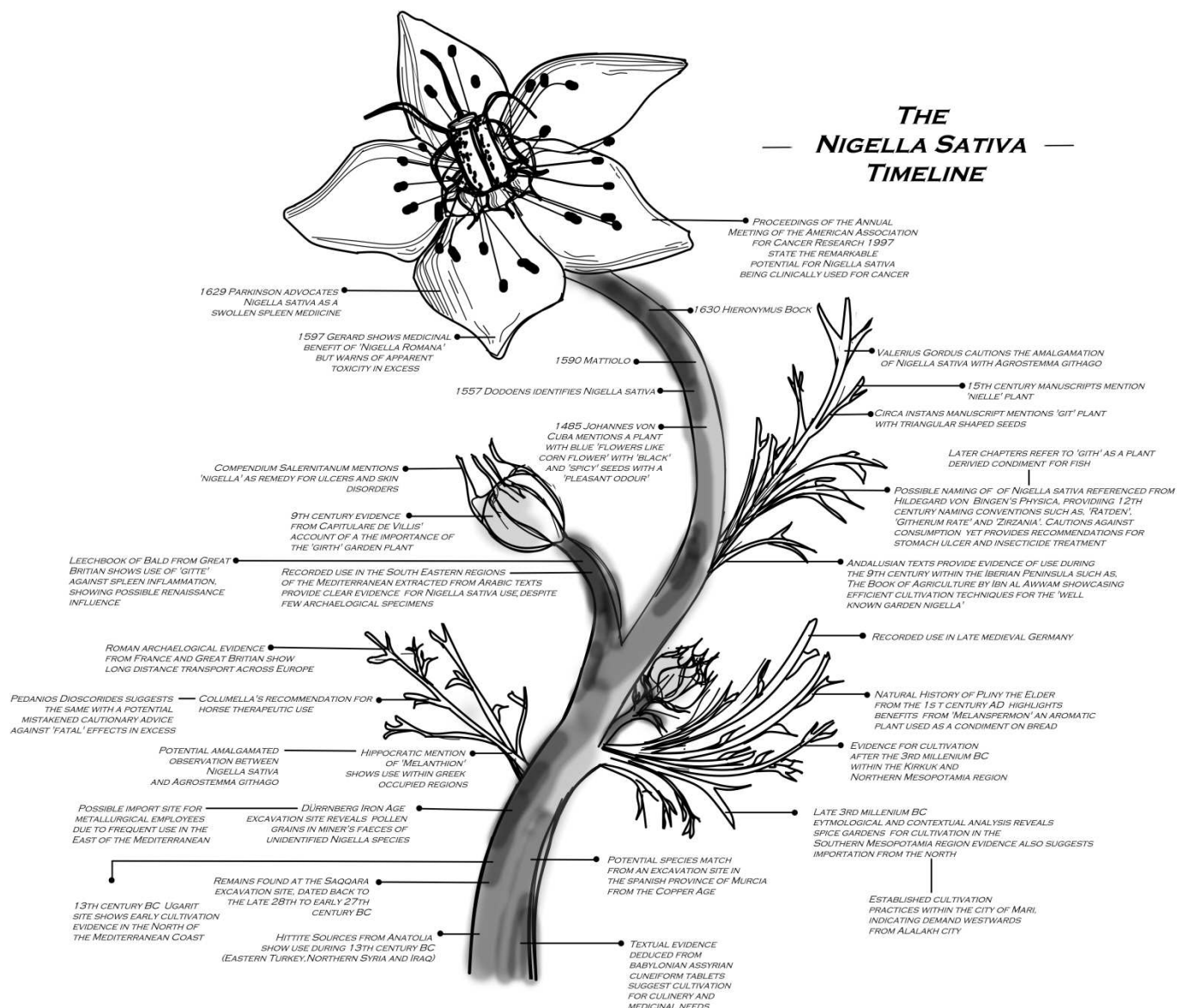


Figure 1: A *Nigella sativa* historical timeline composed from available etymological, archaeological, textual, and illustrative primary resources.

Some key dated points may be subject to interpretation, as they may be supported by illustrative evidence that another may find to be confounding (if identified with another *Nigella* species) for example, '1454 Johannes von Cuba' entry which has been scrutinised for in text contradictory descriptions and illustrative figures (192). See 'Supplementary Material' for further archaeological and textual findings (Source: 192). Abbreviations: CE = Common Era, BCE = Before Common Era (Gregorian calendar system) (Adapted from: 109; 216; 263; 313).

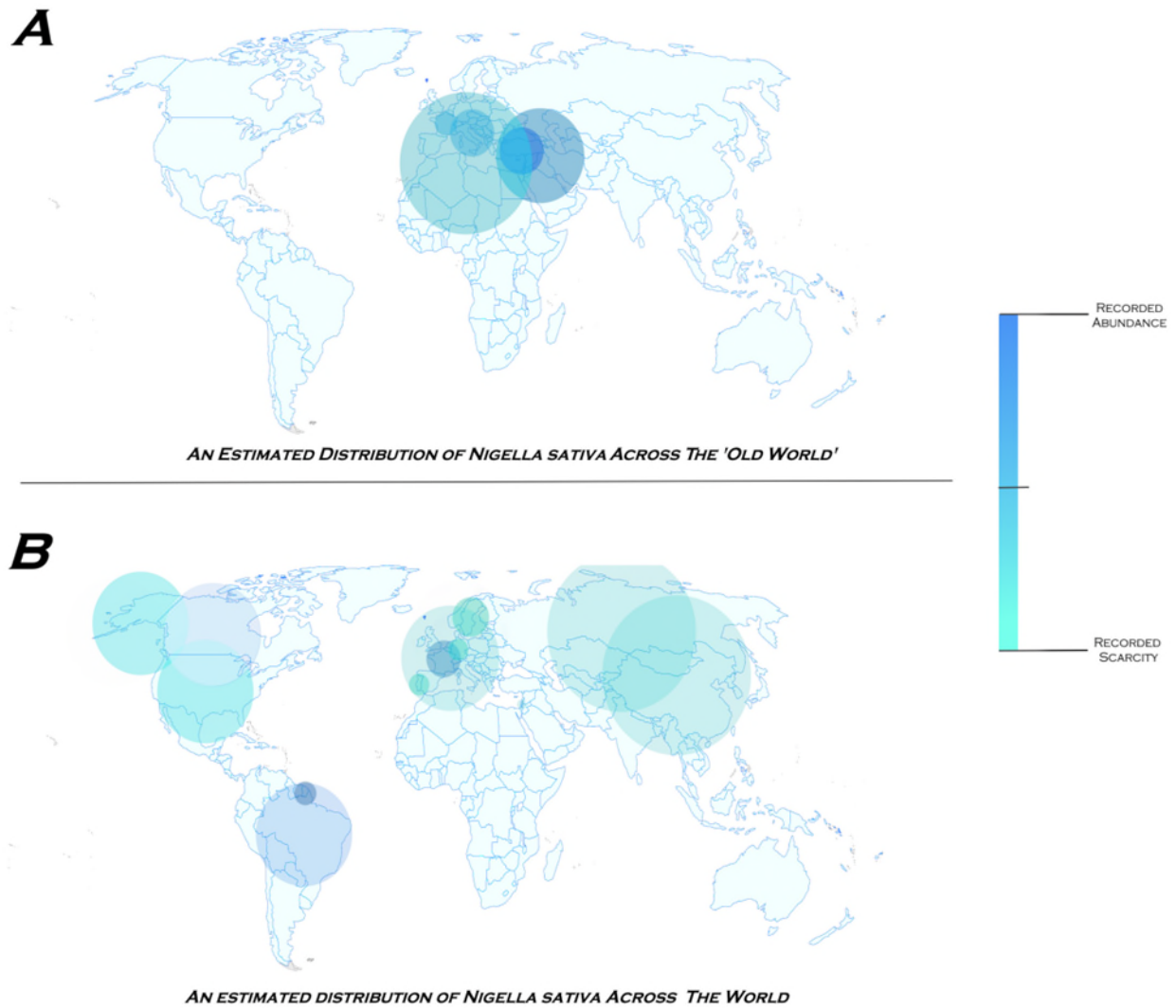


Figure 2: Two heat-maps highlighting the quantitative difference in distribution patterns of *Nigella sativa* at significantly different time-points, across the globe.

Shading and positioning was based upon known recorded evidence of geo-referenced specimen data, archaeological findings, etymological findings and through inference of textual and illustrative manuscript content. Section A (A) highlights the 'Old World' heat mapped distribution of *Nigella sativa* from past records (Source: 192). Section B (B) highlights present global distribution of *Nigella sativa* in which the data suggests a novel introduction of species within 14 different countries to date (Source: 160). Darker shades of blue indicate a greater concentration of recorded location presence, whereas lighter shades indicate scarcity. The map layout used is of a modified Robinson projection (Source: 393) See 'Supplementary Material' for the dataset used for contemporary measurements.

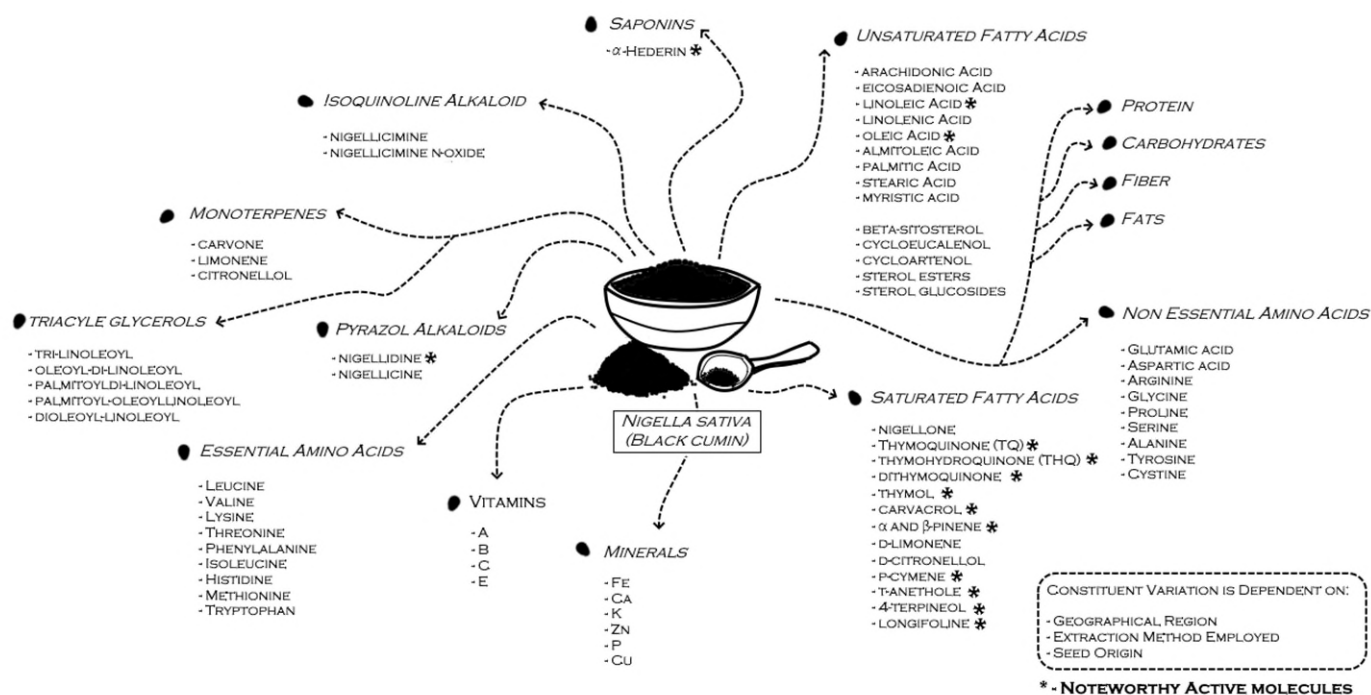


Figure 3: A summary of bio-constituents typically found in *Nigella sativa*.

NS contains fixed oils; terpenes; alkaloids; coumarins; saponins; flavonoids; phenolics; amino-acids; metals; trace-elements, among other important bio-constituents (11). (Source: 147; 358; 318). (Adapted from: 390)

Table 1: The Table of Names. A *Nigella sativa* classifier across various languages.

Language	Name (in Original Script)	Name (Transliteration)
Arabic	حبة السوداء حبة البركة	Habbat Al-Barakah/ Habbat As-Sauda
English	Black Cumin/ Black Caraway	
Urdu	کلوئی	Kalonji
Hindi	कलोजेरा	Kalojera
Tibetan	ཇི་ར་ནག་པོ།	Zi Ra Nag Po
Mandarin	黑種草	Hei Zheng Cao Zi
Greek	Μελάνθιον	Melanthion
French	Cumin noir	
Russian	Чанушка	Chanushka
Persian	زیره کوهی	Zireh kuhi
Burmese	ဆမ္မုန်-နက်	Samon-net

Transliteration data is in a Unicode Common Locale Data Repository (CLDR) Transliterated derivative form. (Adapted from: 230 and 279). To see additional names see 320 (para. 1.2).



Figure 4: A Photographic Overview of *Nigella sativa* and Taxonomic Classification Profile (Source: 305).

A, a field view image of *Nigella sativa* grown in a continental climate (Adapted from: 117). B, a close-up image of a recently bloomed *Nigella sativa* flower (Adapted from: 43). C, a close-up image of the stem structure and formation of leaves of *Nigella sativa*, in its vegetative state (Adapted from: 304). D, a close-up image of a mature seed capsule near the harvest end-stage of *Nigella sativa* (Adapted from 407). E, an image of a small pile of *Nigella sativa* seeds along with a standard measuring ruler with a centimetre (cm) scale showing an approximate seed width of 1 millimetre (mm) (Adapted from: 447). F, a whole seed image observed through light-microscopy, with a scale bar - a quarter ($\frac{1}{4}$) is 1 mm (Adapted from: 44).

1.7 The Inherited Dioscorides Dilemma

One of the first instances towards describing the 'toxic' nature of NS is within Dioscorides' book '*De Materia Medica*' where he writes on '*Melanthion*', which he provides cautionary advice against its excess-consumption, for potential induction of lethal-effects; however, this could be considered foible; a possible product of misidentification, as prior botanical classification-techniques would only considered isolated plant-characteristics as the sole identification parameters, without considering the variability of taxonomically relevant features such as plant-anatomy and flower-morphology.

This ostensible grouping allowed for toxic-species such as *Agrostemma githago*, *Nigella arvensis* and *Nigella damascena* to be homogenised into one, with NS not being exempted - masking its correct therapeutic-ascription (192).

These assumptions can still be seen in the present-day. In Errachidia (Morocco) – an area known for extensive use of herbal-remedies - observations from Tahraoui *et al.* (2006) showed NS, used for arterial-hypertension and diabetes-mellitus treatment, administered in low-doses to circumvent the assumed toxic-effects in excess; Despite, no clinical-reports of toxicity from higher-doses or long-term use, within the province. In addition to the absence of prescription regulations.

1.8 A Contemporary Toxicological Analysis

The safety-profile of NS should be derived from rigorous-testing to ensure its bio-constituents/metabolites do not generate a cascade of harmful reactions after dispensation - hindering any clinical-incorporation prospects.

NS oil-formulations have been reported to be safe at doses of 40mg/kg in both human-adult and child-models. However, caution should be taken with concentrations at/above 80mg/kg for children due to the risk of initiating temporary gastro-intestinal discomfort (218); pregnant-women should take precautions with NS, due to its reported a hypoglycaemic-effects causing possible teratogenic-repercussions (86).

A study with 27 post-menopausal women, with osteopenia, showed no observable harmful side-effects from the daily-intake of 0.05mL/kg of NS oil over a duration of three months (435). However, in an acute toxicity-test with mice, of intraperitoneally administered NS ethanol-extract showed signs of toxicity after 4-6 hours of exposure, inducing tachypnoea; the final median lethal-dose was observed at 471.2mg/kg, suggesting moderate-toxicity.

On the other hand, an oral acute toxicity-test revealed properties of low-toxicity and wide safety-margins for dosage, with toxic-effects only observed at 10000mg/kg of n-hexane NS seed-oil (427); a suitable human equivalent dose can also be extrapolated from the *in-vivo* model by Zaoui *et al.* (2002). Estimates with the body-normalisation method showed 0.41mL/kg orally/0.02mL/kg intraperitoneally as a human-adult dosage with low-risk of adverse side-effects (351).

1.9 The Multifaceted Minute

Ibn Sina (Avicenna) in his monumental work '*Al-Qanun fi'l-tibb*', (The Canon of Medicine) relays the magnitude of NS's curative utilities such as, general psycho-stimulation, relief of nausea and regulation of disposition (394)

The multifarious-potential of the pungent NS seed is not limited to a plethora of ailments (figure 5; table 3) but can be expanded towards the biocidal-field as a pollution-free insecticide (table 2) and a preservative for stored-food (62). NS as a cosmetic has been a well-established practice with reports of monarchs such as Cleopatra and Nefertiti utilising formulations to maintain hair/nail quality (204). Cosmeceutical-applications of NS are still relevant in present global cosmetic-markets including augmentation, aromatherapy, dental-care, photo-aging and sun-protection (28) (29) (133) (144) (145) (266) (150)(161) (324).

Additionally, by directly modulating key-hormone and pro-inflammatory (interleukin-1 β -6) markers and restoring leptin-sensitivity, within obese-adults, NS has the ability to encourage metabolic syndrome/insulin resistance reversal, weight-management, and reductions in body-mass-index (BMI) (349); NS also has the potential to be used within ontological-treatments for acoustic-trauma induced hearing-loss. Quantitative-assessments of *in-vivo* rat models showed thymoquinone's ameliorating-effect upon auditory-brainstem-responses (ABR) (113) (19)

Interestingly, an *in-vitro* study with *Labeo rohita* revealed NS oil's growth promoting character once added with aquatic-feed (248). The statistical increase in growth performance and muscle-protein content observed agrees with Bektaş *et al.* (2019), as treated *Oncorhynchus mykiss* exhibited an increase in the protein-efficiency ratio (PER) indicating clear nutritional value from NS's abundant source of essential amino-acids (figure 3) (137).

Giving rise to the possible stimulation of muscle protein-synthesis and facilitation of heightened muscle turnover-frequency, allowing NS to modulate recovery-times for onset muscle-soreness and exercise induced injury.

1.10 The Current Research Attitude and Environment

NS was first mentioned within scientific research-literature more than one-hundred years ago (172). However significant attention towards NS has only been seen, within contemporary scientific research-literature, in the last two-decades (figure 6D). There is a general upwards-trend towards content being produced that is related to NS or its bio-constituents and their potential therapeutic roles. The literature available originates from a number of countries around the world, with key-contributors from India, Saudi Arabia, and Egypt (figure 6A). Review and research articles each assume an approximate third of the literature established, with book chapters securing the third position of prevalence (figure 6C). NS is a versatile plant which has been incorporated into many sectors from dentistry, veterinary-sciences, environmental-science, chemistry, medicine and pharmacology, the latter being the most popular field to-date. (figure 6B).

Table 2: An overview of noteworthy studies highlighting *Nigella sativa*'s non-polluting biocidal effect.

<i>Leaf miner (Tuta absoluta)</i>	<ul style="list-style-type: none"> ❖ <i>Nigella sativa</i> oil exhibited strong <i>in-vitro</i> repellent activity and insecticidal character against larvae. ❖ In a dose and time dependent manner concentrations of 0.039, 0.079 and 0.157 $\mu\text{l}/\text{cm}^2$ caused 71.25 82.5 and 91.25% repulsion, respectively. ❖ Concentrations of 0.236 $\mu\text{l}/\text{cm}^2$ caused total mortality (100%) ❖ The reported LD₅₀ value of 0.105 $\mu\text{l}/\text{cm}^2$, from a four-hour exposure period, highlights its strong pesticide effect 	(8)
<i>Malaria Mosquito (Anopheles gambiae)</i>	<ul style="list-style-type: none"> ❖ <i>Nigella sativa</i> blends 2 and 6 exerted a concentration-dependent repellent effect, with a significant increase in efficacy in the absence of p-cymene, (-)-α-pinene constituents. ❖ Blend 2 and 6 at 0.1g/ml caused 100% repellence and concentrations causing 50% decrease in respiratory rate (RD₅₀) were 2.84g/ml and 3.29g/ml, respectively. ❖ Constituents (+)-β-pinene(-)-β-pinene, (+)-α-pinene, tetradecane, or 1,2,3,4,5-pentamethylcyclopentane removed from blends 3, 4, 5, 8 and 9 respectively suggest a significant pesticidal contribution due to the subsequent reduction in formation efficacy, in its absence. ❖ The isolated molecule (+)-α-pinene is the most active player in <i>Nigella sativa</i> due to the recorded 99.38% repellence at 0.1g/ml 	(309)
<i>Yellow-Fever Mosquito (Aedes aegyptii)</i>	<ul style="list-style-type: none"> ❖ Results from dual choice bioassay observations showed 64% of females significantly avoided areas concentrated with <i>Nigella sativa</i> crude extract within a duration of 3.2minutes at 5000 parts per million (ppm) ❖ Avoidance behaviour in windy conditions was observed at 1000 and 5000 ppm, however, only 5000ppm induced avoidance in no wind conditions. ❖ Thymoquinone, styrol, p-cymene were suggested to be the most effect constituents modulating behaviour 	(446)
<i>Headlice (Pediculus humanus capitis)</i>	<ul style="list-style-type: none"> ❖ Assessment of <i>Nigella sativa</i>'s anti-pediculicidal activity with the filter paper diffusion method showed 100% mortality after 1 hour of exposure. ❖ Results confirmed the proposition of <i>Nigella sativa</i> being a viable alternative to solve resistance to neurotoxic synthetic pesticides and providing potent anti-lice activity with no adverse side effect from topical use. ❖ Thymoquinone was responsible for majority of the repellent action and could be utilised in combination with established anti-lice products. 	(35)
<i>Red Flour Beetles (Tribolium castaneum)</i>	<ul style="list-style-type: none"> ❖ <i>Nigella sativa</i> oil produced median lethal concentrations (LC₅₀) at 3594, 629, 335 mg/L air (adult stage) and 1040, 416, 227 mg/L air (larval stage) for fumigation exposure periods lasting 3, 5, 7 days, respectively. ❖ Minimal histological changes of mammalian organs were observed at 3 days exposure to fumigant suggesting safe consumption to fumigated produce 	(392)
<i>Lone-star tick (Amblyomma americanum (L.))</i>	<ul style="list-style-type: none"> ❖ All four species were repelled by <i>Nigella sativa</i> extracts. ❖ <i>I. scapularis</i> was 100% repelled by extract 1 and 4 at a concentration of 0.206 mg extract/cm² filter paper. ❖ <i>D.variabilis</i> was repelled at 100%, 90% with a concentration of 0.827 mg extract/cm² using extract 1 and 4, respectively. 	(97)
<i>American dog tick (Dermacentor variabilis)</i>	<ul style="list-style-type: none"> ❖ More than 87% of nymphs from <i>A. americanum</i> and <i>R. sanguineus</i> were repelled with a concentration of 0.413 mg extract/cm² filter paper of extract 1 and 4. 	
<i>Deer tick (Ixodes scapularis)</i>	<ul style="list-style-type: none"> ❖ Repellent longevity was observed against <i>A. americanum</i> was observed at 66.7% at 192 hours 	
<i>Brown dog tick (Rhipicephalus sanguineus)</i>		

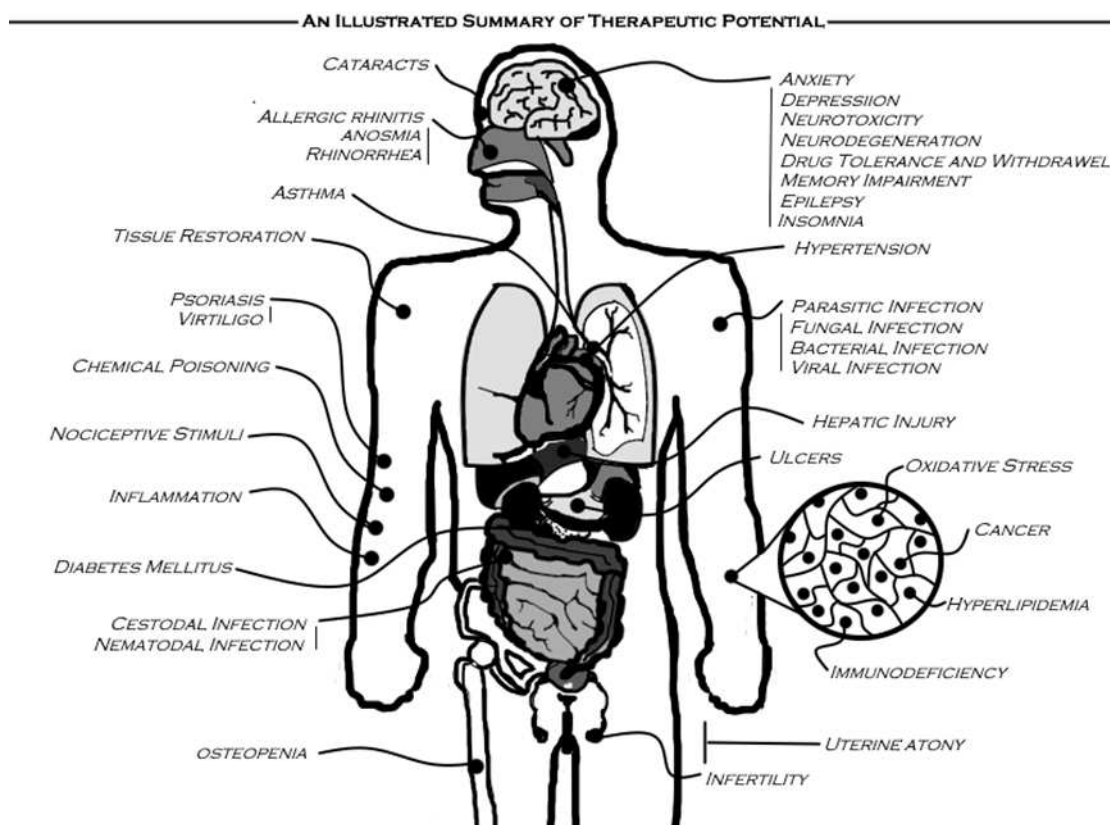


Figure 5: An anatomical diagram highlighting potential therapeutic targets of *Nigella sativa* (Adapted from: 197).

Nigella sativa been reported to exhibit anti-inflammatory, hypotensive, hepatoprotective, anti-oxidant, anti-cancer, hypoglycaemic, aphrodisiac anti-tussive, uricosuric, choleric, anti-fertility, galactogen, prophylaxis and spasmolytic properties, antihypertensive, antidiabetic, anticancer, gastroprotective, antioxidant, anti-inflammatory, anti-histamine, anti-microbial, anti-tumour, anti-hepatotoxic, anti-nephrotoxic, respiratory, immunomodulatory effects, anthropometric indices, anti-dyslipidaemia, anthelmintic, anti-nociceptive, oral ulcerations and mucositis healing, nephroprotective, anti-aging, pro-stamina, hair-growth, pro-vitality, anti-fatigue, anti-convulsant, bone-marrow immune modulating (myelopoiesis), appetite stimulating, liver injury modulating, anti-odorant, digestive modulating, diuretic, renal calculi modulating, flatulence mediatory, anti-polio, diarrhoea, dropsy, amenorrhoea and dysmenorrhoea effects - among a magnitude of others. For a comprehensive list see 'table 1' (204 (p27)).

Table 3: A Comprehensive List of Key *Nigella Sativa* (and Constituents) Induced Observational-Data Across Different Maladies

<i>Anxiety</i>	Oil	❖ ❖ ❖ ❖	Periodical increase of rodent open-field activity (Anxiolytic outcome) Increased attainment efficiency in elevated plus maze test (from increased locomotion) Increase in brain plasma tryptophan levels and 5-HT (serotonin') Reduction in 5-HIAA serotonin metabolite	(329)
<i>Depression</i>	Extract	❖ ❖ ❖ ❖	Overall decrease in LPS-induced depressive behaviour. Decrease in immobility period ('habitualisation' efficiency) Decrease in open-field peripheral crossing number. And an increase in open field central crossing number (open-space aversion mitigation)	(198)
<i>Neurotoxicity</i>	Extract	❖	Reduction in SGD-initiated cytotoxicity through possible anti-oxidant pathways reducing heightened ROS production	(294)
<i>Neurodegeneration</i>	Oil	❖ ❖ ❖	Neural morphology of injured rodent sciatic nerve conserved. Reduction in neuronal darkening and picnotic nuclei (irreversible cell-lysis development) Overall absence of distorted nerve cells	(212)
<i>Drug Dependency and Associated Tolerance</i>	Oil	❖ ❖ ❖	Successful halting of tolerance and withdrawal symptoms through the maintenance of heat pad analysis latency and the suppressive character of naloxone through: Inhibition of nitric oxide excess and malondialdehyde concentration from repeated tramadol use Inhibition of decreased intracellular reduced glutathione concentration and glutathione peroxidase activity	(3)
<i>Memory Impairment</i>	Oil	❖ ❖ ❖	Increase step through latency. Significant alternation behaviour (difference between treated and untreated diabetic rat model groups) Signifies an increase in spatial and established memory recall performance	(211)
<i>Epilepsy</i>	Oil	❖ ❖	Reduction in oxidative stress within 'pilocarpinised' rat's hippocampus Modulation of nitric oxide, glutathione concentration, and catalase activity (back to control conditions)	(5)
<i>Asthma</i>	Extract	❖ ❖ ❖	Observable increase within all pulmonary functional assessments 30-minute broncho-dilatory onset duration Potent anti-asthmatic effect duration lasted for 105 minutes	(82)
<i>Allergic rhinitis</i>	Oil	❖ ❖ ❖	Significant reduction in nasal scratching frequency 85.7% of group members had no inflammatory changes. Eosinophil invasion, cilia reduction, chondrocyte hypertrophy, angiogenesis, and goblet cell increase were all significantly reduced	(175)
<i>Acne vulgaris</i>	Hydrogel	❖ ❖	78% mean reduction in The Investigators Global Assessment Score, resembling significant clearing of pre-formed comedones, papules, pustules after two months of exposure. 63.49% decrease within the acne disability index questionnaire	(396; 397)
<i>Cataracts (Glucose-induced)</i>	Extract	❖ ❖ ❖ ❖ ❖ ❖	Photographic evaluation of goat eye lenses revealed the slowed progression of lens opacification. Biochemical analysis revealed an increase in Na ⁺ /K ⁺ ATPase activity and total proteins (including water soluble) Restoration of K ⁺ concentration to standard conditions Reduction in Na ⁺ ion concentration and MDA Increase in reduced glutathione (GSH)	(15)
<i>Vitiligo</i>	Oil	❖ ❖	A recorded decrease mean score with the VASI system from 4.98 to 3.75. Majority of the improvements were found upon the trunk, head, and neck	(165)

Table 3: Continued

<i>Psoriasis</i>	Oil and capsule	❖	Combination therapy was responsible for completely curing lesions, with 'excellent' graded responses making up 85% of the tested cases within the cohort	(13)
<i>Wound (Burn)</i>	Cream	❖	After histopathological inspection of burnt rat tissue samples; burnt tissue healing was significantly better than that seen within the control group, as indicated by a reduction of several initial inflammatory signs such as, heat, redness, and swelling.	(452)
		❖	Statistical analysis showed significant shortening of the healing process	
<i>Parasitic Infection (Plasmodium yoelii)</i>	Extract	❖	Both oral and intraperitoneal administration methods suppressed parasitic activity independent of extract type.	(4)
		❖	Statistically significant results further highlight the ability to reduce parasitaemia and increase an infected hosts survival-time	
<i>Fungal Infection (Bipolaris sorokiniana)</i>	Defensins	❖	Isolated defensins (Ns-D1 and Ns-D2) exerted potent anti-fungal effect on hyphae which caused 92% inhibition, with the D2 derivative having a higher potency.	(360)
		❖	Both defensins required low concentration amounts to cause maximal observable effect (minimum inhibitory concentration)	
		❖	Spore destruction was present on germinated conidia.	
		❖	Morphological changes on conidia by defensin disruption of cell-wall and membrane structures	
<i>Bacterial Infection (Streptococcus mutans)</i>	Extract	❖	Through paper disc diffusion study an observable inhibition zone was induced with a diameter of 20.3 ± 0.35 for the 800mg/mL concentration	(406)
		❖	The minimum inhibition concentration assay was 380mg/mL	
<i>Viral Infection (SARS-CoV-2)</i>	Oil	❖	TSB was reduced at a considerably faster rate from the recorder 14 th day mark.	(241)
		❖	Lowered the rate of hospitalisation.	
		❖	Significant effect upon anosmia and rhinorrhoea resolution	
<i>Oxidative stress</i>	TOH, TQ and DTQ	❖	Chemiluminescence and spectrophotometer analysis showed key constituents exhibiting scavenging activity upon several ROS such as superoxide anion radical, hydroxyl radical and singlet oxygen	(244)
<i>Hepatic injury</i>	Oil	❖	Ischemia and reperfusion injured hepatic tissue analysis obtained from the treated group highlighted significant reductions in liver enzymes, TOS, OSI and MPO.	(455)
		❖	TAC was significantly higher within the treated group compared to those without	
		❖	Pathological changes were significantly reduced, and histological tissue damage was less pronounced for the treated cohort	
<i>Cancer</i>	Extract	❖	Within <i>in-vivo</i> mouse models the methanolic extract displayed selective toxicity for Elrich ascites carcinoma, Dallon's ascites lymphoma and Scarcoma-180 allowing for ascite tumour inhibition whilst preserving normal lymphocytes.	(371)
		❖	Observable cellular modulation of thymidine incorporation	
		❖	High potency with similar concentration amounts exerting 50% inhibitory effect across the <i>in-vivo</i> study.	
		❖	Within <i>in-vitro</i> cell culture analysis oral carcinoma cells underwent growth inhibition whereas myelogenous leukaemia cell-growth was temporarily halted.	
<i>Diabetes Mellitus</i>	Oil	❖	The seed oil within the alloxan induced diabetic rabbit study induced a significant hypoglycaemic effect in a time dependent manner.	(22)
		❖	No observable changes to basal insulin levels were found suggesting a possible non-insulin mediated hypoglycaemic mechanism being responsible for the results	

Table 3: Continued

<i>Cestodal Infection</i> (<i>Schistosoma mansoni</i>)	Oil	<ul style="list-style-type: none"> ❖ A two-week administration of oil within an infected mice-model causes significant parasitological changes. ❖ Observed reduction in worm-distribution within the liver, changes within oogram patterns (19-20% mature ova reduction, slight immature ova modulation and increased number of dead ova in the intestinal wall), ova count reduction (in both liver and intestines) and a considerable reduction in liver granuloma diameter 	(267)
<i>Nematodal Infection</i> (<i>Aspicularis tetraptera</i>)	Oil	<ul style="list-style-type: none"> ❖ An oral treatment duration of two days caused reduction in the respective nematode and its eggs as observed under stereomicroscopy. ❖ The reduction observed was significantly different compared to the untreated cohort 	(56)
<i>Inflammation</i>	Oil	<ul style="list-style-type: none"> ❖ Within 3 hours of induced inflammation, through carrageenan sodium salt induced paw edema, a significant reduction in mean paw-volume increase was observed. ❖ The reductive effect was comparable to conventional anti-inflammatory medication like aspirin. 	(21)
<i>Nociceptive Stimuli</i>	Oil	<ul style="list-style-type: none"> ❖ Three examination techniques were carried out on rat and mice-models in which observations included changes in hot-plate latency, tail pinch-test tolerance, and acetic acid induced withering. ❖ Results were speculated to be a product of the opioid principle by which was supported through naloxone antagonisation. 	(470)
<i>Ulcers</i>	Extract	<ul style="list-style-type: none"> ❖ Significant reduction in ulcer index score within rat-model of aspiring induced gastric-ulcers ❖ Significant reduction in gastric juice total-volume and overall acidity of gastric secretion 	(346)
<i>Hypertension</i>	Extract	<ul style="list-style-type: none"> ❖ Observed diuretic and hypotensive influence. ❖ Increased diuresis outcome by 17% over the duration of 15 days ❖ Observed chlorine, sodium, and potassium ion content increase in urine excretion. ❖ Observed reduction in mean arterial pressure of 22% outperforming nifedipine - a conventional hypertensive therapeutic 	(462)
<i>Hyperlipidaemia</i>	Powder	<ul style="list-style-type: none"> ❖ The two-month human study saw significant plasma lipid alterations. ❖ Administration of powder after a fasted state, consistently, for the experimental duration allowed for a decrease in total plasma cholesterol, triglycerides, and LDL-cholesterol levels, significantly. ❖ An increase in HDL-cholesterol levels was observed 	(77)
<i>Chemical Poisoning</i>	Extract	<ul style="list-style-type: none"> ❖ The compound across a two-month duration alleviated all apparent respiratory symptoms, chest wheezing along with facilitated PFT improvement across its various types. This was especially seen from second and third visitation records, once compared to the initial interaction showing a 50% reduction and 10% increase, respectively. ❖ Additional therapeutic dependency in the form of an inhaler, oral β-agonists and oral corticosteroids was reduced 	(81)
<i>Immunodeficiency</i>	Extract	<ul style="list-style-type: none"> ❖ T-lymphocyte activation was initiated. ❖ Induced macrophage effect by secretion of inter-leukin species (IL-3 and IL-1β) ❖ CD4-positive T-cell modulatory effect for efficient and adaptive conditioning ❖ Suppressed leukocyte chemiluminescence activity 	(188)
<i>Uterine atony</i>	Oil	<ul style="list-style-type: none"> ❖ Inhibition of spontaneous movements found within isolated uterine horns and oxytocin induced smooth-muscle contraction within the rat and guinea pig model. ❖ Effects observed were reversible and concentration dependent 	(46)
<i>Infertility</i>	Oil	<ul style="list-style-type: none"> ❖ After a two-month duration of consistent administration, significant improvement was seen among the treated cohort of infertile men with abnormal sperm morphology, which included: <ul style="list-style-type: none"> ❖ Count ❖ Motility ❖ Volume ❖ Morphological character ❖ pH ❖ Round-cell conditions 	(239)

Abbreviations: 5-HT = 5-hydroxytryptamine, 5-HIAA = 5-hydroxyindole acetic acid, LPS = lipopolysaccharide, SGD = serum/glucose deprivation, ROS = Reactive oxygen species, % = percentage, Na = Sodium, K = Potassium, ATPase = Adenosine triphosphatase, MDA = Malondialdehyde, GSH = Reduced glutathione, VASI = Vitiligo area scoring index, Ns-D = Nigella sativa derived defensin, TSB = Total symptom burden, TOH = Thymol , TQ = Thymo-quinone, DTQ = Dithym-oquinone, TOS = Total oxidative status, OSI = Oxidative stress index, MPO = Myeloperoxidase, TAC = Total antioxidant capacity, LDL = Low-density lipoprotein, HDL = High-density lipoprotein, PFT = Pulmonary function test, CD4 = Cluster of differentiation 4, pH = Hydrogen activity logarithmic scale

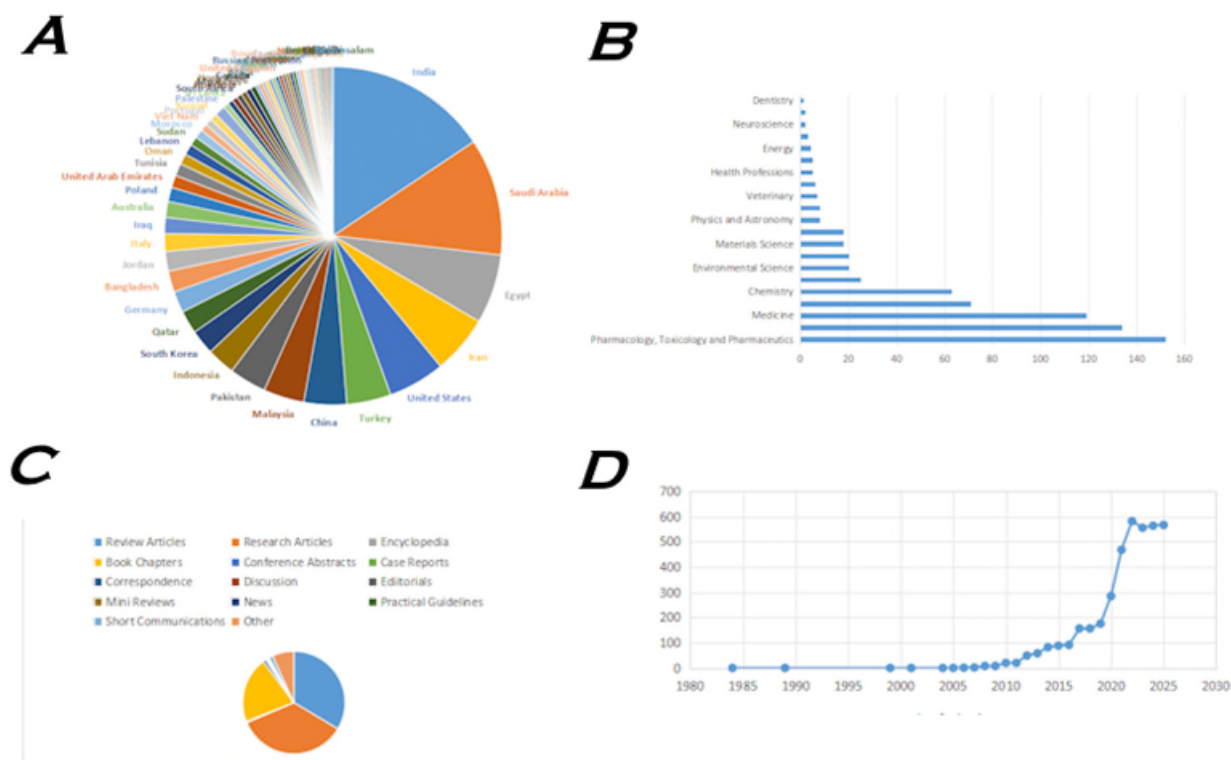


Figure 6: A contemporary summary of available literature regarding *Nigella sativa* and its derivatives.

Graphical representations are derived from a simple search query ‘*Nigella sativa* Anticancer’ across 3 databases, including: PubMed (D), ScienceDirect (C) and Scopus (A and B). Data was accessed during the year 2025. See 'Supplementary Material' for exact statistical values.

2. Materials and Methods

This 'systematised-review' was configured upon the updated guidelines for the preferred reporting items for systematic-reviews and meta-analyses (PRISMA) statement (323).

2.1 Search Strategy

We searched three electronic-databases which included PubMed, ScienceDirect and Scopus using query-terms suitable for each respective database within the 'titles and abstracts' category. Search-terms included Medical Subject Headings (MeSH) to ensure a comprehensive literature scope-search. Three variations of key-terms were utilised to adapt to Boolean and word-count constraints within each respective website, they are based on the following:

('Thymoquinone' OR 'alpha-hederin' OR 'α-Hederin' OR 'α-thujen' OR 'oloeresin' OR 'nigellidine' OR 'nigellimine' OR 'p-cymene' OR 'r-cymene' OR 'thymohydroquinone' OR 'carvacrol' OR 'linoleic acid' OR 'oleic acid' OR 'eicosadienoic acid' OR 'Herbal-melanin' OR 'Nigella sativa' OR 'TQ' OR 'NS' OR 'Black cumin' OR 'black pearl' OR 'kalonji' OR 'Kalojira' OR 'Hasb as sauda' OR 'zi ra nag po' OR 'Hei zhong cao zi' OR 'Melanthion' OR 'Habbat-ul-barakah' OR 'Black caraway' OR 'Black seed' OR 'Nigella sativa' [MeSH] OR 'Nigella sativa oil'[Supplementary Concept]) AND ('Cancer cell line' AND ('MTT' OR '3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide' [Supplementary Concept]))).

The search was not limited to any language or particular time period. One reviewer screened and evaluated the eligible papers against a previously established inclusion and exclusion protocol.

2.2 Inclusion and Exclusion Criteria

Eligible literatures were included if they met the inclusion-criteria which consisted of 1. studies primarily focussing on *in-vitro* cancer cell-line studies, with no cancer-type restriction. 2. Studies involving NS oil/extract formulations alone. 3. Studies utilising a control-parameter to assess formulation-effect on treated-cells. 4. Studies measuring outcomes of anti-cancer effect with an MTT assay.

The following exclusion criteria were also implemented: 1. Studies with no reported use of a control-group 2. Studies using NS in combination with another therapeutic partner-molecule, conventional-therapy, or nano-formulation. 3. Studies not considered primary-research articles. 4. Studies without a clear MTT-assay result. 5. Studies without full-text availability for data-extraction. 6. *in-vivo* studies, due to present quality-standard.

2.3 Data Extraction

Data was iteratively extracted from eligible papers through an established protocol formed by one reviewer. The extraction-parameters specified were author(s) name, cell-line type, administered cell-line number, well-culture plate number, formulation-type administered, treated culture half maximal-inhibitory concentration (IC₅₀) value, untreated culture IC₅₀ value,

treated MTT-assay mean percentage, untreated MTT-assay mean percentage, experiment control-type utilised.

In order to correctly carry out a single means meta-analysis for appropriate effect-size calculations, estimated standard-deviations were derived from the range of MTT-assay values reported from the *in-vitro* study, for both conventionally-treated/untreated/normal and treated cell-cultures (377; 378). If there were multiple cell-viability percentages reported for a given cell-type, due to the administration of different concentrations, a weighted percentage mean was calculated to avoid duplication and redundancy, ensuring a precise and accurate final-estimate. If multiple cancer cell-line types were evaluated the type exhibiting the most significant change were included within the meta-analysis to allow for a comprehensive estimate of anti-proliferative potential. If multiple formulations of seed-types were used only one was included within the meta-analysis to avoid duplication. Lastly, if a paper did not adequately rely on quantitative cytotoxic assay-results, for each cancer cell line type or formulation concentration, an estimate was deduced from the graphical figure display or IC₅₀ value.

In order to extract the necessary data sets authors of the research-articles were contacted by email. When no response was received the research articles were disregarded from the meta-analysis and the 'systematised-review' composition (201).

One investigator examined the quality of each included paper using a modified Newcastle-Ottawa scale (table 5).

2.4 Statistical Analysis

Data was reported as weighted mean-difference (WMDs) and standard-deviations (SDs) as a measure of spread to highlight the effect scale of NS on cell-outcomes. Effects on overall cell-viability were considered primary outcomes, and those on IC₅₀ were regarded as secondary outcomes to compare the potency of NS.

To pool the WMDs, a random effect-model was used as studies used different formulation-types and concentrations as well as the difference in effect-sizes observed within each different cancer cell-line type, in addition to the fixed-model assumption not being met (126). Heterogeneity in the analysis was observed using the I-squared (I²) index. I² values greater than 50% were deemed to suggest the presence of high heterogeneity (444). Existence of publication-bias was assessed through Egger's regression model, trim-and-fill analysis along with three fail safe N-tests: Rosenthal's, Rosenberg's, and Orwin's. All data analytical procedures were performed using R-studio version 4.3.1 (RStudio, 2023).

3. Results

3.1 Study Characteristics

Among the initial 1,012 studies that were identified from the search query (70 duplicates; 266 Secondary literature-articles) 589 were removed during the title screening-process. After further evaluation of the abstract text of the remaining 87 papers, 73 studies were further removed for the following reasons: the study utilised NS in combination with another therapeutic type (n=9) and the study only utilised isolated bio-constituents of NS disregarding the synergistic potential of bio-constituents found in crude extracts or oil formulations (n=64) (figure 7).

The properties of the included studies are summarised in table 4.

In order to carry out a meta-analysis, 9 effect-sizes extracted from 9 *in-vitro* cell-line studies including cancer cell-line types: A-549 (human lung), MCF-7 (human breast), HepG2 (human liver), HCT (human colon), PC3 (human prostate), HeLa (human cervical) and MDA-MB231/435 (human breast). Each cancer cell-line type was analysed in a 96 well plate format, each consisting of 231-50000 cells across the respective studies.

We conducted a single means meta-analysis to evaluate the NS derived anti-cancer therapeutic potential upon a range of different cancerous cell-line types. Our analysis showed that a significant effect of cell-viability was induced ($g=44.864$, $p<0.0001$). The included forest-plot can be seen in figure 8.

We subsequently checked the model for outlier effect-sizes or effect-sizes that highlighted potential significant influence. We found two studies (34; 23/24) that were significantly influential (figure 10).

After looking further at this study, it was decided that the experimental design was not considerably different from the rest of the included study-set; thus, the effect-size was kept as is, from the original analysis.

We also found the presence of significant heterogeneity in the sample $Q(8)=4239.6185$, $p<0.0001$, $I^2=99.67\%$, $\tau^2=15.389$, therefore moderator analysis was conducted.

We examined if the therapeutic type administered upon the given cell-line studies had a significant reduction effect upon the cell-viability results after MTT-assay analysis (table 6). Our analysis indicated a significant effect $Q_m(1)=7.09$, $p=0.008$. Therefore, administering NS as an oil-based therapeutic may be a better formulation to exert a cytotoxic response. $\tau^2=134.0003$ ($SE=72.3597$).

3.2 Publication-Bias

We next checked our sample for publication-bias. After firstly visually reviewing the funnel-plot for asymmetry, which was subsequently observed (figure-9). Egger's regression test for funnel-plot asymmetry also indicated its presence ($b=6.7454$, $z=60872$, $p<0.001$). We next executed

a trim-and-fill analysis, which revealed that two studies could be inputted to the left side of the funnel-plot (figure 9).

Inputting these studies would only have a small effect of the overall meta-analysis effect size, with the effect changing to $g=40.5426$ with the inputted studies; a change of only $g=4.3214$. Lastly, we examined three fail-safe N-tests, Rosenthal's ($n=51479$), Orwin's ($n=9$), and Rosenberg's ($n=27203$). In summary the results of these analyses suggest that publication bias does not appear to be a significant concern in this study.

3.3 The Collation

The majority of the included studies within the systematic review were conducted in Asia: Saudi Arabia (n=3) (23/24) (26) (307), India (n=2) (60/61) (120/121) and Pakistan (n=1) (271/272). Two studies originated from Africa: Sudan (n=1) (179/180) and Egypt (n=1) (34) and from Europe: Turkey (n=1) (422/423); all studies were between 2014-2023.

Four studies included a breast-cancer cell-line type (179/180) (23/24) (120/121) (60/61). Specific breast-cancer cell-lines included MDA-MB-435 (179), MCF-7 (23) (121) (179) and MDA-MB-231 (61) (121). Three studies utilised a HepG2 liver-cancer cell-line (23) (307) (271). Two studies observed anti-proliferative effects on a A-549 lung-cancer cell-line (23) (26). Another two studies observed the cell-viability on a HeLa cervical-cancer cell-line (34) (307) (271). Two studies observed anti-proliferative effects on a HCT colon-cancer cell-line, with Banerjee *et al.*, 2017 specifically using the HCT116 culture (34). One study involved the use of a PANC pancreas-cancer cell-line (34)

Most of the NS derived formulations were extracted from raw local-market seed produce (n=5) (26) (23) (34) (121) (179) (423), however Banerjee, *et al.*, made no mention of the origin of the seeds utilised. Hago, *et al.*, also obtained seeds from a private-garden vendor, leaving a combination of commercially refined seeds and organic grown produce. One study derived their extract formulations from NS leaves of unknown origin (271).

The extraction techniques employed included: cold-pressing (n=2) (34) (121), Soxhlet (n=4) (23) (26) (61) (423) and ultrasonic-extraction (n=1) (179)

NS was administered in the form of unspecified oil-medium (n=1) (34), ethanol oil (n=1) (121), petroleum-ether oil (n=3) (23) (26) (271), unspecified alcoholic-extract (n=1) (26), ethanol-extract (n=4) (61) (179) (271) (307), water-extract (n=1) (271) and methanol-extract (n=1) (423). The concentration of formulations administered mostly lied within the range of 10-50 ug/ml, with 6 *in-vitro* test including concentrations beyond the upper-bound value 500ug/ml (34), 250ug/ml (23), 1mg/ml (26), 320ug/ml (61), 10000ug/ml (179) 200ug/ml (307), along with 4 including concentrations below the lower bound value, such as 0.24ug/ml (34), 1ug/ml (179), 2.5 ug/ml (307), 5ug/ml (121).

All of the studies explicitly mentioned NS's effect upon cell-viability was of a concentration-dependent manner, except for (179).

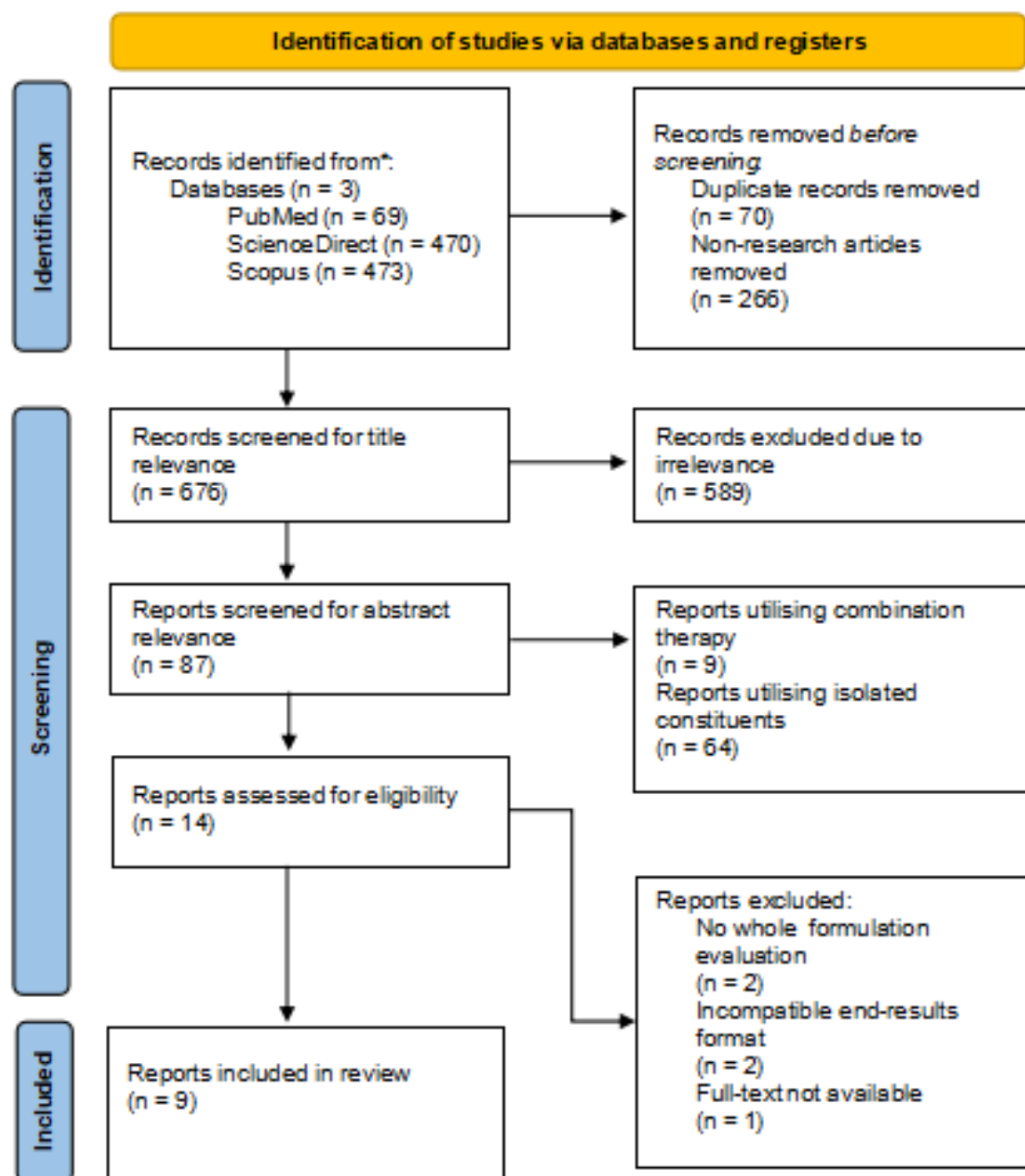


Figure 7: PRISMA flow-diagram of the literature search and study selection-process.

(Adapted from: 323)

Table 4: Summary tables of the nine included research articles within the 'systematised-review' and meta-analysis from extracted effect-sizes (n=9).

Study	Cancer Cell-Line Type	Administered Cell Line Number	Well-Culture Plate Number	Type of NS Product Administered	Therapy IC ₅₀ (µg/mL) 2 d.p.
Al-Sheddi <i>et al.</i> (26)	A-549 (H.Lung)	10000	96	Extract	500.00
Das <i>et al.</i> (121)	MCF-7 (H.Breast)	231	96	Oil	25.00
Al-Oqail <i>et al.</i> (23)	HepG2 (H.Liver)	10000	96	Oil	44.60
Ali <i>et al.</i> (34)	HCT (H.Colon)	NOT REPORTED	96*	Oil	4.24
Hago <i>et al.</i> (179)	MCF-7 (H.Breast)	NOT REPORTED	96	Extract	10.07
Nawaz <i>et al.</i> (307)	HepG2 (H.Liver)	NOT REPORTED	96	Extract	25.91
Toy <i>et al.</i> (423)	PC3 (H.Prostate)	6000	96	Extract	12.16
Maqbool <i>et al.</i> (271)	HeLa (H.Cervical)	NOT REPORTED	96	Extract	495.21
Banerjee <i>et al.</i> (61)	MDA-MB231 (H.Breast)	50000	96	Extract	125.80

Therapy MTT Assay Mean (%) 2 d.p.	Estimated Therapy MTT Assay Standard Deviation 2d.p.**	Control Type	Control IC50 (µg/mL) 2 d.p.	Control MTT Assay Mean (%) 2 d.p.
50.33	12.25	Untreated	0.00	95.00
49.00	10.50	Untreated	0.00	100.00
30.00	6.50	Normal (HEK293)	1136.00	94.33
12.00	3.88	Treated (Doxorubicin)	101.00	16.22
50.00	12.5	Untreated/Normal (HUVEC)	830.90	100.00
56.17	10.75	Untreated and Normal (BHK)	89.30	100.00
42.67	8.50	Untreated	0.00	100.00
50.00	13.75	Untreated	0.00	100.00
63.97	17.16	Untreated	0.00	100.00

Statistical reports on inter-differential NS potency across cancer-cells lines were observed by Al-Oqail *et al.* (2017) (HepG2 > MCF-7; A-549); Das *et al.* (2023) (MCF-7 > MDA-MB-231); Hago *et al.* (2023) (MCF-7 > MDA-MB-435); Maqbool *et al.* (2019) (HeLa > HepG2); Nawaz *et al.* 2022 (HepG2 > HeLa)*, Although not explicitly reported by Ali *et al.* (2023) the well-culture plate number was inferred from references of utilising protocols by Ghasemi *et al.* (2021) within their 'Material and Methods' section. **, To compensate for some literature not reporting standard-deviations within their data-set, the range was used as another measure of dispersion (Standard-deviation = range/4). Our decision was supported by our condensed data-set number that displayed minimal outliers across the cohort. However, researchers should take caution when deducing any conclusions, due to potential instability. (Source: 377/378). Abbreviations: HEK293=Human embryonic kidney 293, HUVEC=Human umbilical vein endothelial cells, BHK=Baby Hamster Kidney fibroblasts, H.=Human, d.p.=decimal-place. Future cell-line studies should address blood cancers; skin cancers; fibrosarcoma; renal cancers.

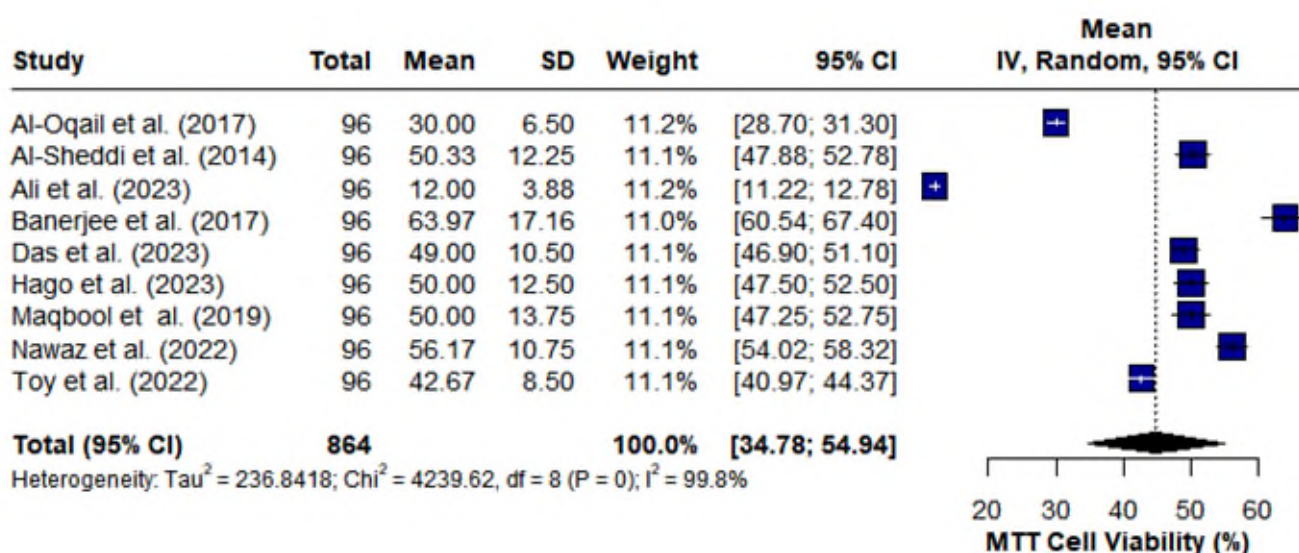
Table 5: A methodological analysis of scientific research-article quality, based upon a modified Newcastle-Ottawa Scale.

Study	Selection			Comparability		Outcome			Total (out of 13)	AHRQ standards (good, fair, and poor)
	Representativeness of the exposed cohort (Maximum: **)	Selection of the non-exposed cohort (Maximum: *)	Ascertainment of exposure (Maximum: **)	Demonstration that outcome of interest was not present at start of study. (Maximum: *)	Comparability of cohorts on the basis of the design or analysis (Maximum: **)	Assessment of outcome (Maximum: **)	Was follow-up long enough for outcomes to occur? (Maximum:*)	Adequacy of follow up of cohorts. (Maximum:**)		
Al-Sheddi <i>et al.</i> (26)		*	*	*	**		*	*	**** **** * (7)	Good
Das <i>et al.</i> (121)		*	*	*	**		*	*	**** **** * (7)	Good
Al-Oqail <i>et al.</i> (23)	*	*	*	*	**		*	*	**** **** ** (8)	Good
Ali <i>et al.</i> (34)	*	*	*	*	**		*	*	**** **** ** (8)	Good
Hago <i>et al.</i> (179)		*	*	*	**		*	*	**** **** * (7)	Good

Table 5: Continued

Nawaz <i>et al.</i> (307)	✱	✱	✱	✱	✱✱		✱	✱	✱✱✱ ✱✱✱ ✱✱ (8)	Good
Toy <i>et al.</i> (423)		✱	✱	✱	✱✱		✱	✱	✱✱✱ ✱✱✱ ✱ (7)	Good
Maqbool <i>et al.</i> (271)	✱	✱	✱	✱	✱✱		✱	✱	✱✱✱ ✱✱✱ ✱✱ (8)	Good
Banerjee <i>et al.</i> (61)	✱	✱	✱	✱	✱✱		✱	✱	✱✱✱ ✱✱✱ ✱✱ (8)	Good

(Adapted from: 321)

Figure 8: A forest-plot of the cytotoxic effects of *Nigella sativa* upon various cancer cell-lines using mean-values and 95% confidence intervals (CIs).

Nine effect sizes were utilised within a random effects-model. Left: a list included studies within the meta-analysis denoted by the first author of the publication and the year the research paper was published. Middle: Respective study effect-estimates are displayed with values for the mean, standard-deviation (SD), weight (power) and 95% CIs. Overall, the included studies have similar influences on the overall effect the random effects-model. Right: a forest-plot visually representing respective effect-estimates. Bottom: Data for the overall-statistics such as overall-effect and total high variability by heterogeneity (I^2). $P=0$ ($p < 0.0001$); high-significance.

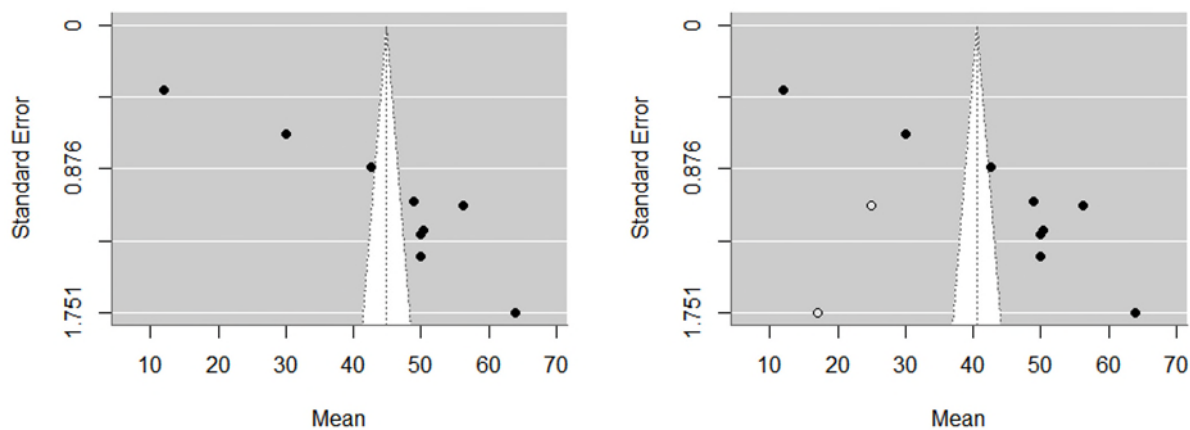


Figure 9: A funnel-plot and trim-and-fill funnel plot.

Left: Asymmetrical distribution of studies (filled-dots) necessitated our fail-safe tests to indicate statistical absence of publication-bias. Right: A graph highlighting the adjusted effect-sizes, with two inputted studies for absence-estimates (hollow dots) after considering potential publication-bias. Both plots are subject to interpretation and utilising techniques to show potential sensitivity to a certain type of publication-bias rather than validity (416/417). Trim-and-fill notes: Estimated number of missing studies on the left side: 2 (SE = 2.1189), Random-Effects Model (k = 11; tau² estimator: REML), tau² (estimated amount of total heterogeneity): 284.5633 (SE = 127.9175), tau (square root of estimated tau² value): 16.8690, I² (total heterogeneity / total variability): 99.70%, H² (total variability / sampling variability): 328.46, Test for Heterogeneity: Q(df = 10) = 4296.7500, p-val < .0001, Model Results include estimate: 40.5426, se: 5.0993, zval: 7.9506, pval <.0001, ci.lb: 30.5481, ci.ub: 50.5371, ***, p<0.001. See table 6 abbreviations.

Table 6: A table showcasing mean effect-sizes from moderator-analysis tests conducted upon the relationship between respective variables, modified by a categorical dichotomous moderator variable (formulation-type).

	THERAPY_ TYPE_	NEXP	NCTRL	K	ESTIMATE (2 D.P)	SE (2 D.P)	ZVAL (2 D.P)	PVAL (2 D.P)	CI.LB (2 D.P)	CI.UB (2 D.P)	
FACTOR (THERAPY_ TYPE_) EXTRACT	Extract	576	576	6	52.16	4.76	10.97	5.41e-28	42.84	61.48	***
FACTOR (THERAPY_ TYPE_) OIL	Oil	288	288	3	30.29	6.70	4.52	6.12e-06	17.16	43.42	***

	ESTIMATE	SE	ZVAL	PVAL	CI.LB	CI.UB	
INTERCEPT	52.1619	4.7556	10.9685	<.0001	42.8411	61.4827	***
FACTOR(THERAPY_TYPE_)OIL	-21.8741	8.2144	-2.6629	0.0077	-37.974	-5.7743	**

Abbreviations: REML, Restricted maximum likelihood. DF, Degrees of freedom. K, Study number for each category of the moderator. SE, Standard error. CI, confidence interval (95%). UB, Upper-bound. LB, Lower-bound. NEXP, Number for experiment. NCTR, Number for control. ***, p<0.001.**, p<0.01. Notes: Mixed-Effects Model (k = 9; tau² estimator: REML). tau² (estimated amount of residual heterogeneity): 134.0003 (SE = 72.3597). tau (square root of estimated tau² value): 11.5758. I² (residual heterogeneity / unaccounted variability): 99.35%. H² (unaccounted variability / sampling variability): 154.61. Test for Residual Heterogeneity: QE(df = 7) = 1533.3712, p-val < .0001. Test of Moderators (coefficients 1:2): QM(df = 2) = 140.7581, p-val < .0001. Iterative-model notes: Mixed-Effects Model (k = 9; tau² estimator: REML), tau² (estimated amount of residual heterogeneity): 134.0003 (SE = 72.3597), tau (square root of estimated tau² value):11.5758, I² (residual heterogeneity / unaccounted variability): 99.35%, H² (unaccounted variability / sampling variability): 154.61, R² (amount of heterogeneity accounted for): 43.42%, Test for Residual Heterogeneity: QE(df = 7) = 1533.3712, p-val < .0001, Test of Moderators (coefficient 2): QM(df = 1) = 7.0911, p-val = 0.0077.

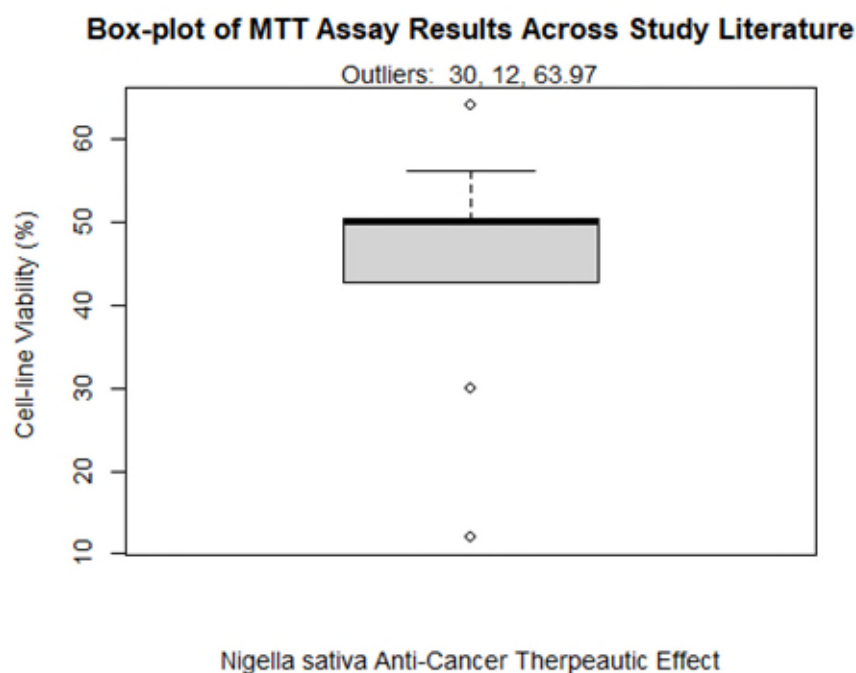


Figure 10: A Box plot utilising the mean cell-viability percentages across the collated studies.

Potential outliers warranting further investigation are shown as hollow dots. Al-Oqail *et al.*, 2017 (30.00%); Banerjee *et al.*, 2017(63.97%); Ali *et al.*, 2023 (12.00%). Potential hypotheses as to why this may be the case are discussed within the 'replicates' paragraph within the 'discussion' section of this paper; upon statistical evaluation utilising the Grubb's test the significance for Banerjee *et al.*, 2017 was marginal with the p-value 'teetering on the brink of insignificance' ($p=0.04261$); however, once considered with Al-Oqail *et al.*, the influence become more severe ($p=0.02114$). Thus, a possible less than 5% mathematical chance of encountering these outliers compared to other data-sets by chance alone (63) (205). For this reason, we decided to look further into the experimental design of the two, despite the possibility of the statistical test being hindered by the Type I error rate, due to our low sample size ($n=9$) (88); considering the experimental design it was decided to keep such data-values. Ali *et al.*, 2023 was considered statistically insignificant ($p=0.9369$). However, researchers should take precautions when deducing conclusions through the subjective statistical-thresholds (228). Notes: data: (Results for Ali *et al.*, 2023) `cbind_df$therapy_mtt_assay_mean_percentage_`, $G = 1.23766$, $U = 0.78459$, $p\text{-value} = 0.9369$, alternative hypothesis: highest value 63.97 is an outlier. (Results for Banerjee *et al.*, 2017) data: `cbind_df$therapy_mtt_assay_mean_percentage_`, $G = 2.13603$, $U = 0.35838$, $p\text{-value} = 0.04261$, alternative hypothesis: lowest value 12 is an outlier. (Results for Banerjee *et al.*, 2017; Al-Oqail *et al.*, 2017) data: `cbind_df$therapy_mtt_assay_mean_percentage_`, $U = 0.14065$, $p\text{-value} = 0.02114$, alternative hypothesis: lowest values 12 , 30 are outliers.

4. Discussion

Inappropriate cell-proliferation, a hallmark of cancer and a leading cause of cellular genomic-aberrations; potentiating the induction of aggressive adaptations, therapeutic-resistance, limitless-replication, and sustained growth-signalling. All aiding in the transition of a normal-cell to a metastatic neoplastic-mass (185). For this reason, our study reviewed and statistically analysed the available *in-vitro* research for the effect NS may have in preventing this end-result.

Previous studies have prospected the efficacious nature of NS with anti-proliferative *in-vitro* results being reported for cervical (131), colon (370), prostate (454), pancreatic (103), lung (265), breast (146), and liver (411) cancer cell-lines.

Thus, our collation included MTT-assays evaluating cell-lines from a range of common cancer types including Hela; HCT; PANC; A-549; MDA-MB-231/435; MCF-7; PC3; HepG2 in order to scrutinise if this result holds true through the use of NS in oil/extract form.

4.1 A Viable Union with Current Therapeutics

The study by Ali *et al.* (2023) may provide quantitative evidence pertaining to unrefined organic-oil having a higher cytotoxic-potential than anthracyclines. The clinical relevance of this finding is centred towards the potential of developing a more efficacious approach with current antineoplastic-treatments; its synergistic employment with present therapies could reduce the administered doses of routinely used chemo-agents, as NS is able to reduce cancer cell-viability further within each respective concentration. Furthermore, if this approach was to utilise pre-treatments (freeze-thawing) to stabilise, retain and also heighten key bio-constituents, exacerbation of the oil's potent cytotoxic results and reduction in dose-dependent side-effects (doxorubicin-induced cardiotoxicity) may become a reality; thus, NS could elevate a cancer-patient's quality-of-life whilst also further reducing cell-viability - halting unwanted side-effects that often hinder the efficacy of well-established therapeutics. Through further research, this less toxic alternative may allow for synthetic novel-therapeutics to be developed, 'spring-boarding' manufactured replacements to current toxic chemotherapy-agents (89) (78) (178).

The research undertaken by Ali *et al.*, (2023) showed MTT-assay IC_{50} results for HeLa; HCT; PANC cells to be 5.51, 4.24 and 22.7 $\mu\text{g/ml}$, respectively; with doxorubicin showing consistent reductions in potency, in comparison to NS oil, with IC_{50} values of 101, 188 and 244 $\mu\text{g/ml}$, respectively. This disparity agrees with previous studies with only higher-concentrations of doxorubicin consistently being seen to exert any significant effect upon cell-viability (57). Additionally, the cold-press extraction method may have been crucial in determining the overall make-up of the compound; directly influencing its anti-cancer efficacy through the preservation of thermal-sensitive bio-active components (figure 11) by eliminating exposure of contents to higher-temperatures, as seen in Soxhlet-extraction. Therefore, this may provide some explanation towards Al-Sheddi *et al.* (2014) lower A-549 cell-line viability percentages (0.25mg/ml; 0.5mg/ml; 1mg/ml doses administered) found within their oil-medium compared

to their NS extract (oil: 52%, 41% and 13%; extract: 75%, 50% and 20%, respectively); as well as, the higher IC₅₀ values observed by Al-Oqail *et al.* (2017) compared to Ali *et al.* (2023) despite both utilising an oil-medium (348).

If this proposal is true, key active-constituents would have efficiently exerted their anti-neoplastic effects through adequate exposure and retained functional-properties, such as saponin α -hederin which has been observed to reduce of Lewis-lung carcinoma *in-vivo* (203); considering the potential effects thymoquinone alone upon cancer cell-lines may provide explanation towards the significant effects witnessed at concentrations starting from as little as 0.1mg/ml and the consistent dose-dependent effects on cell-proliferation *in-vitro*, within all of the collated studies. However, further research of molecular-signalling mechanism would need to be addressed in the future to back this point (26) (209) (223). Research undertaken by Wei Keat Ng and colleagues may show supportive-evidence for this proposal with thymoquinone exhibiting higher MTT IC₅₀ cytotoxicity in human-cervical squamous carcinoma cells (SiHa), displaying another example of increased potency, when compared to a widely used chemo-agent, cisplatin.

4.2 Fallible Steps in Quantifying Cytotoxicity

However, NS MTT-assays need to be considered holistically with each step leading to respective IC₅₀ results scrutinised, including its preliminary-stages. For example, Das *et al.* (2023) and Hago *et al.* (2023) were the exclusive few to include data for the supply-origin of NS seeds locally purchased; additionally, only Al-Oqail *et al.* (2017), Nawaz *et al.* (2022), Maqbool *et al.* (2019), Toy *et al.* (2022) and Das *et al.* (2023) made mention of the amount of replicates established for each cell-line. Thus, such factors may culminate uncertainty towards whether NS oils/extracts produced could be implemented as a consistent efficacious treatment for synergistic-use, with previous studies indicating a clear stratification of bio-constituent composition, especially in regard to thymoquinone, across NS seeds of different origins (75) (102).

Mass-spectrometry used by Ali *et al.* (2023), Das *et al.* (2023) and Hago *et al.* (2023) may not be sufficient to indicate the seed's origin, with chemical fingerprints within the same species being too similar. Additionally, as our results show a location-specific trend for this area of research another potential for reduced accuracy, within this technique, may occur due to NS metabolites being too 'nuanced' for adequate bio-chemical identification within international protein databases (UniProtKB) remaining incomplete - leading to critical data being absent; a potential product of the 'Canalization-of-Research-Areas-on-the-Principle-of-Training-Inertia' theory (110) (240) (255).

Hence, Al-Oqail *et al.* (2017), Al-Sheddi *et al.* (2014) relying solely on taxonomical-evaluation for species deciphering would be an even weaker approach and a considerable pitfall within the experimental-analysis, due to the high possibility of same-species seed-origin conflation. Thus, if this factor is neglected, this may lead to potential reduced potency within a given dose,

underrepresenting NS's clinical potential. Banerjee *et al.* (2017) and Das *et al.* (2023) also utilised targeted phytochemical-screening negating the possibility of fertiliser contamination within their medium (325). However, the same cannot be said for other researchers, possibly allowing for heavy-metals (cadmium) causing mitochondrial-dysfunction induced low cell-viability instead of NS within their doses (87).

Moreover, an alternative explanation for the low IC₅₀ synergistic potential of NS oil, may also be a product of unknown pre-processing of raw seeds rather than the extraction technique itself. With no mention from Ali *et al.* (2023), Al-Oqail *et al.* (2017); Al-Sheddi *et al.* (2014) of the state in which the NS seeds were stored before purchase. The modulation of the post-harvest and the absence of seed-quality screening by Ali *et al.* (2023) allows multiple variables to be introduced for the low-dose high-potency properties found within their cancer cell-lines (92).

Arslan and Acar (2024) found refrigerated (unroasted) NS seeds stored under shade and in cloth-bags caused a composition with lower free fatty-acids, reducing a potential independent lipid-peroxidation pathway leading to carcinogenic modulation of cells within the *in-vitro* study. Additionally, roasting the same seeds produced oils with higher amounts of phenolic-compounds and hydrogen-peroxide concentrations, allowing for apoptosis and receptor signalling, enzyme-modulation, gene-expression regulation, epigenetic-regulation, and metabolic-dysfunction within cancer-cells (58).

The MTT assay procedure itself is an often-overlooked factor that may inflate cell-viability data within this study, despite its wide use. Reagents within the preparation are known to also exhibit cytotoxic effects, with dramatic changes to morphology during formazan-crystal formation (355/356); the use of acidified-isopropanol is crucial in the crystal-solubilisation step, however, Al-Oqail *et al.* (2017) use of organic solvents and Toy *et al.* (2022) use of dimethyl-sulfoxide run the risk of precipitating proteins within the medium causing light-scattering and misleading results (283). Additionally, tetrazolium reduction reflects the general metabolic activity of the cell, which could be easily skewed by the amount of glucose in the medium, the morphology of the cell, and the cell monolayer dividing rates - leading to poor dose representation.

Ali *et al.* (2023), Hago *et al.* (2023), Maqbool *et al.* (2019) and Nawaz *et al.* (2022) also made no mention of the number of cells seeded per well, in regard to the MTT assay for each cancer cell-type. Hence, conflation between the significant reduction in cell-viability compared to the chemo-agent from NS medium alone or due to external factors still stands; moreover, overcrowding within the well may lead to less proliferation space and reduced nutrient-availability affecting cell viability, leading over-estimated results.

Lastly, no 'untreated' control cell-line was established by Ali *et al.* (2023) and Toy *et al.* (2022); consequently, any reduced proliferation from plate-environment limitations would not have been evaluated, nor any cell-number titration tests established, thus, providing another avenue to potentially create highly-potent MTT results. As a result, the IC₅₀ values should be met with caution and confirmed *in-vivo*.

4.3 'Don't Use a Cannon to Kill a Fly'

The current research-landscape within oncology has yet to provide a viable novel-alternative that displays efficacious therapeutic-potential, across multiple cancer-types, whilst also lacking toxicity towards normal cell-types; furthermore, chemo-therapeutics, radiotherapy and surgical treatments are frequently employed towards those diagnose despite their non-selectivity and high-toxicity towards healthy-cells; these treatments often lead to a high-risk of tumour re-occurrence or cause irreversible consequences towards the patients quality-of-life (286). The study by Al-Oqail *et al.* (2017) is potential quantitative evidence supporting the possibility of NS oil exhibiting selective-cytotoxicity across a broad range of cancers whilst preserving the viability of normal cells within the same dose. *In-vitro* results for the IC₅₀ value pertaining to HepG2, MCF-7, A-549 cell-lines displayed considerable cytotoxicity, agreeing with Ali *et al.* (2023); in addition to lower-values found in the control Human-embryonic-kidney-293 cell-line (44.6; 46.2; 245 ug/ml compared to 1136ug/ml, respectively). The selective-cytotoxicity agrees with findings from El-Najjar *et al.* (2009), Das *et al.* (2023), Banerjee *et al.* (2017), Nawaz *et al.* (2022), Maqbool *et al.* (2019) and Hago *et al.* (2023). However, contradicting selective-cytotoxicity results have been displayed with NS extracts (51) (118) (405); Such conflating results may suggest crucial heat-sensitive bioactive constituents being degraded by calefaction during extraction-processes along with possible formation of polycyclic-aromatic-hydrocarbons (222) (221).

Thus, these findings suggest oil-refining techniques may better preserve bio-constituents necessary for the anti-cancer effect of NS, such as thymoquinone, flavonoids and polyphenols (361) (402). However, selectivity may also be a product of differential pre-processing, with Hago *et al.* (2023), Nawaz *et al.* (2022) and Banerjee *et al.* (2014) shade-drying their seed-sample before extraction, a technique known to increase monoterpene-hydrocarbons, such as carvacrol, within the main composition of essential-oils (190); hence, possibly allowing for a limited cytotoxic effect on normal cell-lines but a highly cytotoxic paradigm within HepG2, HeLa, HCT and MDA-MB-231/435 *in-vitro* and *in-vitro* experiments (121) (177).

The decision by Al-Oqail *et al.* (2017) and Maqbool *et al.* (2019) to assess cell-viability by the direct trypan blue-dye exclusion end-point assay with cell-lines cultured in Dulbecco's Modified Eagle Medium (DMEM) may provide more confidence towards the true potency of NS oil within different cancer-types, as well as its non-cytotoxicity in normal-cells. Studies have indicated a substantial drawback with indirect (MTT) formazan-reduction assays, used by Ali *et al.* (2023); Al-Sheddi *et al.* (2014); Banerjee *et al.* (2017), as flavonoids reduce MTT in the absence of cells - statistically heightened in DMEM (409).

Fetal Bovine Serum (FBS) within the cell-culture medium may affect the quality of the cells assessed. FBS can interfere with cell-proliferation, cell-viability, and cytokine interleukin (IL)-6 production causing potential biases within the cell-culture; however, this behaviour can vary significantly depending on the supplier which only Al-Sheddi *et al.* (2014) and Hago *et al.* (2023) specified (Liu *et al.*, 2023); protocols using 10% FBS have been reported to proliferate more

than serum-free media, creating ambiguity with the cytotoxic-potential of NS, possibly causing higher IC₅₀ readings found in Al-Sheddi *et al.* (2014); Hago *et al.* (2023); Maqbool *et al.* (2019); Nawaz *et al.* (2022) compared to protocols *in-absentia*, supporting our proposal for future experimental use of multiple end-point assays (338) (374).

A crucial intermediary step established by Al-Oqail *et al.* (2017); Al-Sheddi *et al.* (2014) was the manual-screening of seeds which may have allowed for an overall higher carbon-conversion efficiency within the oil-metabolism pathways allowing for adequate production of medium-chain triacylglycerols (MCTs); damaged seeds are known to cause specific enzymes to be mobilised which reduce the overall oil-content through lipids-reservoirs used as energy by degradation. Moreover, improved MCTs may suggest a reduction in the chronic-inflammation hallmark which often leads to further DNA-damage and genomic instability, as MCTs have the ability to lower cytokines and chemokines in the cell, potentially halting sustained NF-κB; IL-6/Jak/Stat3; NLRP3 proliferative signalling found in inflammatory tumour-microenvironments (185) (439). However, further research through metabolic-flux analysis should be employed to elucidate the validity of this proposal.

4.4 Connections. The Modulatory Role of Extraction Upon Anti-Cancer Effect

Cold pressing is known to retain thermally-sensitive bio-active molecules from NS seed-filaments, allowing for the presence of phenolic-compounds and tocopherols in the medium, which have been reported to contribute to some oxidative stability in oils (229). This may suggest that low-heat is essential in maintaining the low-dose high-potency character observed by Ali *et al.* (2023).

On the other hand, solvent extraction techniques despite their use of high-heat still retain a higher proportion of tocopherol content compared to cold-pressing techniques (466). These naturally occurring antioxidants may reduce the formation of peroxides and linolenic-acids. Thus, despite the reduced abundance of thermal-sensitive bio-constituents the resulting medium may compensate for this loss through higher-oxidative stability and improved radical-scavenging activity, allowing for Banerjee *et al.* (2017), Toy *et al.* (2022) and Hago *et al.* (2023) to observe high-cytotoxicity within their cancer cell-lines (258) (373).

Furthermore, petroleum-ether based solvents allow for further extraction of non-polar compounds through the use of aliphatic-hexane and derivatives which could contribute to the selectivity found by Al-Oqail *et al.* (2017), Maqbool *et al.* (2019) and Das *et al.* (2023). Interestingly Maqbool *et al.* (2019) differed by utilizing NS leaves within their extract, yet the medium still displayed considerable selective-cytotoxic results, possibly owing to the preserved non-polar bio-constituents.

Non-polar carotenoids such as lutein and zeaxanthin along with alpha/gamma/delta-tocopherols have been previously reported to display considerable selective anti-cancer effects *in-vitro*, supporting our premise (167) (401).

However, as Das *et al.* (2023) used Roswell Park Memorial Institute (RPMI)-1640 as the growth medium for the peripheral-blood mononuclear cells (PBMC), normal-control, but DMEM for both breast-cancer cell lines, the selective-cytotoxic effect observed may have been a result of increasing interferon-stimulated gene (ISG)-56 expression increasing cell-proliferation within the control cohort – inflating IC₅₀ results (256) (330).

The use of ultrasonic-assisted extraction by Hago *et al.* (2023) may have allowed for a higher increase in total alkaloid-content, such as nigellidine (9). Such constituents have been reported to regulate cholesterol-metabolism through modulation of the enzyme HMG-CoA reductase (HMGCR), lowering the total low-density lipoprotein (LDL) cholesterol within cancer-cells (16) (242). As reports suggest cholesterol-lowering molecules are able to overcome chemotherapy-resistance in triple-negative breast-cancer, this technique may have allowed an anti-cancer effect in both oestrogen-positive and negative-breast cancer cell-lines, whilst maintaining low-toxicity in the normal cell-line as seen by Das *et al.* (2023); suggesting extraction methods have a huge role in dictating the synergistic use, chemo-sensitisation and future stand-alone prospects of plant-based therapeutics (237).

Thus, from our observations cell-viability seems to also be highly influenced by the extraction-technique used, due to the resulting medium-composition (166). However, further research should be undertaken to find the molecular-mechanisms of the retained bio-constituents antineoplastic effect and improve extraction-techniques to preserve thermal-sensitive components.

4.5 The Importance of Multiple End-Point Assays

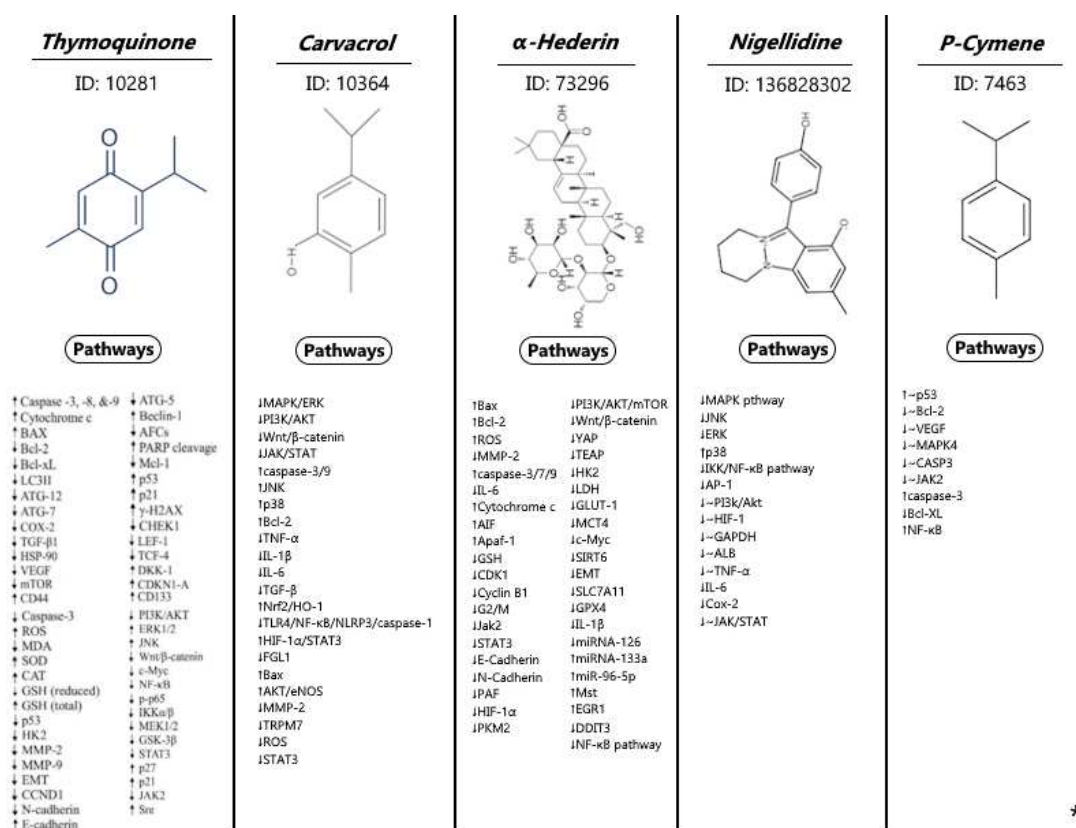
The use of only formazan MTT-assays by the majority of researchers within the collation provides clear IC₅₀ index values to compare the potency of NS oils/extracts on cell-viability, across different cancer cell-types. However, it only indirectly indicates cell-viability, due to its lack of consideration towards the direct cell-number; the obtained results could be considered misleading towards the true induction of apoptosis, within a given cell-line. Factors such as transient metabolic-impairment may be wrongly interpreted as an apoptotic state, a given dose may also have induced significant cell-cycle growth arrest but due to the cell still being metabolically-active it is perceived as viable - despite future loss of proliferation or induction of apoptosis occurring.

To address this issue future research should utilise multiple end-point assays as seen by Maqbool *et al.* (2019); a combination such as the trypan blue-dye exclusion and formazan MTT tests would quantify the number of cells subject to apoptosis, whilst any increase in cell-damage during trypsinisation, would be compensated by the metabolic-function evaluation that is less exacerbated. Thus, allowing for a holistic overview that may be truer to the oil/extract's actual anti-neoplastic effect.

4.6 A Statistical Overview

The majority of researchers within the collated literature reached a statistical conclusion that NS has high therapeutic-potential, based upon the data-sets published of treated cells which displayed significant difference from their control counterparts, suggesting the correlation between NS exposure and reduced cell-viability is very unlikely to be due to random noise ($p < 0.05$); further supported by their respective post-hoc tests (figure 12).

However, the use one-way ANOVAs assume observations of each cell-line sample were drawn from a normal-distribution; thus, as none of the researchers have explicitly reported to have used any normality-tests with their study there is the possibility of the reported p-values being inaccurate, due to samples having heavier tail-ends - leading to less statistical-power compared to other permutation tests. Therefore, future *in-vitro* studies (figure 13) should utilise normality-test such as the Kolmogorov Smirnov test or graphical tools for general-linear-model assumptions (utilising the central-limit theory) test upon samples belonging to a single-condition, such as 96 wells of the untreated control for each respective cell-type (163).



*

Figure 11: Key thermal-sensitive bio-active constituents of *Nigella sativa* and their molecular impacts.

Thymoquinone: The molecular structure and the molecular and cellular mechanisms and targeted signalling pathways possibly explaining its anti-metastatic, pro-apoptotic/anti-proliferative, and antioxidant activities (*in-vitro*). **Carvacrol:** The molecular structure and the cellular mechanisms and targeted signalling pathways possibly explaining its ability to induce cell cycle arrest, apoptosis induction, inhibited phosphorylation of kinases, reduce angiogenesis and inhibition of cell-proliferation (*in-vitro*). **α -Hederin:** The molecular structure and the molecular and cellular mechanisms and targeted signalling pathways possibly explaining the induction of apoptosis; cell-cycle arrest; autophagy suppression; cell-proliferation inhibition; cell-migration, invasion, and metastasis inhibition; ATP metabolism reduction; inflammation inhibition and angiogenesis halting (*in-vitro*). **Nigellidine:** The molecular structure and the reported and *in-silico* predicted (~) cellular mechanisms and targeted signalling pathways possibly explaining its ability to reduce inflammation; reduce cell proliferation; reduce cell-growth (*in-vitro*). **P-Cymene:** The molecular structure and the reported and *in-silico* predicted (~) cellular mechanisms and targeted signalling pathways possibly explaining its ability to reduce inflammation; reduce cell-proliferation; reduce metastasis; reduce cell-growth; lipid metabolism; angiogenesis (*in-vitro*). Abbreviations can be determined from the list provided by Cell Signalling Technology, 2026; ID=PubChem Compound CID. *This list may be subject to change. (Adapted from: 7; 27; 45; 73; 74; 104; 142; 268; 382; 210; 280; 314; 336).

No Statistical Analysis
Reported For:

Ali *et al.*, 2023
Banerjee *et al.*, 2017
Hago *et al.*, 2023

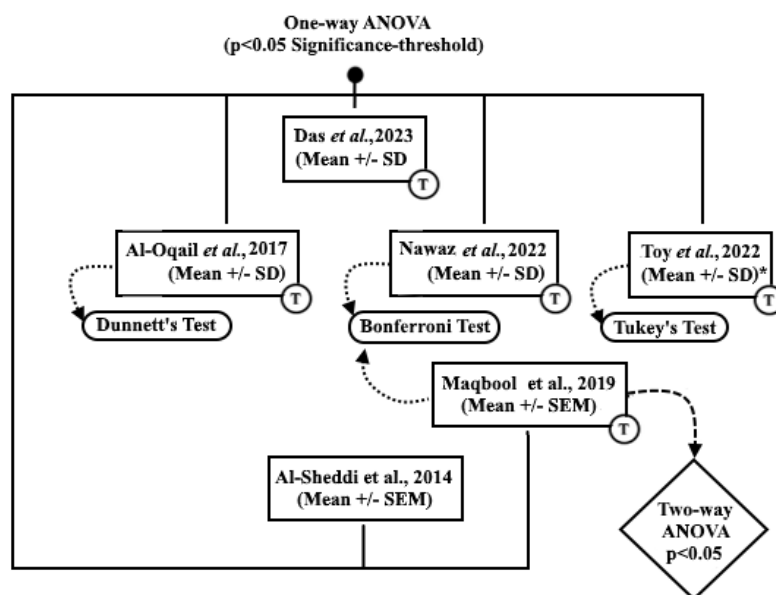


Figure 12: A visual overview of the statistical-analysis and post-hoc analytical approaches employed across the research-papers.

The statistical difference found within the cohort utilising a one-way ANOVA indicated a significant effect of NS upon cancer-cell line in terms of their cell-viability ($p < 0.05$). The use of *post-hoc* tests within experiments utilising multiple cancer cell-lines may have allowed for statistical evaluation of any possible stratification of NS effect upon a given type (141). Caution should be applied when interpreting statistical results due to the occurrence of false positives within smaller sample sizes, leading to unreliable results (50) (121). Das *et al.* (2023)'s and Al-Sheddi *et al.* (2014)'s statistical approach indicated a rejection of the null hypothesis as the means were statistically different showing statistical difference on cell-viability when NS was administered to the cell-line. Das *et al.* (2023) did not use a *post-hoc* test, thus, statistical inter-difference between cell-type may be inconclusive. Al-Oqail *et al.* (2017) used a Dunnett's test allowing for multiple cancer cell-lines (and NS efficacy, respectively) to be compared to the control cell-line, however inter-difference between cancer cell-types was not determined statistically, leaving inconclusively (251); this method provided a lower risk for Type-I error induction when comparing treatments to control (with equal variance; normal distribution assumed). Nawaz *et al.* (2022)'s; Maqbool *et al.* (2019)'s use of a Bonferroni test may be due to the presence of unequal cell-line sampling within the different cell-types. Caution should be employed, due to multiple cell-lines used, due to the increase in Type-II errors through possible additional inter-difference hypotheses (173); this method provided a low-risk for Type-I error when comparing treatments to control (with normal distribution assumed). Toy *et al.* (2022)'s of a Tukey's test may be due to the use of one treated PC-3 cell-line and control cell-line. Tukey's test would have allowed for statistical comparison of cell-lines with equal sample sizes and a low risk of significant difference by 'chance' alone, in regard to mean comparison of the end-results within this technique (418). Statistical analysis from Al-Sheddi *et al.* (2014) has the risk of invalidation (by not employing replicates) if the assumption of equal distribution of error within the SEM is not met. Maqbool *et al.* (2019) utilised different extract-mediums (ethanol, petroleum-ether, and water) possibly encouraging the use of a two-way ANOVA to determine inter-differential relationships of each extract within the given cell-line. (Adapted from: 49). Dotted-lines=*post-hoc* analysis, dashed-lines=secondary statistical-analysis, solid-line=initial employed statistical analysis. Abbreviations: *et al.*= *et alia*, SD=Standard-deviation, SEM=Standard-error of the mean, ANOVA=Analysis of variance, *=triplicate graphically inferred, T=triplicate. See Twisk, (2024) for statistical fallibility (426).

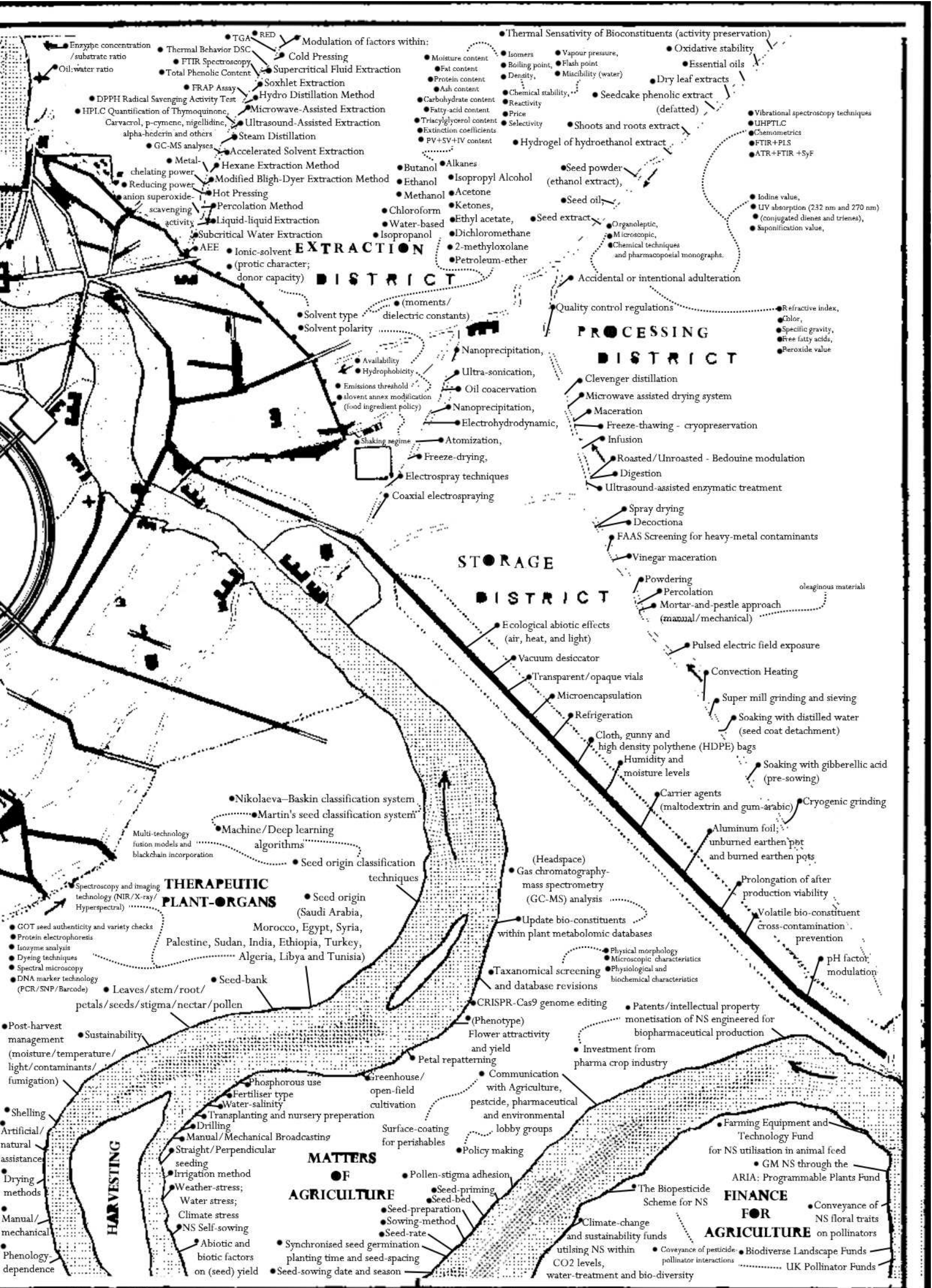


Figure 13: A visual representation of the multiple avenues potentially leading to 'The House of Medicine' – a map for *Nigella sativa*'s clinical incorporation in oncological treatments.

Many of the factors shown should be considered within future research-pipelines and scrutinised within systematic-reviews and meta-analysis articles. Abbreviations: GM=Genetically modified, NS=*Nigella sativa*, ARIA=the UK's Advanced Research and Innovation Agency, GOT=Grow-Out Tests by the International Association for Seed Inspection, NIR=Near-Infrared, SNP=Single nucleotide polymorphism, PCR=Polymerase Chain Reaction, DNA=Deoxyribonucleic acid, FAAS=Flame Atomic Absorption Spectrometry, nm=nanometres. UHPTLC= Ultra high-performance thin-layer chromatography, FTIR=Fourier transform infrared spectroscopy, PLS=partial least squares, ATR=attenuated total reflection, SyF=synchronous fluorescence, +=combination, FTIR=Fourier-transform infrared, TGA=Thermogravimetric Analysis, FRAP=Ferric Reducing Antioxidant Power Assay, DPPH=1, 1-Diphenyl-2-picrylhydrazyl, DSC=Differential scanning calorimetry, PV=peroxide value, IV=Iodine value, SV=saponification, RED=Hansen relative energy difference criterion, AEE=Aqueous enzymatic extraction, DMSO=Dimethyl sulfoxide, TM=Trade-mark, ZIL=zwiterionic liquid, DMEM-HG=Dulbecco's Modified Eagle's-High Glucose medium, SBF= Simulated body fluid, RPMI=Roswell Park Memorial Institute 1640 medium, FCS=fetal calf serum, BGS=HyClone Bovine Growth Serum, HS=Horse serum, GF-AFC =glycylphenylalanyl-aminofluorocoumarin, LCC=Lowest cytotoxic concentration, IC₅₀ Inhibitory Concentration (50% cell-viability), SOD=Superoxide dismutase assay, CAT=Catalase assay, (TAC=Total Antioxidant Capacity Assay, D=dimensional, FISH=Fluorescent *in-situ* hybridisation, LDH=lactate dehydrogenase, PI=phosphatidylserine, BrdU =5-bromodeoxyuridine, ELISA=enzyme-linked immunosorbent assay, HTRF=Homogeneous Timer-Resolved FRET, CDX=cell line-derived xenograft; patient-derived tumour, ATP=Adenosine triphosphate, MTT=3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, MTS Assay = A Colorimetric Method, XTT=Cell Viability Assay, WST-1=Roche Cell Proliferation Reagent, MM-GBSA =Molecular Mechanics combined with the Generalized Born Surface Area , OPLS=optimized potentials for liquid simulations, ANCOVA=Analysis of covariance, PEG=Polyethylene glycol, BAX=Bcl-2 Associated X-protein, PI3K=phosphatidylinositol 3-kinases, Akt1=RAC(Rho family)-alpha serine/threonine-protein kinase, mTOR=mechanistic target of rapamycin, RNA=Ribonucleic acid, PCRq=polymerase chain reaction with Taq DNA Polymerase, NPK=nitrogen, phosphorus and potassium, MMP-9=matrix metalloproteinase-9. This list may be subject to change. Material and methods for respective techniques should be scrutinised and evaluated for a comprehensive conclusion of NS endpoint results. Additional avenues of research that may catalyse NS incorporation clinically are as follows: combination-therapy (dates: ajwa/khidri/kholas/medjool+alternatives); saffron; yemeni ('sidr'/'marai') honey; quince; senna leaves; agar wood; Zamzam water; *Withania somnifera*; *Asphaltum punjabinum*; asafoetida; frankincense; potassium aluminium sulphate; '*hindi costus*'; *Pimpinella anisum*; moringa leaf; turmeric; black pepper; ginger; *Salvadora persica*; *Olea europaea* derivatives; agriculture disease prevention (foot and root disease - *Fusarium* induced); floral abundance/richness on seed yield; medical supply-chain variables (manufacturer/geopolitical stability/ demand (misinformation)); hydrogel bioavailability; hydrophobicity management for reductions in metabolic rate; deducing molecular mechanism of key anti-cancer bio-constituents in NS not only focussing on thymoquinone as a 'poster-molecule'; NS's effect on Bcl-2 protein expression; effect of extract pH upon thymoquinone stability; removing adulterations in cold-press extractions; standardising quality regulations of NS oil/extracts, globally; reducing genus seed 'mix-ups'; optimising the 'Bedouin thermal-effect' of NS seeds (bio-constituent optimisation); penetration ability of cold-press oils compared to other extraction methods and its effect on topically treating skin/oral cancers; statistics for oil extract type consumed around the world and potential relationship with individual cancer risk; NS (thymoquinone/carvacrol/trans-anethole) effect on migratory cancer-cells; NS effect upon p53 and caspase 7/9 *in-vitro/in-vivo*; NS modulation of DNA fragmentation/PI3K/Akt1 pathway/Bax levels/mTOR pathway, NS regulation of micro-RNA; potential links between medicinal herb accessibility and location and its effect upon clinical trial prognosis; effects of anhydrous sodium sulphate and flash-evaporation upon NS bio-constituent composition (within oils/extracts); Network Pharmacology and derivatives to deduce predictive models for mechanisms of key NS constituents (target identification and foundations for synthetic therapeutic developments); developing portable, affordable and sustainable extraction/storage machinery for use internationally; anticancer efficacy/bioavailability *in-vivo/in-vitro* of solid lipid nanoparticles/pickering emulsions/double emulsions/hexosomes/cubosomes/self-nano-emulsifying-drug-delivery -systems/nano lipid carriers; niosomal vehicles/biopolymeric nanocarriers; NS formulation type and its protective effect against ionizing radiation induced oxidative stress; long term NS side effect *in-vivo* analysis and longer *in-vivo* clinical trial duration observance; residual water effect of NS formulation yield and efficacy; NS cytotoxicity assessment by neutral red uptake assay/morphological analysis by phase contrast microscope/glutathione content/ reactive oxygen species generation/ mitochondrial membrane potential/quantitative real-time PCRq analysis of apoptotic marker genes/ Transwell migration and wound healing assays for NS effect on metastasis; NS serum toxicity assay; NS Catalase Assay; Lactate Dehydrogenase Assay; Gene Expression Profiling for respective cancer cell-line studies *in-vitro*; NS Colony formation assay; western blotting assays (expression levels of TIGAR/LC3

; different treatments of cattle manure/biofertilisers on NS total chlorophyll content/carbohydrate percentages/volatile/fixed oil contents/NPK percentages; seed yield link with nitrate/nitrite; reducing NS production costs by minimising chemical fertilizers/environmental hazards/improving soil structure/using leveraging agriculture; NS effect on cell migration and MMP-9 expression using scratch wound-healing assay/gelatine zymography; stratification within different ethnic descents towards NS antitumor compounds (α -hederin); potential carcinogenesis of contraceptive pills and NS replacement, to lower risk; addressing potential propaganda undermining NS utility, through mis-use of standard deviation and standard error bars within graphical displays of research papers; seed identification protocols for *N. sativa*, *N. damascena*, and *N. arvensis* (*Sesamum indicum*, *Allium cepa*, *Argemone mexicana*, *Corchorus*, and *Clitoria guianensis*); mitigating NS high commodity inflation value, and its price per litre; addressing NS oil dilution with known vegetable oils such as palm (*Elaeis guineensis*, *Arecaceae*) oil/ refined corn (*Zea mays*, *Poaceae*)/sunflower (*Helianthus annuus*, *Asteraceae*)/soybean (*Glycine max*, *Fabaceae*)/ grape (*Vitis vinifera*, *Vitaceae*) seed/canola (*Brassica napus*, *Brassicaceae*)/walnut (*Juglans regia*, *Juglandaceae*) oil and the challenges of detecting such adulteration; present day remnants of the historical NS seed confusion with toxic and black-seeded corn cockle (*Agrostemma githago*); NS affordability - market dynamics within countries of origin (stability/fair trade); whole seed adulteration by *N. damascena* or with similar coloured/sized seeds such as *Sesamum radiatum*/*Caryophyllaceae*/*Pedaliaceae*/*Allium cepa*/*Liliaceae*/*Argemone mexicana*/*Papaveraceae*/*Malvaceae*/*Clitoria guianensis*/*Fabaceae*; addressing adulterations due to grass seeds coated with charcoal dust, cumin/fennel seeds covered with marble dust, and peanut butter powder or shell material; improve reporting standards in NS cell culture research; hormonal contents within NS Seeds of different origins; weed management within NS cultivation; NS modulation of haematocrit levels. (Adapted from 1; 6; 10; 14; 24; 25; 27; 30; 32; 33; 36-39; 40-49; 52-54; 59; 60; 64-74; 79; 80; 85; 90; 93-96; 98-101; 104; 106-109; 112; 114-117; 119; 120; 122-125; 127-130; 132; 135; 136; 138-140; 142; 143; 147; 149; 151-154; 157; 158; 160; 162; 164; 169-171; 174; 180-183; 186; 187; 189; 191; 194; 195; 197; 199; 200; 206-208; 210; 213; 215-217; 219; 220; 224-227; 231-236; 238; 243; 245-247; 249; 252-254; 257; 259-263; 268-270; 272; 276-282; 284; 285; 287-293; 296; 297; 299-306; 310; 311; 313-315; 318; 320-322; 326-328; 331-333; 335-337; 339-345; 347; 350; 353-359; 362-368; 372; 372; 375-386; 388-390; 393; 395-398; 403; 404; 407; 410; 412-414; 416; 417; 419-422; 424; 425; 426; 428-433; 436-438; 440-433; 436-438; 440-442; 447-450; 453; 456-458; 460; 461; 462-465; 468; 469; 471).

5. Future Prospects

5.1 Black Gold

Globally, NS products have the potential to be a key economical asset within the investment-portfolios of pharmaceutical companies. Financial estimates indicate an exponential growth from \$19.68-\$41.56 billion (2023-2033), with the highest growth within the pharmaceutical sector (399). This projection may incentivise investors to fund a commodity with a low-risk and high-profit return. This is further supported by the establishment of five patented formulations targeting various diseases (table 7), giving confidence towards a legally defensible therapeutic that could dominate an ever-growing oncology combination-therapy market and become a widespread commodity through pilot-partnerships (415) (334). If additional human-trials are established and are proven to be significantly effective, pharmaceutical-companies may be encouraged to fund research towards refining current extraction-techniques, incentivising projects that may increase the likelihood of this therapeutic progressing through clinical-trial stages and future clinical-incorporation.

5.2 The Damascena Discernment Issue: Misconstruction-in-a-mist. A Potential Pre-requisite Stunting Clinical-Trial Progression

Although NS's medicinal properties are well known, remaining a household name across a plethora of countries, NS across the Atlantic, despite being approved for safe-use and consumption, is not commonly associated with any healing potentiality but rather as an annual ornamental-piece (91) (298) (434).

The geographical location of the few clinical-trials incorporating NS around the world (table 8) further highlights the lack of pharmaceutical consideration, especially within the United States, who currently dominates the international clinical research landscape (443). Thus, the implications stemming from this partial understanding have the potential to inhibit future global research-trajectories, as essential funding is relocated to 'safer' therapeutics that have a higher-probability to follow-through and yield a profitable return (156) (202) (264).

The reason for the emancipation, within the perception of the general public, of NS from any medicinal benefit may be a direct result of commonly misidentifying *Nigella damascena* (Love-in-a-mist) for *Nigella sativa* (Black cumin) (figure 14), with the former having the potentially toxic alkaloid damascenine, which has the possibility to induce vomiting and diarrhoea if ingested (83) (84) (192) (391). Therefore, the potential effects of conflating the two species within the minds of the public, may deter many from participating within critical clinical-trials, due to concerns over adverse side-effects.

This in turn would halt the translation process of preclinical studies to clinical-trial phases as worries over side effects are the leading factors dictating trial attendance (295).

A proposed solution would be to ensure that there is adequate education available for researchers, therapeutic providers and the general-public, especially in the United-States to instil the correct understanding of NS's use within healthcare.

Contradictory figures showcasing morphological observations with

amalgamated identifiers of both species should also be rectified lest millions of researchers stay mis-informed (11 p.2; fig.2) (230) (224 p.173; fig.1) (287 p.8; fig.1) (317 p.1624; fig.1), along with contemporary literature such as, published books and journal-articles, especially within widely accepted and reputable databases such as PubMed - which receives 8.5 million monthly-visits (50% from the United States) (17).

Furthermore, the confusion within the vernacular classification both commercially and within research articles needs to be addressed as currently 'love-in-a-mist' is inappropriately being used for both species, blurring the ability to discern between the two, as seen by the specimen-gallery of GBIF Secretariat (2023) (11) (105) (316) (387). As both species have formulations available to the public with *Nigella damascena* used for perfume and NS used for oral and topical administration (193) (196).

This small discrepancy could have real life consequences for patients around the world if not monitored, with cases similar to Fang-Ji's (*Stephania tetrandra*) which was confused for Guang-Fang-Ji (*Aristolochia fangchi*) causing more than 100 women to experience carcinogenic consequences (134).

Table 7: Scarcity of cancer related *Nigella sativa* related clinical-trials.

CLINICAL STUDY STATUS	TRIAL NUMBER
COMPLETED	23
CURRENTLY ACTIVE	2
NOT RECRUITING YET	3
RECRUITING	2
WITHDRAWN	1

The present condition of *in-vivo* clinical-trials involving *Nigella sativa* (n=31). Presently, none are related to cancer-treatment. Pakistan=5; Egypt=6; Saudi Arabia=5; Turkey=4; Indonesia=2; Bangladesh=2; Tunisia=1; Italy=2; Iran=1; United States of America=1; Unknown=2. (Source: 108). See 'Supplementary Materials' for exact profiling.

Table 8: Food and Drug Administration (FDA) Established patents of *Nigella sativa* in the United States (US) of America.

PATENT IDENTIFIER	DESCRIPTION	INVENTOR(S)
US 5,653,981	Inhibition of cancer cell growth	R. D. Medenica.
US 6,042,834	Diabetes	Wasif Baraka
US 5,482,711	Improvement of the Immune System	R. D. Medenica
US 6,841,174	Viral Infections	S. I. A. Shalaby and E. M. A. H. Allah
US 6,531,164	Psoriasis	H. H. R. Credé

(Adapted from: 224).

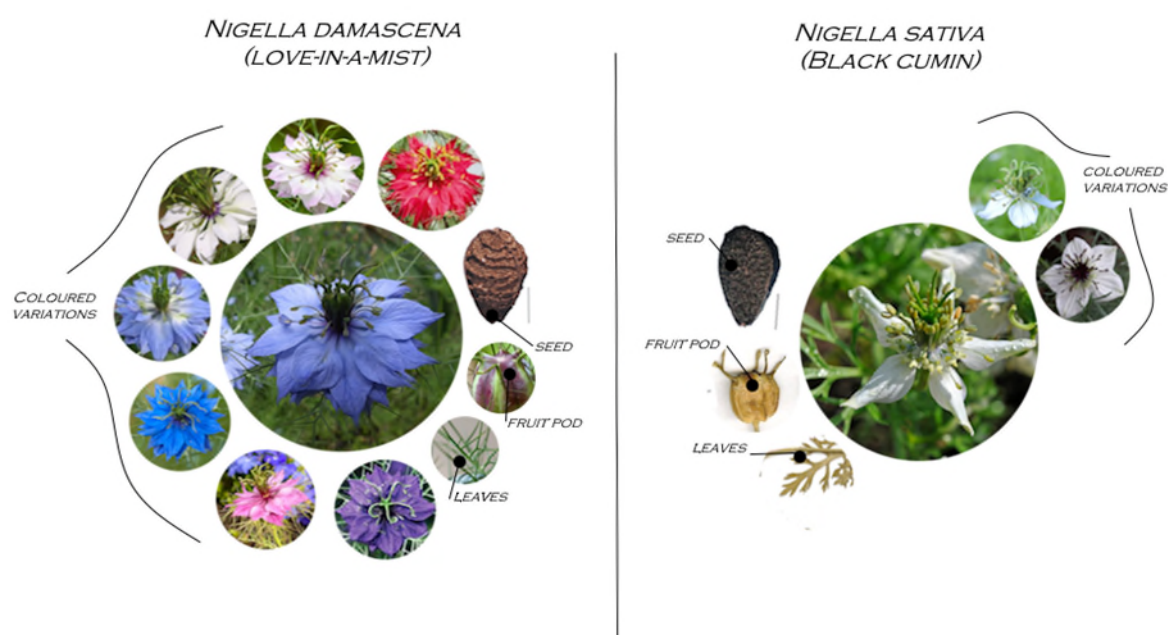


Figure 14: A phenotypic comparison between the oft-mistaken *Nigella sativa* (Black cumin) with *Nigella damascena* (Love-in-a-mist).

Discernment resources should be focused primarily upon botanic morphology such as, their dissimilar leaves, presence of conspicuous thin tract-structures (involucre) that surround the flower-head as well as the range of colours that each typically display - with *Nigella damascena* producing vivid coloured variants such as 'Persian rose' and deep blues with violet undertones, which is vastly different to the generally muted colour-tones of *Nigella sativa* (90, 2018) (352). (Adapted from: 79; 162; 169; 187; 243; 375; 335; 381; 448; 449; 353; 354).

6. Conclusion

To the best of our knowledge, the present study is the first meta-analysis and 'systematised-review' in regard to NS oil/extract-based mediums on cell viability modulation *in-vitro*. The data across the collated studies for both compounds suggest that NS has promising therapeutic potential, substantial dose-dependent reductions in cell-viability and a high-potency across many different cancer-types, shown by its ability to significantly reduce cancer-cell proliferation in all MTT-assays. Furthermore, our findings within the meta-analysis also suggests that within these potent-mediums the oil based types may be superior, compared to studies using extract-mediums; additionally the repeated selective-cytotoxic properties found within such oils supports the idea of it being a promising non-toxic 'stepping stone' for novel therapeutic derivatives that may both be utilised synergistically with present anti-cancer agents or possibly as a standalone clinical treatment in the future. However, further research is needed *in-vivo* to confirm the efficacious nature and elucidate the most appropriate method of administration to preserve its bioavailability; along with *in-vitro* evaluations for the most appropriate pre/post storage methods, molecular mechanisms of isolated molecules and refining-techniques - in regard to NS oil and its overall bio-composition on cell-viability on both normal and cancerous cell-lines - utilising multiple end-point assays and statistical approaches.

Word-count

7487 words are included (including the title, abstract, in-text-citations, necessary headings/sub-headings, introduction, and main body of text).

Ethical consideration

The following study was carried out and reported based on PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) with attention to its main guiding principles. The protocols used for performing the present study can be found under registration in the following website: PROSPERO website, www.crd.york.ac.uk/PROSPERO (PROSPERO registration number = CRD42022358471).

Funding

None.

Declaration of Competing Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Use of Artificial Intelligence

No generative AI or AI-assisted technologies were used in any stage of curation within this analytical project.

Data Availability

The dataset supporting the conclusions of this article is included within the article.

Author Contribution

The author read and approved the final manuscript.

Consent for Publication

Yes.

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All praise be to Allah, Lord of the worlds, Who Colours my perspective with true colouring. May peace and blessings be upon the noblest of the Prophets and Messengers, His final Prophet and Messenger Muhammad, and upon all his family and companions. To proceed:

I express my gratitude to my mother whose love, patience, strength, compassion and cultivation has left me with eternal debt; to my siblings, who continue to remind me of the multifaceted nature of intelligence; to my brothers and sisters around the world who remain steadfast, despite external and internal struggles; to my supervisor Dr. Robert Britton for his mentorship during the preliminary-stages of this project; to my tutor Dr. Celia May for her continuous academic-support; to my lecturers for imparting me knowledge; to all of the staff at The University of Leicester and to the bus-drivers for getting me to campus and back home again.

Allah Knows Best.

References

1. A. SMM, A. HE, A MJ, A MA. Response of *Nigella sativa* L. Plants to Humic acid and Seaweed extract treatments. IJCBS [Internet]. 2023 [cited 2026 June 9];24(12):828–37. Available from: <https://iscientific.org/wp-content/uploads/85-ijcbs-23-24-12-85n.pdf>
2. Abdalbasit Mariod. Biochemistry, Nutrition, and Therapeutics of Black Cumin Seed. Elsevier; 2022.
3. Abdel-Zaher AO, Abdel-Rahman MS, ELwasei FM. Protective effect of *Nigella sativa* oil against tramadol-induced tolerance and dependence in mice: Role of nitric oxide and oxidative stress. *NeuroToxicology*. 2011 Dec;32(6):725–33.
4. Abdulelah H, Zainal-Abidin B. In Vivo Anti-malarial Tests of *Nigella sativa* (Black Seed) Different Extracts. *American Journal of Pharmacology and Toxicology* [Internet]. 2007 [cited 2026 Jan 7];2(2):46–50. Available from: <https://thescipub.com/pdf/ajptsp.2007.46.50.pdf>
5. Aboul Ezz HS, Khadrawy YA, Noor NA. The Neuroprotective Effect of Curcumin and *Nigella sativa* Oil Against Oxidative Stress in the Pilocarpine Model of Epilepsy: A Comparison with Valproate. *Neurochemical Research*. 2011 July 13;36(11):2195–204.
6. Abubakar A, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences* [Internet]. 2020 Jan 29;12(1):1–10. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7398001/>
7. Acikgul FC, Duran N, Kutlu T, Ay E, Tek E, Bayraktar S. The therapeutic potential and molecular mechanism of Alpha-pinene, Gamma-terpinene, and P-cymene against melanoma cells. *Heliyon*. 2024 Sept;10(17):e36223.
8. Adil B, Tarik A, Kribii A, Ounine K. The Study Of The Insecticidal Effect Of *Nigella Sativa* Essential Oil Against *Tuta Absoluta* Larvae [Internet]. 2015 Oct [cited 2026 Jan 22]. Available from: <https://www.ijstr.org/final-print/oct2015/The-Study-Of-The-Insecticidal-Effect-Of-Nigella-Sativa-Essential-Oil-Against-Tuta-Absoluta-Larvae.pdf>
9. Aguilar-Hernández G, Zepeda-Vallejo LG, García-Magaña M de L, Vivar-Vera M de Á, Pérez-Larios A, Girón-Pérez MI, et al. Extraction of Alkaloids Using Ultrasound from Pulp and By-Products of Soursop Fruit (*Annona muricata* L.). *Applied Sciences* [Internet]. 2020 Jan 1;10(14):4869. Available from: <https://www.mdpi.com/2076-3417/10/14/4869>

10. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pacific Journal of Tropical Biomedicine [Internet]. 2013 May;3(5):337–52. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3642442/>

11. Ahmad MF, Ahmad FA, Ashraf SA, Saad HH, Wahab S, Khan MI, et al. An updated knowledge of Black seed (*Nigella sativa* Linn.): Review of phytochemical constituents and pharmacological properties. Journal of Herbal Medicine. 2021 Feb;25:100404.

12. Ahmad MF, Ahmad FA, Ashraf SA, Saad HH, Wahab S, Khan MI, et al. An updated knowledge of Black seed (*Nigella sativa* Linn.): Review of phytochemical constituents and pharmacological properties. Journal of Herbal Medicine. 2021 Feb;25:100404.

13. Ahmed JH, Ibraheem AY, Al-Hamdi KI. Evaluation of efficacy, safety and antioxidant effect of *Nigella sativa* in patients with psoriasis: A randomized clinical trial. Journal of Clinical and Experimental Investigations. 2014 June 11;5(2).

14. Ahmed M, Zakaria M, Mousumi M, Ami J. Effect of sowing time on growth and yield of black cumin ARTICLE HISTORY ABSTRACT. Int J Innov Res [Internet]. 2024 [cited 2026 June 12];9(2):2520–5919. Available from: [https://www.irsbd.org/papers/3._IJIR_9\(2\)_August_2024_4.pdf](https://www.irsbd.org/papers/3._IJIR_9(2)_August_2024_4.pdf)

15. Ahmed S, Ahmed N, Waheed A, Jaffar A. Anticataract Activity of Ethanolic Extract of *Nigella Sativa* on Glucose Induced Cataract in Goat Eye Lens. 2011 Dec.

16. Ahmed S, Ali MS, Nishigaki Y, Das R, Shiblu SA, Akter S, et al. Black Cumin Seed (*Nigella sativa*) Confers Anti-Adipogenic Effects in 3T3-L1 Cellular Model and Lipid-Lowering Properties in Human Subjects. Food Science & Nutrition. 2025 Sept;13(9).

17. Ahrefs. Organic traffic of <https://pubmed.ncbi.nlm.nih.gov/> [Internet]. Ahrefs. 2024 [cited 2026 Jan 16]. Available from: <https://ahrefs.com/traffic-checker/?input=https%3A%2F%2Fpubmed.ncbi.nlm.nih.gov%2F&mode=subdomains>

18. Akerele O. Medicinal Plants: Policies and Priorities. Conservation of Medicinal Plants [Internet]. 1991 July 26 [cited 2026 Jan 9];3–12. Available from: <https://resolve.cambridge.org/core/books/abs/conservation-of-medicinal-plants/medicinal-plants-policies-and-priorities/8CD839FAE87F74FA1888877787B51A27>

19. Aksoy F, Dogan R, Yenigun A, Veyseller B, Ozturan O, Ozturk B. Thymoquinone treatment for inner-ear acoustic trauma in rats. *The Journal of Laryngology & Otology* [Internet]. 2015 Jan 1 [cited 2026 Jan 21];129(1):38–45. Available from: <https://www.cambridge.org/core/journals/journal-of-laryngology-and-otology/article/thymoquinone-treatment-for-innerear-acoustic-trauma-in-rats/D5E25B4F46D21C9294EBCEFC9567C0A3>
20. Al-Amri AM, Bamosa AO. Phase I Safety and Clinical Activity Study of Thymoquinone in Patients with Advanced Refractory Malignant Disease. *Shiraz E-Medical Journal* [Internet]. 2009 Aug 31 [cited 2026 Jan 15];10(3):107–11.e76453. Available from: <https://brieflands.com/journals/semj/articles/76453>
21. Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. *Journal of Ethnopharmacology*. 2001 June;76(1):45–8.
22. Al-Hader A, Aqel M, Hasan Z. Hypoglycemic Effects of the Volatile Oil of *Nigella sativa* Seeds. *International Journal of Pharmacognosy*. 1993 Jan;31(2):96–100.
23. Al-Oqail MM, Al-Sheddi ES, Al-Massarani SM, Siddiqui MA, Ahmad J, Musarrat J, et al. *Nigella sativa* seed oil suppresses cell proliferation and induces ROS dependent mitochondrial apoptosis through p53 pathway in hepatocellular carcinoma cells. *South African Journal of Botany*. 2017 Sept;112:70–8.
24. Al-Oqail MM, Al-Sheddi ES, Al-Massarani SM, Siddiqui MA, Ahmad J, Musarrat J, et al. *Nigella sativa* seed oil suppresses cell proliferation and induces ROS dependent mitochondrial apoptosis through p53 pathway in hepatocellular carcinoma cells. *South African Journal of Botany*. 2017 Sept;112:70–8.
25. Al-Rawashde FA, Al-wajeeh AS, Vishkaei MN, Saad HKM, Johan MF, Taib WRW, et al. Thymoquinone Inhibits JAK/STAT and PI3K/Akt/ mTOR Signaling Pathways in MV4-11 and K562 Myeloid Leukemia Cells. *Pharmaceuticals* [Internet]. 2022 Sept 1;15(9):1123. Available from: <https://www.mdpi.com/1424-8247/15/9/1123>
26. Al-Sheddi ES, Farshori NN, Al-Oqail MM, Musarrat J, Al-Khedhairi AA, Siddiqui MA. Cytotoxicity of *Nigella Sativa* Seed Oil and Extract Against Human Lung Cancer Cell Line. *Asian Pacific Journal of Cancer Prevention*. 2014 Jan 30;15(2):983–7.
27. Alamri AM, Assiri AA, Khan NU. Nigellidine from *Nigella sativa* Targets AKT1 and GAPDH in rectal cancer: Insights from network pharmacology and molecular dynamics simulations. *Current Pharmaceutical Analysis*. 2026 Mar;22(2):117–33.

28. AlAttas S, Zahran F, Turkistany S. *Nigella sativa* and its active constituent thymoquinone in oral health. *Saudi Medical Journal*. 2016 Mar 1;37(3):235–44.
29. Alaufi OM, Noorwali A, Zahran F, Al-Abd AM, Al-Attas S. Cytotoxicity of thymoquinone alone or in combination with cisplatin (CDDP) against oral squamous cell carcinoma in vitro. *Scientific Reports*. 2017 Oct 13;7(1).
30. Alberts A, Moldoveanu ET, Niculescu AG, Grumezescu AM. *Nigella sativa*: A Comprehensive Review of Its Therapeutic Potential, Pharmacological Properties, and Clinical Applications. *International Journal of Molecular Sciences* [Internet]. 2024 Dec 14;25(24):13410–0. Available from: <https://www.mdpi.com/1422-0067/25/24/13410#sec2-ijms-25-13410>
31. Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D, et al. Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. *Cancer Cell International* [Internet]. 2015 July 15;15(1). Available from: <https://cancer-ci.biomedcentral.com/articles/10.1186/s12935-015-0221-1>
32. Ali B, Amin S, Ahmad J, Ali A, Ali M, Mir SR. Bioavailability enhancement studies of amoxicillin with *Nigella* [Internet]. *Nih.gov*. 2024 [cited 2026 June 11]. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC3385243/>
33. Ali E, Hassan F. Bio-production of *Nigella sativa* L. seeds and oil in Taif area | كلية الزراعة [Internet]. *Aun.edu.eg*. 2021 [cited 2026 June 12]. Available from: <https://b.aun.edu.eg/agriculture/ar/node/35701>
34. Ali M, Ahmed H, Abdelbaset S, Bakry S. The cytotoxic potential for *Syzygium aromaticum* and *Nigella sativa* essential oil compared to doxorubicin against pancreatic, colonic, and cervical cancer cell lines. *Egyptian Journal of Chemistry*. 2023 Aug 12;0(0):0–0.
35. Ali M, Saeed A, Bamaga O. Effectiveness of Six Selected Medicinal Plants Against Headlice, *Pediculus humanus capitis*: an in Vitro Study. *Hadramout University Journal of Natural & Applied Science*. 2025;22(1):2025.
36. Alkharfy KM, Ahmad A, Rao KMA, Al-Shagha WM. Pharmacokinetic plasma behaviors of intravenous and oral bioavailability of thymoquinone in a rabbit model. *European Journal of Drug Metabolism and Pharmacokinetics*. 2014 June 13;40(3):319–23.
37. Alshwyyeh HA, Aldosary SK, Ilowefah MA, Shahzad R, Shehzad A, Bilal S, et al. Biological Potentials and Phytochemical Constituents of Raw and Roasted *Nigella arvensis* and *Nigella sativa*. *Molecules* [Internet]. 2022 Jan 16;27(2):550. Available from:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8779992/#:~:text=The%20phytochemicals%20present%20in%20the>

38. Alta AH, Ewadh MJ, Zaidan HK. Hormonal Contents of Two Types of Black Seed (*Nigella sativa*) Oil: Comparative Study. *Medical Journal of Babylon* [Internet]. 2006 June 30;3(1–2):17–22. Available from:
https://www.researchgate.net/publication/232808421_Hormonal_Contents_of_Two_Types_of_Black_Seed_Nigella_sativa_Oil_Comparative_Study
39. Alzahrani MA, Abunohaiah I, Altuwaijry Y, Alahmadi M, Qasim O, Alzahrani A, et al. Effect of Black Cumin (*Nigella sativa*) on Prostate Health: Narrative Review. *F1000Research* [Internet]. 2025 Feb 21 [cited 2025 May 28];13:229–9. Available from:
<https://f1000research.com/articles/13-229>
40. Amjed S, Junaid K, Jafar J, Amjad T, Maqsood W, Mukhtar N, et al. Detection of antibacterial activities of Miswak, Kalonji and Aloe vera against oral pathogens & anti-proliferative activity against cancer cell line. *BMC Complementary and Alternative Medicine*. 2017 May 15;17(1).
41. Amman Panorama Art Gallery. رَبَائِيْ آلاءَ رَبِّكُمَا تُكَذِّبَانِ Details - Amman Panorama Art Gallery [Internet]. ammanpanoramaartgallery.com. 2026 [cited 2026 June 7]. Available from:
<https://ammanpanoramaartgallery.com/en/Products/Single/%D9%81%D9%8E%D8%A8%D9%90%D8%A3%D9%8E%D9%8A%D9%91%D9%90-%D8%A2%D9%84%D9%8E%D8%A7%D8%A1%D9%90-%D8%B1%D9%8E%D8%A8%D9%91%D9%90%D9%83%D9%8F%D9%85%D9%8E%D8%A7-%D8%AA%D9%8F%D9%83%D9%8E%D8%B0%D9%91%D9%90%D8%A8%D9%8E%D8%A7%D9%86%D9%90>
42. Amooaghaie R, Saeri MR, Azizi M. Synthesis, characterization and biocompatibility of silver nanoparticles synthesized from *Nigella sativa* leaf extract in comparison with chemical silver nanoparticles. *Ecotoxicology and Environmental Safety*. 2015 Oct;120:400–8.
43. Andre H. *Nigella sativa* flower [Internet]. 2010 [cited 2026 Jan 13]. Available from:
<https://commons.wikimedia.org/wiki/File:Nsativa001Wien.jpg>
44. Andreas G. H. Fig 3 [Internet]. 2011 [cited 2026 Jan 13]. Available from:
https://www.researchgate.net/figure/Light-microscope-images-of-Nigella-species-investigated-A-Nigella-nigellastrum-B_fig3_230745173

45. Anwar N, Malik MNH, Atif M, Alanzi AR, Alharbi HA, Younis W, et al. p-Cymene Targets Multiple Oncogenic Pathways in Hepatocellular Carcinoma: Insights From Network Pharmacology and In Vitro Studies. *Food Science & Nutrition*. 2025 Oct;13(10).
46. Aqel M, Shaheen R. Effects of the volatile oil of *Nigella sativa* seeds on the uterine smooth muscle of rat and guinea pig. *Journal of Ethnopharmacology*. 1996 May;52(1):23–6.
47. Araya M, Tesfay GB, Altaye SZ, Mengesha B, Beyene G, Gebremeskel Y. Evaluation of pollination modes on *Nigella sativa* yield and seed quality, with analysis of farmer practices in Northern Ethiopia. *Discover Agriculture*. 2026 May 29;4(1).
48. Arif R, Bukhari SA, Mustafa G, Ahmed S, Albeshr MF. Network Pharmacology and Experimental Validation to Explore the Potential Mechanism of *Nigella sativa* for the Treatment of Breast Cancer. *Pharmaceuticals*. 2024 May 10;17(5):617.
49. Armstrong RA. POST-HOC TESTING IN OPHTHALMIC RESEARCH [Internet]. 2021. Available from: https://www.researchgate.net/publication/355394415_POST-HOC_TESTING_IN_OPHTHALMIC_RESEARCH
50. Arrive Guidelines. Sample size | ARRIVE Guidelines [Internet]. arriveguidelines.org. Available from: <https://arriveguidelines.org/arrive-guidelines/sample-size/2b/explanation>
51. Arslan B, Isik F, Gur H, Ozen F, Catal T. Apoptotic effect of *Nigella sativa* on human lymphoma U937 cells. *Pharmacognosy Magazine [Internet]*. 2017 [cited 2025 May 17];13(51):628. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC5669107/>
52. Arslan D, Acar A. Effects of different postharvest storage conditions of black cumin seeds on the oxidative stability of cold pressed oil [Internet]. 2024 Feb [cited 2026 May 25]. Available from: https://www.innovhub-ssi.it/kdocs/2169009/2024_risg_3_arслан_p165.pdf
53. AS Deena H, Musharraf Hussain M. Prophetic Chemo Is the Safest, Cheapest and Most Effective Alternative to Modern Chemotherapy for the Cure of all Forms of Cancers – A Review. *Journal of Cancer Science & Therapy*. 2018;10(05).
54. Asritha C, Kambrekar DN, Kandakoor SB, Hiremath SM, Ramesha NM. Comparative Analysis of Pollinator Diversity, Abundance, and Foraging Activity Patterns on Fennel (*Foeniculum vulgare*) and Black Cumin (*Nigella sativa*) in the Northern Transitional Zone of Karnataka, India. *International Journal of Bio-resource and Stress Management [Internet]*.

2025 Oct 17 [cited 2026 June 9];16(Oct, 10):01–7. Available from:
<https://doi.org/10.23910/1.2025.6299>

55. ATCC. MTT Cell Proliferation Assay [Internet]. 2011 [cited 2026 Jan 15]. Available from:
<https://www.atcc.org/-/media/product-assets/documents/instruction-sheets/multicomponent-products/mtt-cell-proliferation-assay.pdf?rev=b028c9a0390841afa2b4599e604e9839>
56. Ayaz E, Yilmaz H, Ozbek H, Tas Z, Orunc O. The effect of *Nigella sativa* oil against *Aspiculuris tetraptera* and *Hymenolepis nana* in naturally infected mice. 2007 June.
57. Baig WA, Alwosaibai K, Al-Jubran KM, Chaudhry TM, Al-Dowish N, Alsaffar F, et al. Synergistic anti-cancer effects of *Nigella sativa* seed oil and conventional cytotoxic agent against human breast cancer. *Drug Metabolism and Personalized Therapy*. 2022 Apr 11;37(3):315–21.
58. Bakrim S, El Omari N, El Hachlafi N, Bakri Y, Lee LH, Bouyahya A. Dietary Phenolic Compounds as Anticancer Natural Drugs: Recent Update on Molecular Mechanisms and Clinical Trials. *Foods*. 2022 Oct 23;11(21):3323.
59. Balbay A, Kaya Y, Sahin O. Drying of black cumin (*Nigella sativa*) in a microwave assisted drying system and modeling using extreme learning machine. *Energy*. 2012 Aug;44(1):352–7.
60. Banerjee S, Pandey S, Mukherjee P, Sayeed A, Pandurangi AV, George S, et al. Investigation of cytotoxicity induced by *Nigella sativa* and *Azadirachta indica* using MDA-MB-231, HCT 116 and SHSY5Y cell lines. *Pharmacognosy Journal*. 2017 Mar 5;9(2):192–5.
61. Banerjee S, Pandey S, Mukherjee P, Sayeed A, Pandurangi AV, George S, et al. Investigation of cytotoxicity induced by *Nigella sativa* and *Azadirachta indica* using MDA-MB-231, HCT 116 and SHSY5Y cell lines. *Pharmacognosy Journal*. 2017 Mar 5;9(2):192–5.
62. Barmanray A, Kaushik N, Nyemb JN, Yadav A, Luther M. Study on extraction and surface coating of black cumin (*Nigella sativa* L.) seed oil on the shelf life of lemons. *Food Materials Research*. 2024 Jan 1;4(1):0–0.
63. Barnett V, Lewis T. *Outliers in statistical data*. Chichester: Wiley; 1994.

64. Barut M, Cavdar AS, Tansı LS, Karaman Ş. Yield and Quality Traits of Black Cumin (*Nigella sativa* L.) Genotypes in Response to the Different Sowing Dates. *Turkish Journal of Agriculture - Food Science and Technology*. 2023 Dec 27;11(12):2276–87.
65. Bashyal R, Roberts DL. Assessing the identification uncertainty in plant products traded as traditional Asian medicines. *Journal for Nature Conservation*. 2023 June;73:126410.
66. Baskin CC, Baskin JM. A revision of Martin's seed classification system, with particular reference to his dwarf-seed type. *Seed Science Research*. 2007 Mar;17(1):11–20.
67. Baskin JM, Baskin CC. The great diversity in kinds of seed dormancy: a revision of the Nikolaeva–Baskin classification system for primary seed dormancy. *Seed Science Research*. 2021 Dec 1;1–29.
68. Begum S, Mannan A. A Review on *Nigella sativa*: A Marvel Herb. *Journal of Drug Delivery and Therapeutics*. 2020 Mar 15;10(2):213–9.
69. Beheshti F, Majid Khazaei, Hosseini M. Neuropharmacological effects of *Nigella sativa*. *Avicenna Journal of Phytomedicine* [Internet]. 2016 Jan;6(1):104. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC4884225/>
70. Bekalu Y, Kitaw SL, Manayia AH, Asmare NH. Analysis of *Nigella sativa* L. (Black Cumin) seeds for levels of heavy metals using FAAS: geospatial profiling and regional safety implications. *Discover Chemistry* [Internet]. 2025 Sept 25;2(1). Available from: https://www.researchgate.net/publication/395871287_Analysis_of_Nigella_sativa_L_Black_Cumin_seeds_for_levels_of_heavy_metals_using_FAAS_geospatial_profiling_and_regional_safety_implications
71. Bekinwari BM R., Boate U R. Insecticidal Property of Black Seed (*Nigella sativa*) Powder as an Eco-friendly Management of Skin Beetle *Dermestes maculatus* (Coleoptera: Dermestidae) in Atlantic Codfish *Gadus morhua* (Gadiformes: Gadidae). *Asian Journal of Advances in Agricultural Research*. 2020 June 27;47–56.
72. Bektaş ZH, Savaşer S, Akçimen U, Ceylan M, Yener O, Bulut C. Using of Black Cumin Seed Powder (*Nigella sativa*) as Immunostimulant and Growth Promoter in Rainbow Trout, *Oncorhynchus mykiss* (Walbaum). *Turkish Journal of Fisheries and Aquatic Sciences* [Internet]. 2019 May 28 [cited 2026 Jan 21];19(12):987–99. Available from: <https://www.trjfas.org/abstract.php?lang=en&id=1403>
73. benchchem. Nigellidine: A Technical Guide to its Discovery, Isolation, and Characterization from *Nigella sativa* [Internet]. 2026 [cited 2026 June 6]. Available from:

https://pdf.benchchem.com/12853/Nigellidine_A_Technical_Guide_to_its_Discovery_Isolation_and_Characterization_from_Nigella_sativa.pdf

74. benchchem. Thymoquinone: A Technical Guide to its Discovery, Isolation from *Nigella sativa*, and Molecular Mechanisms Discovery and Historical Context [Internet]. 2026 [cited 2026 June 5]. Available from: https://pdf.benchchem.com/1261/Thymoquinone_A_Technical_Guide_to_its_Discovery_Isolation_from_Nigella_sativa_and_Molecular_Mechanisms.pdf
75. Berkkan A, Süntar I, Erdem SA, Şenol Deniz FS, Atak M. Fatty Acid Profiling and Thymoquinone Quantification of Cold-Pressed Oils from Turkish- and Syrian-Originated *Nigella sativa* Seeds. *Natural Products Analysis*. 2025 Nov 13;1(1).
76. Bharadwaj M, Mounica Vallurupalli, Huang FW. Global Precision Oncology: A Call to Action on Expanding Access to Targeted Cancer Therapies. *The Oncologist* [Internet]. 2021 Feb 5;26(5):353–5. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8100554/?utm_source=chatgpt.com
77. Bhatti IU, Rehman FU, Khan MA, Marwat SK. Effect of prophetic medicine kalonji (*Nigella sativa* L.) on lipid profile of human beings. An in vivo approach. *World Applied Sciences Journal* [Internet]. 2009 Jan 1;6(8):1053–7. Available from: https://www.researchgate.net/publication/284561690_Effect_of_prophetic_medicine_kalonji_Nigella_sativa_L_on_lipid_profile_of_human_beings_An_in_vivo_approach
78. Bhutani V, Varzideh F, Wilson S, Kansakar U, Jankauskas SS, Santulli G. Doxorubicin-Induced Cardiotoxicity: A Comprehensive Update. *Journal of Cardiovascular Development and Disease* [Internet]. 2025 May 30;12(6):207. Available from: <https://www.mdpi.com/2308-3425/12/6/207>
79. Bishy Barnabees Cottage Garden. *Nigella Mulberry Rose (Love-in-a-mist)* [Internet]. Bishy Barnabees Cottage Garden Ltd. 2025 [cited 2026 Jan 25]. Available from: <https://www.bishybarnabeescottagegarden.com/products/nigella-mulberry-rose-seeds>
80. Blair RH, Trichler DL, Gaille DP. Mathematical and Statistical Modeling in Cancer Systems Biology. *Frontiers in Physiology*. 2012;3.
81. Boskabady MH, Farhadi J. The Possible Prophylactic Effect of *Nigella sativa* Seed Aqueous Extract on Respiratory Symptoms and Pulmonary Function Tests on Chemical War Victims: A Randomized, Double-Blind, Placebo-Controlled Trial. *The Journal of Alternative and Complementary Medicine*. 2008 Nov;14(9):1137–44.

82. Boskabady MH, Mohsenpoor N, Takaloo L. Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients. *Phytomedicine*. 2010 Aug;17(10):707–13.
83. Botanical-Online. *Nigella* Properties and Toxicity [Internet]. Botanical online. Botanical-online; 2019 [cited 2026 Jan 5]. Available from: <https://www.botanical-online.com/en/medicinal-plants/aranuela-toxicity>
84. Bouguezza Y, Khettal B, Tir L, Boudrioua S. Damascenine induced hepatotoxicity and nephrotoxicity in mice and *in vitro* assessed human erythrocyte toxicity. *Interdisciplinary Toxicology* [Internet]. 2015 Sept 1 [cited 2026 Jan 5];8(3):118–24. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC4961907/>
85. Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Marzouk B. Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. *Comptes Rendus Biologies*. 2008 Jan;331(1):48–55.
86. Braak EWMT, Evers IM, Willem Erkelens D, Visser GHA. Maternal hypoglycemia during pregnancy in type 1 diabetes: maternal and fetal consequences. *Diabetes/Metabolism Research and Reviews*. 2002;18(2):96–105.
87. Branca JJV, Pacini A, Gulisano M, Taddei N, Fiorillo C, Becatti M. Cadmium-Induced Cytotoxicity: Effects on Mitochondrial Electron Transport Chain. *Frontiers in Cell and Developmental Biology*. 2020 Nov 30;8.
88. Bryan N. Is Outlier Misidentification a Problem in Bioassay? - Quantics Biostatistics [Internet]. Quantics Biostatistics. 2025 [cited 2026 June 5]. Available from: <https://www.quantics.co.uk/blog/outlier-misidentification/>
89. Bulathsinhala H, Srimali N, Rajapaksha S. Enhanced Thymoquinone Recovery from Cold-Pressed Black Seeds (*Nigella sativa* L.) through Freeze-Thaw and Microwave Pretreatment Techniques. *Turkish Journal of Agriculture - Food Science and Technology* [Internet]. 2025 Apr 27;13(4):927–33. Available from: <https://agrifoodscience.com/index.php/TURJAF/article/view/7351>
90. Bumbleseeds. Cumin From Confusion to Clarity on Black Cumin Botanicals [Internet]. Bumbleseeds. 2018 [cited 2026 Jan 5]. Available from: <https://bumbleseeds.com/blogs/cumin-from-confusion-to-clarity-on-black-cumin-botanicals/tagged/edible>

91. Burdock GA. Assessment of black cumin (*Nigella sativa* L.) as a food ingredient and putative therapeutic agent. *Regulatory Toxicology and Pharmacology*. 2022 Feb;128:105088.
92. Bustamam MSA, Hadithon KA, Mediani A, Abas F, Rukayadi Y, Lajis N, et al. Stability Study of Algerian *Nigella sativa* Seeds Stored under Different Conditions. *Journal of Analytical Methods in Chemistry*. 2017 Jan 1;2017:1–12.
93. Butt MS, Imran M, Imran A, Arshad MS, Saeed F, Gondal TA, et al. Therapeutic perspective of thymoquinone: A mechanistic treatise. *Food Science & Nutrition*. 2021 Jan 28;9(3):1792–809.
94. Cahyo SD, Kurniawati A, Faridah DN, Ghulamahdi M. Growth, Production and Bioactive Content of Several Black Cumin (*Nigella sativa* L.) Accessions With Different Harvesting Times in a D3 Type Climate Regime. *Journal of Tropical Crop Science* [Internet]. 2020 Oct 15 [cited 2026 June 12];7(03):104–12. Available from: <https://pdfs.semanticscholar.org/74fa/b77f02c8c44b02d3aef2c6f32ca36ea49e3d.pdf>
95. Camp JE, Nyamini SB, Scott FJ. Cyrene™ is a green alternative to DMSO as a solvent for antibacterial drug discovery against ESKAPE pathogens. *RSC Medicinal Chemistry*. 2020;11(1):111–7.
96. CARLONI M. In Vitro Assays to Study The Hallmarks of Cancer [Internet]. QIMA Life Sciences. 2023. Available from: <https://qima-lifesciences.com/blog/blog-pharmacology/in-vitro-assays-hallmarks-of-cancer/>
97. Carroll JF, Babish JG, Pacioretty LM, Kramer M. Repellency to ticks (Acari: Ixodidae) of extracts of *Nigella sativa* (Ranunculaceae) and the anti-inflammatory DogsBestFriend™. *Experimental and Applied Acarology*. 2016 July 9;70(1):89–97.
98. Carré P, Berthold S, Piofczyk T, Bothe S, Hadjiali S. Solvent solutions: comparing extraction methods for edible oils and proteins in a changing regulatory landscape. Part 1: Physical-properties. *OCL* [Internet]. 2024 [cited 2025 Mar 11];31(OCL):31. Available from: https://www.ocl-journal.org/articles/ocl/full_html/2024/01/ocl240033/ocl240033.html
99. CD Genomics. Traditional vs. Modern Seed Identification Methods: An Overview [Internet]. Cd-genomics.com. 2021. Available from: <https://www.cd-genomics.com/agri/resource-seed-identification-traditional-modern-method.html>

- 10 Cell Signaling Technology. Protein and Pathway Abbreviations [Internet]. Cell Signaling Technology. 2026 [cited 2026 June 5]. Available from: <https://www.cellsignal.com/learn-and-support/reference-tables/protein-and-pathway-abbreviations>
- 10 Center for Food Safety. Center for Food Safety | Pharmaceutical Crops | | About
1. Pharmaceutical Crops [Internet]. Center for Food Safety. 2026 [cited 2026 June 9]. Available from: <https://www.centerforfoodsafety.org/issues/311/ge-foods/pharmaceutical-crops#>
- 10 Chathoth S, Nawaz M, Amir M, Ahmed R, Aldholmi M, Al-Mofty S, et al. Assessment of the
2. effect of solvent polarity on *Nigella sativa* extracts of different origin. *Journal of Pharmacy & Pharmacognosy Research*. 2025 May 19;13(5):1345–55.
- 10 Chehl N, Chipitsyna G, Gong Q, Yeo CJ, Arafat HA. Anti-inflammatory effects of the *Nigella*
3. *sativa* seed extract, thymoquinone, in pancreatic cancer cells. *HPB*. 2009 Aug;11(5):373–81.
- 10 Cheméo. p-Cymene (CAS 99-87-6) [Internet]. Cheméo. 2026 [cited 2026 June 5].
4. Available from: <https://www.chemeo.com/cid/70-441-4/p-Cymene>
- 10 Chiltern Seeds. *Nigella sativa* Seeds [Internet]. Chilternseeds.co.uk. 2023 [cited 2026 Jan
5. 18]. Available from: https://www.chilternseeds.co.uk/item_903e_nigella_sativa_seeds
- 10 Christie's. ABU 'ALI AL-HUSAYN IBN 'ABDULLAH IBN SINA (AVICENNA) (D. AH 428/1037
6. AD): AL-QANUN FI AL-TIBB, ABBASID BAGHDAD, 12TH CENTURY | Christie's [Internet]. Christies.com. Christie's; 2020 [cited 2026 June 12]. Available from: <https://www.christies.com/en/lot/lot-6162843>
- 10 Cleveland Clinic. Serotonin [Internet]. Cleveland Clinic. 2022. Available from:
7. <https://my.clevelandclinic.org/health/articles/22572-serotonin>
- 10 ClinicalTrials.gov. Search Results [Internet]. Clinicaltrials.gov. 2026 [cited 2026 Jan 9].
8. Available from: <https://clinicaltrials.gov/search?cond=nigella%20sativa>
- 10 CONN Herbarium, University of Connecticut Herbarium . *Nigella sativa*. [Internet].
9. <https://gobotany.nativeplanttrust.org/species/nigella/sativa/>. 2026 [cited 2026 Jan 11]. Available from: <https://gobotany.nativeplanttrust.org/species/nigella/sativa/>
- 11 Cooper BT, Yan X, Simón-Manso Y, Tchekhovskoi DV, Mirokhin YA, Stein SE. Hybrid Search:
0. A Method for Identifying Metabolites Absent from Tandem Mass Spectrometry Libraries.

Analytical Chemistry [Internet]. 2019 Oct 10 [cited 2026 May 31];91(21):13924–32. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC7299168/>

11 CR UK. All cancers combined statistics [Internet]. Cancer Research UK. CRUK; 2025.

1. Available from: <https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/all-cancers-combined>

11 Crown Bioscience. Crown Bioscience [Internet]. Crownbio.com. 2026 [cited 2026 June

2. 10]. Available from: <https://www.crownbio.com/technologies/in-vitro-high-content-imaging/3d-ex-vivo-patient-tissue-platform>

11 Culhaoglu B, Erbek SS, Erbek S, Hizal E. Protective Effect of *Nigella sativa* Oil on Acoustic

3. Trauma Induced Hearing Loss in Rats. *Audiology Research*. 2017 July 18;7(2):181.

11 Cytiva. Cytiva [Internet]. Cytivalifesciences.com. 2022 [cited 2026 June 10]. Available

4. from: <https://www.cytivalifesciences.com/en/us/products/category/cell-culture-and-fermentation/sera/engineered-serum-and-alternatives>

11 Dabeer S, Rather MA, Rasool S, Rehman MU, Alshahrani S, Jahan S, et al. History and

5. traditional uses of black seeds (*Nigella sativa*). *Pharmacological and Therapeutic Applications*. 2022 Jan 1;1–28.

11 Dagtas AS, Griffin RJ. *Nigella sativa* extract kills pre-malignant and malignant oral

6. squamous cell carcinoma cells. *Journal of Herbal Medicine*. 2021 Oct;29:100473.

11 Dam Seeds. Cumin, Black Cumin [Internet]. [cited 2026 Jan 13]. Available from:

7. <https://www.damseeds.com/products/black-cumin>

11 Daneshyar R, Zandi M, Sharifnia F, Sanjabi MR, Ghaedrahmati A. Evaluation of Cytotoxic

8. Effects and Selectivity of *Nigella sativa* Extracts on MCF-7 and HDF Cell Lines. *Gene, Cell and Tissue* [Internet]. 2026 Feb 18 [cited 2026 May 26];13(1). Available from: <https://brieflands.com/journals/gct/articles/169056>

11 Darakhshan S, Bidmeshki Pour A, Hosseinzadeh Colagar A, Sisakhtnezhad S.

9. Thymoquinone and its therapeutic potentials. *Pharmacological Research*. 2015 May;95–96:138–58.

12 Das S, Ghosh A, Upadhyay P, Sarker S, Bhattacharjee M, Gupta P, et al. A mechanistic

0. insight into the potential anti-cancerous property of *Nigella sativa* on breast cancer through micro-RNA regulation: An in vitro & in vivo study. *Fitoterapia*. 2023 Sept;169:105601.

- 12 Das S, Ghosh A, Upadhyay P, Sarker S, Bhattacharjee M, Gupta P, et al. A mechanistic insight into the potential anti-cancerous property of *Nigella sativa* on breast cancer through micro-RNA regulation: An in vitro & in vivo study. *Fitoterapia*. 2023 Sept;169:105601.
- 12 Davis JM. Assisting Farmers to Produce High-quality Medicinal Herbs. *HortScience*. 2012 Aug;47(8):976–8.
- 12 Department for Environment, Food & Rural Affairs. Biodiverse Landscapes Fund [Internet]. 3. GOV.UK. 2021. Available from: <https://www.gov.uk/government/publications/biodiverse-landscapes-fund>
- 12 Department for Environment, Food & Rural Affairs. Farming Equipment and Technology Fund now open – The Farming Blog [Internet]. *Blog.gov.uk*. 2025 [cited 2026 June 9]. Available from: <https://defrafarming.blog.gov.uk/2025/05/29/farming-equipment-and-technology-fund-now-open/>
- 12 Desai A, Vishwanath YC, Narayanapur VB, Reddi SG, Naik JN. EFFECT OF SOWING DATES AND PLANT SPACING ON GROWTH AND YIELD PARAMETERS OF BLACK CUMIN (*NIGELLA SATIVA* L.) UNDER NORTHERN DRY ZONE OF KARNATAKA INDIA. *Plant Archives*. 2024 Oct 5;25(1).
- 12 Dettori JR, Norvell DC, Chapman JR. Fixed-Effect vs Random-Effects Models for Meta-Analysis: 3 Points to Consider. *Global Spine Journal*. 2022 June 20;12(7):219256822211105.
- 12 Doğan S, Özek T, Aytar Çelik P. Impact of various extraction and storage conditions on black cumin seed oil. *Food chemistry* [Internet]. 2025;496(Pt 1):146701. Available from: <https://pubmed.ncbi.nlm.nih.gov/41076879/>
- 12 Dugé de Bernonville T, Papon N, Clastre M, O'Connor SE, Courdavault V. Identifying Missing Biosynthesis Enzymes of Plant Natural Products. *Trends in Pharmacological Sciences*. 2020 Mar;41(3):142–6.
- 12 Durazzo A, Ramadan MF, Stefano V Di, Lucarini M. Editorial: Cold Pressed Oils: A Green Source of Specialty Oils, volume II. *Frontiers in Nutrition*. 2023 June 9;10.
- 13 Edelman LB, Eddy JA, Price ND. In silico models of cancer. *Wiley interdisciplinary reviews Systems biology and medicine* [Internet]. 2010;2(4):438–59. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3157287/>

- 13 Effenberger KE, Breyer S, Schobert R. Terpene Conjugates of the *Nigella sativa* Seed-Oil
1. Constituent Thymoquinone with Enhanced Efficacy in Cancer Cells. *Chemistry & Biodiversity*. 2010 Jan 1;7(1):129–39.
- 13 Eguren-Santamaria I, Melero I, Rodríguez I, Ortego I, Armero M, Galvez-Cancino F, et al.
2. Preclinical ex vivo and in vivo models to study immunotherapy agents and their combinations as predictive tools toward the clinic. *Journal for ImmunoTherapy of Cancer* [Internet]. 2025 Oct;13(10):e011279. Available from: <https://jitc.bmj.com/content/13/10/e011279>
- 13 Eid AM, Elmarzugi NA, Abu Ayyash LM, Sawafta MN, Daana HI. A Review on the
3. Cosmeceutical and External Applications of *Nigella sativa*. *Journal of Tropical Medicine* [Internet]. 2017 [cited 2021 Jan 3];2017. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5735686/#sec7title>
- 13 Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and
4. challenges in monitoring safety. *Frontiers in Pharmacology* [Internet]. 2014 Jan 10;4(177). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3887317/>
- 13 El-Dakhakhny M, Mady N, Lember N, Ammon HPT. The Hypoglycemic Effect of *Nigella*
5. *sativa* Oil is Mediated by Extraparacrine Actions. *Planta Medica*. 2002 May;68(5):465–6.
- 13 El-Mahrouk ME, Maamoun MK, Dewir YH, El-Banna AN, Rihan HZ, Salamh A, et al.
6. Synchronized Seed Germination and Seedling Growth of Black Cumin. *HortTechnology*. 2022 Apr;32(2):182–90.
- 13 El-Naggar S, Abou-Ward G, El-Badawi A, Ali A. Commercial oil of *Nigella sativa* as growth
7. promoter in lambs rations سعاد ١. النجار زيت حبة البركة التجاري كمحفز للنمو في علائق الاغنام .*Journal of Veterinary Sciences* [Internet]. 2018 [cited 2026 Jan 23];32(2):199–204 <https://pdfs.semanticscholar.org/9a48/2dfa789daa9fd0989236cd7f3a5d820ab9c4.pdf>
- 13 El-Najjar N, Chatila M, Moukadem H, Vuorela H, Ocker M, Gandesiri M, et al. Reactive
8. oxygen species mediate thymoquinone-induced apoptosis and activate ERK and JNK signaling. *Apoptosis*. 2009 Nov 1;15(2):183–95.
- 13 Elharrif MG, Abdelhamid OM, Abdel-Maksoud HA, Alhusseini NF, Amer NE. Therapeutic
9. potential of black seed oil in treating rheumatoid arthritis: Insights on biochemical and genetic analyses of inflammatory signaling transducer. *Advances in Biomarker Sciences and Technology* [Internet]. 2024 Sept 27;6:217–25. Available from:

<https://www.sciencedirect.com/science/article/pii/S2543106424000188?via%3Dihub#tbl1>

- 14 Elias AA. Hadith on Medicine: Healing properties of blackseed [Internet].
0. www.abuaminaelias.com. 2012. Available from:
<https://www.abuaminaelias.com/dailyhadithonline/2012/10/14/blackseed-cure-except-death/>
- 14 Elliott HL. Post hoc analysis: use and dangers in perspective. *Journal of Hypertension*.
1. 1996 Sept;14(Supplement 2):S21–5.
- 14 El-Shiekh RA, Atwa AM, Elgindy AM, Ibrahim KM, Senna MM, Ebid N, et al. Current
2. Perspective and Mechanistic Insights on α -Hederin for the Prevention and Treatment of Several Noncommunicable Diseases. *Chemistry & Biodiversity*. 2024 Dec 12;22(4).
- 14 Engels G, Brinckmann J. *Nigella* - *Nigella sativa*. 2017 May 1;(114):8–16. Available from:
3. https://www.researchgate.net/publication/320859228_Nigella_-_Nigella_sativa
- 14 Evanti A, Wargasetia T, Gunadi J. Role of *Nigella sativa* L. seed (black cumin) in preventing
4. photoaging (Review). *Biomedical Reports*. 2025 June 4;23(2):1–13.
- 14 EWG's Skin Deep. Products That Contain NIGELLA SATIVA (BLACK CUMIN) SEED
5. EXTRACT || Skin Deep® Cosmetics [Internet]. EWG. 2020 [cited 2026 Jan 21]. Available from: https://www.ewg.org/skindeep/browse/ingredients/720933-NIGELLA_SATIVA_BLACK_CUMIN_SEED_EXTRACT/
- 14 Farah IO, Begum RA. Effect of *Nigella sativa* (N. sativa L.) and oxidative stress on the
6. survival pattern of MCF-7 breast cancer cells. *Biomedical sciences instrumentation* [Internet]. 2003;39:359–64. Available from: <https://pubmed.ncbi.nlm.nih.gov/12724920/>
- 14 Fatemeh Forouzanfar, Sedigheh B, Hosseinzadeh H. Black cumin (*Nigella sativa*) and its
7. constituent (thymoquinone): a review on antimicrobial effects. *Iranian Journal of Basic Medical Sciences* [Internet]. 2014 Dec [cited 2026 Jan 6];17(12):929. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC4387228/#sec1-2>
- 14 Fatfat Z, Fatfat M, Gali-Muhtasib H. Therapeutic potential of thymoquinone in combination
8. therapy against cancer and cancer stem cells. *World Journal of Clinical Oncology*. 2021 July 24;12(7):522–43.

- 14 Fatfat Z, Fatfat M, Gali-Muhtasib H. Therapeutic potential of thymoquinone in combination therapy against cancer and cancer stem cells [Internet]. Nih.gov. World Journal of Clinical Oncology; 2026 [cited 2026 Jan 4]. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC8317652/pdf/WJCO-12-522.pdf>
- 15 Fattah IMR, Ashrafur RSM, Ahmed A. Black cumin (*Nigella sativa* L.) essential oil and aroma quality. Elsevier eBooks [Internet]. 2022 Nov 11 [cited 2024 Oct 27];71–87. Available from: <https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/nigella-sativa>
- 15 Felicione E. I tried to design a clinical trial for a supplement. 5 things got in the way 1. [Internet]. STAT. 2026 [cited 2026 June 10]. Available from: <https://www.statnews.com/2026/02/26/nutraceutical-clinical-trials-5-barriers-nutritional-supplement-research/>
- 15 Feller G, Kugel A, Moonshine D, Chalifa-Caspi V, Scholz M, Prüfer D, et al. African Descents 2. Are More Sensitive Than European Descents to the Antitumor Compounds α -Hederin and Kalopanaxsaponin I. *Planta Medica*. 2010 June 24;76(16):1847–51.
- 15 Ferrando AA, Wolfe RR, Hirsch KR, Church D D, Kviatkovsky SA, Roberts M D, et al. 3. International society of sports nutrition position stand: essential amino acid supplementation on skeletal muscle and Performance. *Journal of the International Society of Sports Nutrition*. 2023 Oct 6;20(1).
- 15 Fletcher J. What Is Heterogeneity and Is It important? *BMJ : British Medical Journal* 4. [Internet]. 2007 Jan 13;334(7584):94–6. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC1767262/>
- 15 Fogel DB. Factors associated with clinical trials that fail and opportunities for improving the 5. likelihood of success: A review. *Contemporary Clinical Trials Communications* [Internet]. 2018 Sept;11(11):156–64. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6092479/>
- 15 Franz C, Bozorgmehr K. US divestment in global health: disruption, uncertainty and 6. response. *BMJ Global Health*. 2025 Nov;10(11):e019990.
- 15 Freepik. Illuminated manuscript border [Internet]. Freepik. [cited 2026 Jan 16]. Available 7. from: https://www.freepik.com/free-vector/hand-drawn-art-nouveau-frame_31222577.htm

15 Ganesan K, Sukalingam K, Xu B. Impact of consumption of repeatedly heated cooking oils on the incidence of various cancers- A critical review. *Critical Reviews in Food Science and Nutrition*. 2017 Oct 20;59(3):488–505.

15 GBIF. *Nigella sativa* L. [Internet]. Gbif.org. 2015. Available from:

9. <https://www.gbif.org/species/165712447>

16 GBIF Secretariat. *Nigella sativa* L. [Internet]. www.gbif.org. 2023. Available from:

0. <https://www.gbif.org/species/5371700>

16 Genders R. *Scented Flora of the World*. Robert Hale Limited; 1994.

1.

16 geograph. SS9769 : Seed pod of *Nigella damascena* (love-in-a-mist) [Internet]. 2010 [cited

2. 2026 June 12]. Available from: <https://www.geograph.org.uk/photo/1967388>

Taken 16 years ago, near to Boverton, The Vale of Glamorgan/Bro Morgannwg, Wales

16 Ghasemi A, Zahediasl S. Normality Tests for Statistical Analysis: a Guide for Non-

3. Statisticians. *International Journal of Endocrinology and Metabolism*. 2012 Apr 20;10(2):486–9.

16 Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT Assay: Utility, Limitations, Pitfalls,

4. and Interpretation in Bulk and Single-Cell Analysis. *International Journal of Molecular Sciences* [Internet]. 2021 Nov 26;22(23):12827. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC8657538/>

16 Ghorbanibirgani A, Khalili A, Rokhafrooz D. Comparing *Nigella sativa* Oil and Fish Oil in

5. Treatment of Vitiligo. *Iranian Red Crescent Medical Journal*. 2014 June 5;16(6).

16 Gong S, Fajar P, De Vries-Idema J, Huckriede A. Comparison of media for a human

6. peripheral blood mononuclear cell-based *in vitro* vaccine evaluation system. *Clinical and experimental vaccine research* [Internet]. 2023 Jan 1;12(4):328–8. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10655156/>

16 Gong X, Smith J, Swanson H, Rubin L. Carotenoid Lutein Selectively Inhibits Breast Cancer

7. Cell Growth and Potentiates the Effect of Chemotherapeutic Agents through ROS-Mediated Mechanisms. *Molecules*. 2018 Apr 14;23(4):905.

- 16 Gouda YA, Kassab DK, Ali M, Badary OA. Thymoquinone and therapeutic potentials: Updated evidences from clinical trials. *Pharmacological Research - Natural Products*. 2025 Sept;8:100281.
- 16 Grand Prismatic Seed. Grand Prismatic Seed [Internet]. Grand Prismatic Seed. 2019 [cited 2026 June 12]. Available from: <https://www.grandprismaticseed.com/herbs/black-cumin>
- 17 Grand View Research. Nanotechnology Market Size, Share & Growth Report, 2030 [Internet]. www.grandviewresearch.com. [cited 2026 Jan 17]. Available from: <https://www.grandviewresearch.com/industry-analysis/nanotechnology-market-report>
- 17 GraphPad Prism. Is it better to plot graphs with SD or SEM error bars? (Answer: Neither) - FAQ 201 - GraphPad [Internet]. www.graphpad.com. Available from: <https://www.graphpad.com/support/faq/is-it-better-to-plot-graphs-with-sd-or-sem-error-bars-answer-neither/>
- 17 Greenish HG. Beiträge zur Chemie von *Nigella sativa*. *Fresenius' Zeitschrift für analytische Chemie*. 1882 Dec;21(1):462–2.
- 17 Groenwold RHH, Goeman JJ, Cessie S Le, Dekkers OM. Multiple testing: when is many too much? *European Journal of Endocrinology*. 2021 Mar;184(3):E11–4.
- 17 Guenette P. Flower Stem with Leaf of *Nigella Sativa* vintage illustration. [Internet]. Vecteezy. 2022 [cited 2026 June 3]. Available from: <https://www.vecteezy.com/free-vector/nigella-sativa>
- 17 Gul R, Hasan Deniz Tansuker, Abdurrahman Bugra Cengiz, Gul M, Alper Tabaru, Funda Emre, et al. Effects of *nigella sativa* oil on allergic rhinitis: an experimental animal study. *Brazilian Journal of Otorhinolaryngology* [Internet]. 2022 Sept 21 [cited 2026 Jan 7];88(S5):S148–55. Available from: <https://www.bjorl.org/en-effects-nigella-sativa-oil-on-articulo-S1808869422001185>
- 17 Gupta A, Eisenhauer EA, Booth CM. The Time Toxicity of Cancer Treatment. *Journal of Clinical Oncology*. 2022 Mar 2;40(15).
- 17 Gönül Geyik Ö, Hasoğlu İ, Metin AS, Aktar Kiremitci S. Combined Cytotoxic Effects of Carvacrol-Based Essential Oil Formulations. *Plants*. 2026 Jan 9;15(2):211.
- 17 Hagag AA, Badraia IM, El-Shehaby WA, Mabrouk MM. Protective role of black seed oil in doxorubicin-induced cardiac toxicity in children with acute lymphoblastic leukemia.

Journal of oncology pharmacy practice : official publication of the International Society of Oncology Pharmacy Practitioners [Internet]. 2020 Sept;26(6):1397–406. Available from: <https://pubmed.ncbi.nlm.nih.gov/31964219/>

- 17 Hago S, Lu T, Abdelgadir AA, Yassin S, Ahmed EM, Xu H. Phytochemical Constituents and
9. In-Vitro Anticancer Activity of some Medicinal Plant Extracts against MCF-7 and MDA-MB-435 Human Breast Cancer Cell L. Tropical Journal of Natural Product Research. 2023 Apr 1;7(3):2506–15.
- 18 Hago S, Lu T, Abdelgadir AA, Yassin S, Ahmed EM, Xu H. Phytochemical Constituents and
0. In-Vitro Anticancer Activity of some Medicinal Plant Extracts against MCF-7 and MDA-MB-435 Human Breast Cancer Cell L: <http://www.doi.org/10.26538/tjnpr/v7i3.6> [Internet]. Doi.org. 2023 [cited 2026 May 31]. Available from: <http://www.doi.org/10.26538/tjnpr/v7i3.6>
- 18 Hajimehdipoor H, Choopani R, Ara L, Hamzeloo-Moghadam M. Alteration in Thymoquinone
1. Content of Nigella sativa Seeds After Processing by a Traditional Method and Stability Assessment of Raw and Processed Seeds. DOAJ (DOAJ: Directory of Open Access Journals). 2018 Sept 1;5(4).
- 18 Hall R, Beale M, Fiehn O, Hardy N, Sumner L, Bino R. Plant Metabolomics. The Plant Cell.
2. 2002 July;14(7):1437–40.
- 18 Hamadain E, Benghuzzi H, Tucci M. HISTORICAL DEVELOPMENT OF THE USE AND THE
3. EFFECTIVENESS OF BLACK SEED (NIGELLA SATIVA) OIL AND ITS ACTIVE INGREDIENT THYMOQUINONE AS ANTI- CANCER AGENT: A COMPREHENSIVE REVIEW [Internet]. 2019 Jan [cited 2026 June 12]. Available from: https://iaexpress.ca/wp-content/uploads/2020/04/24_Hamadain_-Biomed-Sci-Instrum-Jan_-Vol-55_1-2019_pg-145.pdf
- 18 Hamza N. Study and Investigations of archaeobotanical remains from Tutankhamun tomb
4. [Internet]. Academia.edu. 'Universidade de Evora'; 2020 [cited 2026 Jan 6]. Available from: https://www.academia.edu/85847954/Study_and_Investigations_of_archaeobotanical_remains_from_Tutankhamun_tomb
- 18 Hanahan D, Weinberg RA. The Hallmarks of Cancer. Cell. 2000 Jan 7;100(1):57–70.
5.

- 18 Hannan MdA, Rahman MdA, Sohag AAM, Uddin MdJ, Dash R, Sikder MH, et al. Black Cumin (*Nigella sativa* L.): A Comprehensive Review on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. *Nutrients*. 2021 May 24;13(6):1784.
- 18 Happy Green Shop. Love in a mist Persian Jewels red *Nigella damascena*| Happy Green Shop [Internet]. Happy Green Shop. 2026 [cited 2026 June 12]. Available from: <https://www.happygreenshop.com/flower-seeds-bulbs/annual-seeds-bulbs/love-in-a-mist-persian-jewels-red-seeds-nigella-damascena>
- 18 Haq A, Abdullatif M, Lobo PI, Khabar KSA, Sheth KV, Al-Sedairy ST. *Nigella sativa*: effect on human lymphocytes and polymorphonuclear leukocyte phagocytic activity. *Immunopharmacology*. 1995 Aug;30(2):147–55.
- 18 Harika Özkaya Yurttadur. What is Black Cumin? What are the Benefits of Black Cumin Oil? [Internet]. What is Black Cumin? What are the Benefits of Black Cumin Oil? Yeditepe University Hospitals; 2025. Available from: <https://yeditepehastaneleri.com/en/health-guide/healthy-nutrition/what-black-cumin-what-are-benefits-black-cumin-oil>
- 19 Hazrati S, Lotfi K, Govahi M, Ebadi MT. A comparative study: Influence of various drying methods on essential oil components and biological properties of *Stachys lavandulifolia*. *Food Science & Nutrition*. 2021 Mar 8;9(5):2612–9.
- 19 Health and Safety Executive. The Biopesticide Scheme - HSE [Internet]. Hse.gov.uk. 2025. Available from: <https://www.hse.gov.uk/pesticides/active-substances/biopesticide-scheme.htm>
- 19 Heiss AG, Stika HP, Zorzi N De, Jursa M. *Nigella* in the Mirror of Time: A Brief Attempt to Draw a Genus' Ethnohistorical Portrait [Internet]. Research Gate. 2013. p. 147–69. Available from: https://www.researchgate.net/publication/249027594_Nigella_in_the_Mirror_of_Time_A_Brief_Attempt_to_Draw_a_Genus
- 19 Hermitage Oils. *Nigella Damascena Absolute* from hermitageoils.com [Internet]. Hermitage Essential Oils. 2025 [cited 2026 Jan 18]. Available from: <https://hermitageoils.com/product/nigella-damascena-absolute/>
- 19 Hess DR. How to Write an Effective Discussion. *Respiratory Care* [Internet]. 2023 Dec 4. 1;68(12):1771–4. Available from: <https://rc.rcjournal.com/content/68/12/1771>

- 19 Higgins J. Missing data [Internet]. [cited 2026 June 12]. Available from:
5. https://methods.cochrane.org/statistics/sites/methods.cochrane.org/statistics/files/uploads/SMG_training_course_cardiff/2010_SMG_training_cardiff_day1_session3_higgins.pdf
- 19 Holland and Barrett. Fushi Fresh-Pressed Organic Black Cumin Seed Oil 100ml [Internet].
6. Hollandandbarrett.com. 2020 [cited 2026 Jan 18]. Available from: <https://www.hollandandbarrett.com/shop/product/fushi-fresh-pressed-organic-black-cumin-seed-oil-60046369>
- 19 Home Health UK. Male Body Diagram [Internet]. Home Health UK. 2015. Available from:
7. <https://homehealth-uk.com/malebodydiagram/>
- 19 Hosseini M, Khoshdast S, Rastegar M, Kahdouee S, Rakhshandeh H, Zakeri S, et al. The
8. effects of *Nigella sativa* hydro-alcoholic extract and thymoquinone on lipopolysaccharide - induced depression like behavior in rats. *Journal of Pharmacy and Bioallied Sciences* [Internet]. 2012 [cited 2020 Jan 13];4(3):219. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3425171/>
- 19 Hosseini N, Emam-Djomeh Z, Askari G, Hashemi Nejad A, Takbirgou H, Lobenberg R, et al.
9. Black cumin (*Nigella sativa*) oleosome as a natural delivery system for curcumin: characterization, preparation, and in vitro digestive behavior. *Frontiers in Nutrition*. 2026 May 4;13.
- 20 Hosseinzadeh H, Shahroudi MJ, Mehri S. Anti-Aging Effect of *Nigella Sativa* Fixed Oil on D-
0. Galactose-Induced Aging in Mice. *Journal of Pharmacopuncture*. 2017 Mar 31;20(1):29–35.
- 20 Hovhan L. Data Duplication and Its Hidden Costs: Why It’s a Threat to Your Business
1. [Internet]. Sapien.io. Sapien; 2025. Available from: <https://www.sapien.io/blog/data-duplication-and-its-hidden-costs>
- 20 Hu Y, Scherngell T, Man SN, Wang Y. Is the United States Still Dominant in the Global
2. Pharmaceutical Innovation Network? Larivière V, editor. *PLoS ONE*. 2013 Nov 5;8(11):e77247.
- 20 Huat BTK, Swamy SMK. Intracellular glutathione depletion and reactive oxygen species
3. generation are important in α -hederin-induced apoptosis of P388 cells. *Molecular and Cellular Biochemistry*. 2003;245(1/2):127–39.

- 20 Hussain D, Musharraf Hussain M. Nigella sativa (black seed) is an effective herbal remedy
4. for every disease except death -a Prophetic statement which modern scientists confirm
unanimously: A review [Internet]. 2016 Apr [cited 2025 Mar 23]. Available from:
<https://www.netjournals.org/pdf/AMPR/2016/2/16-008.pdf>
- 20 Iglewicz B, Hoaglin DC. How to Detect and Handle Outliers. ASQ Quality Press; 1993.
5.
- 20 Imran M, Rauf A, Khan IA, Shahbaz M, Qaisrani TB, Fatmawati S, et al. Thymoquinone: A
6. novel strategy to combat cancer: A review. Biomedicine & Pharmacotherapy. 2018
Oct;106:390–402.
- 20 International Alliance of Dietary/Food Supplements Associations . A quick guide to food
7. supplement dosage forms | IADSA [Internet]. iadsa.org. IADSA; 2024. Available from:
<https://www.iadsa.org/dosage-forms>
- 20 Irawan R, Wahyudi H, Bawono M, Bakti A, Tualeka AR. Nigella Sativa Supplementation
8. Modulates Exercise-Induced Oxidative Stress and Inflammation: A Randomized Controlled
Trial. Pamukkale Journal of Sport Sciences [Internet]. 2025 Aug 30 [cited 2026 Jan
21];16(2):347–67. Available from: <https://dergipark.org.tr/en/pub/psbd/article/1652627>
- 20 Isa NSM, Ng JS, Ahmad FT, Kassim MNI, Norhayati H, H.M. Yusof. Antioxidant potential and
9. low-density lipoprotein cholesterol (LDL-c) uptake of the black seed and honey mixture on
human hepatocellular carcinoma (HepG2) cells. Food Research. 2021 Dec 5;5(6):141–9.
- 21 J-Global. Nigellidine | Chemical Substance Information | J-GLOBAL [Internet]. Jst.go.jp.
0. 2026 [cited 2026 June 5]. Available from:
https://jglobal.jst.go.jp/en/detail?JGLOBAL_ID=200907039830557869
- 21 Jalali M, Roghani M. The Effect of Nigella Sativa on Learning and Memory in Male Diabetic
1. Rats. 2009 Sept.
- 21 Javanbakht J, Hobbenaghi R, Hosseini E, Bahrami AM, Khadivar F, Fathi S, et al.
2. Histopathological investigation of neuroprotective effects of Nigella sativa on motor
neurons anterior horn spinal cord after sciatic nerve crush in rats. Pathologie-biologie
[Internet]. 2013 Dec;61(6):250–3. Available from:
<https://pubmed.ncbi.nlm.nih.gov/23751360/>
- 21 JCAD Online Editor. Nigella Sativa (Black Cumin) Oil: A Case Report and Analysis Exploring
3. Severe Cutaneous Manifestations and Implications for Patient Care | JCAD - The Journal of

Clinical and Aesthetic Dermatology [Internet]. JCAD - The Journal of Clinical and Aesthetic Dermatology. 2024. Available from: <https://jcadonline.com/nigella-sativa-black-cumin-oil/>

- 21 Jehan S, Huang J, Farooq U, Basheer I, Zhou W. Combinatorial effect of thymoquinone with
4. chemo agents for tumor therapy. *Phytomedicine* [Internet]. 2022 Apr 1 [cited 2022 Mar 24];98:153936. Available from:
<https://www.sciencedirect.com/science/article/pii/S0944711322000149>
- 21 Jehan S, Zhong C, Li G, Syed ZB, Li D, Sui G. Thymoquinone Selectively Induces
5. Hepatocellular Carcinoma Cell Apoptosis in Synergism With Clinical Therapeutics and
Dependence of p53 Status. *Frontiers in Pharmacology*. 2020 Sept 15;11.
- 21 Johannes Z. Afbeelding der Artseny-Gewassen - Nigelle cultivates or black cumin (Nigella
6. sativa) [Internet]. 1796 [cited 2026 Jan 11]. Available from:
<https://www.bridgemanimages.com/en/noartistknown/nigelle-cultivates-or-black-cumin-nigella-sativa-lithograph-from-afbeelding-der-artseny-gewassen-by/nomedium/asset/4732975>
- 21 Kale E, Kale A, Bozali K, Gulgec AS, Ozdemir M, Yalcin B, et al. TQ-Ox, a novel synthetic
7. derivative of thymoquinone on ovarian cancer cells *in vitro*. *Natural Product Research*.
2022 Nov 22;37(18):3015–24.
- 21 Kalus U, Pruss A, Bystron J, Jurecka M, Smekalova A, Josef Lichius J, et al. Effect of Nigella
8. sativa (black seed) on subjective feeling in patients with allergic diseases. *Phytotherapy
Research*. 2003 Jan 1;17(10):1209–14.
- 21 Kang SW. Role of Reactive Oxygen Species in Cell Death Pathways. *Hanyang Medical
9. Reviews*. 2013;33(2):77.
- 22 KARA N, GÜRBÜZER G, BIYIKLI M, BAYDAR H. Effect on Yield and some Quality
0. Characteristics of Seed Harvest at Different Stages of Maturity in Nigella sativa L. *Tarım
Bilimleri Dergisi*. 2021 Dec 4;27.
- 22 Karaman K. Characterization of *Saccharomyces cerevisiae* based microcarriers for
1. encapsulation of black cumin seed oil: Stability of thymoquinone and bioactive properties.
Food Chemistry. 2020 May;313:126129.
- 22 Karimi Z, Mirza Alizadeh A, Ezzati Nazhad Dolatabadi J, Dehghan P. Nigella sativa and its
2. Derivatives as Food Toxicity Protectant Agents. *Advanced Pharmaceutical Bulletin*. 2019
Feb 21;9(1):22–37.

- 22 Kaskoos RA, Ahamad J. Phytochemical analysis and Anticancer Potency of Black Cumin Oil (*Nigella sativa* Linn.) Against Osteosarcoma. *Journal of Angiotherapy*. 2024 May 1;8(5).
- 22 Kazmi A, Khan MA, Ali H. Biotechnological approaches for production of bioactive secondary metabolites in *Nigella sativa*: an up-to-date review. *International Journal of Secondary Metabolite*. 2019 June 11;6(2):172–95.
- 22 Kałużna-Czaplińska J, Gątarek P, Chirumbolo S, Chartrand MS, Björklund G. How important is tryptophan in human health? *Critical Reviews in Food Science and Nutrition*. 2017 Sept;59(1):72–88.
- 22 Keilhack H, Chang P. In Vitro Long-Term Proliferation Assays to Study Antiproliferative Effects of PARP Inhibitors on Cancer Cells. *Methods in Molecular Biology*. 2017;321–36.
- 22 Kemp J. Seed Doodle Template 005 graphic [Internet]. *DigitalScrapbook.com*. 2018 [cited 2026 June 3]. Available from: <https://www.digitalscrapbook.com/janet-scott/designs/seed-doodle-template-005-graphic-element-embellishment-illustration-drawing-hand>
- 22 Kennedy-Shaffer L. Before $p < 0.05$ to Beyond $p < 0.05$: Using History to Contextualize p -Values and Significance Testing. *The American Statistician*. 2019 Mar 20;73(sup1):82–90.
- 22 Ketenoglu O, Kiralan SS, Kiralan M, Ozkan G, Ramadan MF. Cold pressed black cumin (*Nigella sativa* L.) seed oil. *Cold Pressed Oils*. 2020;53–64.
- 23 Khan A, Rehman MU. *Black Seeds (Nigella sativa)*. Elsevier; 2021.
- 23 Khan I, JD Shaikh. Anticancer Effects of *Nigella sativa* Seeds in Cell Line. *International Research Journal of Multidisciplinary Scope* [Internet]. 2025 [cited 2025 Nov 11];06(01):1198–211. Available from: https://www.irjms.com/wp-content/uploads/2025/01/Manuscript_IRJMS_02438_WS.pdf?utm_source=chatgpt.com
- 23 Khoddami A, Ghazali HM, Yassoralipour A, Ramakrishnan Y, Ganjloo A. Physicochemical Characteristics of *Nigella* Seed (*Nigella sativa* L.) Oil as Affected by Different Extraction Methods. *Journal of the American Oil Chemists' Society*. 2010 Oct 9;88(4):533–40.
- 23 Kim S. Overview of Clinical Study design. *Clinical and Experimental Emergency Medicine* [Internet]. 2023 June 2;11(1). Available from: <https://typeset.io/papers/overview-of-clinical-study-design-i8h0sabo>

- 23 Kintzios SE, Barberaki MG. PLANTS THAT FIGHT CANCER [Internet]. 2004 [cited 2026 4. June 11]. Available from:
https://www.almanaqueacoriano.com/images/biblioteca/ingles/Plants_That_Fight_Cancer.pdf
- 23 Kiralan M. Volatile Compounds of Black Cumin Seeds (*Nigella sativa* L.) from Microwave-Heating and Conventional Roasting. *Journal of Food Science*. 2012 Apr;77(4):C481–4.
- 23 Klatt EC. Histotechniques [Internet]. Utah.edu. 2019. Available from:
6. <https://webpath.med.utah.edu/HISTHTML/HISTOTCH/HISTOTCH.html>
- 23 Ko D, Park S, Park M, Kim S, Park JM, Seo J, et al. Pitavastatin is a novel Mcl-1 inhibitor that overcomes paclitaxel resistance in triple-negative breast cancer. *Experimental hematology & oncology* [Internet]. 2025;14(1):125. Available from:
<https://pubmed.ncbi.nlm.nih.gov/41126317/>
- 23 Koklesova L, Liskova A, Samec M, Buhrmann C, Samuel SM, Varghese E, et al. Carotenoids in Cancer Apoptosis—The Road from Bench to Bedside and Back. *Cancers* [Internet]. 2020 Aug 26 [cited 2020 Dec 1];12(9). Available from:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7563597/>
- 23 Kolahdooz M, Nasri S, Modarres SZ, Kianbakht S, Huseini HF. Effects of *Nigella sativa* L. seed oil on abnormal semen quality in infertile men: A randomized, double-blind, placebo-controlled clinical trial. *Phytomedicine*. 2014 May;21(6):901–5.
- 24 Kondratyeva L, Alekseenko I, Chernov I, Sverdlov E. Data Incompleteness May form a Hard-to-Overcome Barrier to Decoding Life’s Mechanism. *Biology* [Internet]. 2022 Aug 1 [cited 2023 July 22];11(8):1208. Available from: <https://www.mdpi.com/2079-7737/11/8/1208>
- 24 Koshak AE, Koshak EA, Mobeireek AF, Badawi MA, Wali SO, Malibary HM, et al. *Nigella sativa* for the treatment of COVID-19: An open-label randomized controlled clinical trial. *Complementary Therapies in Medicine*. 2021 Sept;61:102769.
- 24 Kostner GM, Gavish D, Leopold B, Bolzano K, Weintraub MS, Breslow JL. HMG CoA reductase inhibitors lower LDL cholesterol without reducing Lp(a) levels. *Circulation*. 1989 Nov;80(5):1313–9.
- 24 Kroll HJ. Von Sylt bis Kastanas : Festschrift für Helmut Johannes Kroll. Wachholtz Verlag; 3. 2013.

- 24 Kruk I, Michalska T, Lichszteld K, Kładna A, Aboul-Enein HY. The effect of thymol and its derivatives on reactions generating reactive oxygen species. *Chemosphere*. 2000 Oct;41(7):1059–64.
- 24 Kumar SPJ, Prasad SR, Banerjee R, Agarwal DK, Kulkarni KS, Ramesh KV. Green solvents and technologies for oil extraction from oilseeds. *Chemistry Central Journal* [Internet]. 2017 Jan 23;11(1). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5258651/>
- 24 Kumar V, Aydav PSS, Minz S. Crop Seeds Classification Using Traditional Machine Learning and Deep Learning Techniques: A Comprehensive Survey. *SN Computer Science*. 2024 Nov 6;5(8).
- 24 Kuroda K, Komori T, Ishibashi K, Uto T, Kobayashi I, Kadokawa R, et al. Non-aqueous, zwitterionic solvent as an alternative for dimethyl sulfoxide in the life sciences. *Communications Chemistry* [Internet]. 2020 Nov 11;3(1):1–7. Available from: <https://www.nature.com/articles/s42004-020-00409-7>
- 24 Latif M, Faheem M, Asmatullah, Hoseinifar SH, Van Doan H. Dietary Black Seed Effects on Growth Performance, Proximate Composition, Antioxidant and Histo-Biochemical Parameters of a Culturable Fish, Rohu (*Labeo rohita*). *Animals*. 2020 Dec 29;11(1):48.
- 24 Laukagalis V, Tarasevičienė Ž, Visockis M, Kazancev K, Sendžikienė E, Kiettyka-Dadasiewicz A, et al. Impact of Pretreatment Methods on Yield and Composition of Cold-Pressed Black Cumin (*Nigella sativa* L.) Seed Oil. *Foods*. 2025 Dec 9;14(24):4234–4.
- 25 Launert E. *The Hamlyn Guide to Edible and Medicinal Plants of Britain and Northern Europe*. 1981.
- 25 Lee S, Lee DK. What Is the Proper Way to Apply the Multiple Comparison test? *Korean Journal of Anesthesiology*. 2018 Oct 1;71(5):353–60.
- 25 Leipzig University Library. 03_Ebers Papyrus: Universitätsbibliothek Leipzig [Internet]. *Uni-leipzig.de*. 2024. Available from: <https://www.ub.uni-leipzig.de/en/about-us/exhibitions/permanent-exhibition/ebers-papyrus>
- 25 Lenchner JR, Santos C. *Biochemistry, 5 Hydroxyindoleacetic Acid* [Internet]. PubMed. Treasure Island (FL): StatPearls Publishing; 2021. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK551684/>

- 25 Lensen S. When to pool data in a meta-analysis (and when not to)? *Fertility and Sterility*
4. [Internet]. 2023 June 1;119(6):902–3. Available from:
<https://www.sciencedirect.com/science/article/abs/pii/S0015028223002248>
- 25 Lesiak AD, Cody RB, Dane AJ, Musah RA. Plant Seed Species Identification from Chemical
5. Fingerprints: A High-Throughput Application of Direct Analysis in Real Time Mass Spectrometry. *Analytical Chemistry*. 2015 Aug 18;87(17):8748–57.
- 25 Li TH, Zhao BB, Qin C, Wang YY, Li ZR, Cao HT, et al. IFIT1 modulates the proliferation,
6. migration and invasion of pancreatic cancer cells via Wnt/ β -catenin signaling. *Cellular oncology (Dordrecht, Netherlands)* [Internet]. 2024 Aug;47(4):1253–65. Available from:
<https://pubmed.ncbi.nlm.nih.gov/38536650/>
- 25 Li Y, Li S, Meng X, Gan RY, Zhang JJ, Li HB. Dietary Natural Products for Prevention and
7. Treatment of Breast Cancer. *Nutrients*. 2017 July 8;9(7):728.
- 25 Li Y, Ma WJ, Qi BK, Rokayya S, Li D, Wang J, et al. Blending of Soybean Oil with Selected
8. Vegetable Oils: Impact on Oxidative Stability and Radical Scavenging Activity. *Asian Pacific Journal of Cancer Prevention*. 2014 Mar 30;15(6):2583–9.
- 25 Li Z, Wang Y, Xu Q, Ma J, Li X, Yan J, et al. *Nigella sativa* and health outcomes: An overview
9. of systematic reviews and meta-analyses. *Frontiers in Nutrition*. 2023 Mar 28;10.
- 26 Lintang Sari G, Aliyah AN, Maran GG, Hermawan A, Meiyanto E. Black seed oil inhibits the
0. migration of triple-negative breast cancer cells and regulates MMP-9 expression. *Indonesian Journal of Biotechnology*. 2025 Mar 29;30(1):47.
- 26 Lipinutragen. FORMS OF ADMINISTRATION IN NUTRACEUTICS • Lipinutragen [Internet].
1. Lipinutragen. 2021 [cited 2026 June 11]. Available from:
<https://www.lipinutragen.it/forms-of-administration-in-nutraceuticals/>
- 26 Liu S, Yang W, Li Y, Sun C. Fetal bovine serum, an important factor affecting the
2. reproducibility of cell experiments. *Scientific Reports* [Internet]. 2023 Feb 2;13(1):1942. Available from: <https://www.nature.com/articles/s41598-023-29060-7>
- 26 Lonicer A. *Krauterbuch*. Herbal, Frankfurt, 1557 (17th century pirate edition or atlas of
3. illustrations only, with captions in Latin, Greek, French, Italian, German, and in English manuscript) [Internet]. 1557 [cited 2026 Jan 11]. Available from:
<https://www.bridgemanimages.com/en/noartistknown/black-cumin-nigella-sativa->

handcoloured-woodblock-engraving-of-a-botanical-illustration-from-adam/nomedium/asset/4667087

- 26 Love A. The unsustainability of clinical trials and need for transformative change [Internet].
4. Clinical Trials Arena. 2025. Available from:
<https://www.clinicaltrialsarena.com/sponsored/the-unsustainability-of-clinical-trials-and-need-for-transformative-change/>
- 26 Mabrouk GM, Moselhy SS, Zohny SF, Ali EMM, Helal TEA, Amin AA, et al. Inhibition of
5. methylnitrosourea (MNU) induced oxidative stress and carcinogenesis by orally administered bee honey and *Nigella* grains in Sprague Dawley rats. *Journal of experimental & clinical cancer research : CR* [Internet]. 2002 Sept;21(3):341–6. Available from:
<https://pubmed.ncbi.nlm.nih.gov/12385575/>
- 26 Maduratna E, Sari DS, Rahayu RP, Masya RN, Adinar N. The Anti-Inflammatory Effect of
6. *Nigella sativa* Toothpaste on *Porphyromonas gingivalis* Bacteria Through Decreased TNF- α , MMP-9, PGE-2 Expression in Wistar Rats. *European Journal of Dentistry* [Internet]. 2024 May 14 [cited 2026 Jan 23];18(03):808–14. Available from:
<https://pmc.ncbi.nlm.nih.gov/articles/PMC11290913/>
- 26 Mahmoud MR, El-Abhar HS, Saleh S. The effect of *Nigella sativa* oil against the liver
7. damage induced by *Schistosoma mansoni* infection in mice. *Journal of Ethnopharmacology*. 2002 Feb;79(1):1–11.
- 26 Majdalawieh AF, Al-Samaraie S, Terro TM. Molecular Mechanisms and Signaling Pathways
8. Underlying the Therapeutic Potential of Thymoquinone Against Colorectal Cancer. *Molecules*. 2024 Dec 14;29(24):5907.
- 26 Malik S, Singh A, Negi P, Kapoor VK. Thymoquinone: A small molecule from nature with
9. high therapeutic potential. *Drug Discovery Today*. 2021 Nov;26(11):2716–25.
- 27 Manufacturing Chemist. The rise of nutraceuticals in the pharmaceutical industry
0. [Internet]. *Manufacturingchemist.com*. 2024. Available from:
<https://manufacturingchemist.com/the-rise-of-nutraceuticals-in-the-pharmaceutical-industry>
- 27 Maqbool T, Awan SJ, Malik S, Hadi F, Shehzadi S, Tariq K. In-Vitro Anti-Proliferative,
1. Apoptotic and Antioxidative Activities of Medicinal Herb Kalonji (*Nigella sativa*). *Current Pharmaceutical Biotechnology*. 2019 Nov 22;20(15):1288–308.

- 27 Maqbool T, Awan SJ, Malik S, Hadi F, Shehzadi S, Tariq K. In-Vitro Anti-Proliferative, Apoptotic and Antioxidative Activities of Medicinal Herb Kalonji (*Nigella sativa*). *Current Pharmaceutical Biotechnology*. 2019 Nov 22;20(15):1288–308.
- 27 Martos-Benítez FD, Gutiérrez-Noyola A, Echevarría-Víctores A. Postoperative complications and clinical outcomes among patients undergoing thoracic and gastrointestinal cancer surgery: A prospective cohort study. *Revista Brasileira de Terapia Intensiva*. 2016;28(1).
- 27 María L, Kirici S. Phenology of black cumin (*Nigella sativa* L.) under Mediterranean climate. *ResearchGate* [Internet]. 2021 June;. Available from: https://www.researchgate.net/publication/351069195_Phenology_of_black_cumin_Nigella_sativa_L_under_Mediterranean_climate
- 27 Mashayekhi-Sardoo H, Rezaee R, Karimi G. *Nigella sativa* (black seed) safety: an overview. *Asian Biomedicine*. 2020 Aug 1;14(4):127–37.
- 27 Mashhadi Abolghasem F, Kim RH, Park SY, Lim T, Lee H, Hwang KT, et al. Effects of roasting and ultrasound-assisted enzymatic treatment of *Nigella sativa* L. seeds on thymoquinone in the oil and antioxidant activity of defatted seed meal. *Journal of the Science of Food and Agriculture*. 2023 May 21;103(13):6208–18.
- 27 Maulidiani M, Sheikh BY, Mediani A, Wei LS, Ismail IS, Abas F, et al. Differentiation of *Nigella sativa* seeds from four different origins and their bioactivity correlations based on NMR-metabolomics approach. *Phytochemistry Letters* [Internet]. 2015 Sept [cited 2019 Dec 3];13:308–18. Available from: <https://www.sciencedirect.com/science/article/pii/S1874390015300306?via%3Dihub>
- 27 Mbarek LA, Mouse HA, Elabbadi N, Bensalah MK, Gamouh A, Aboufatima R, et al. Anti-tumor properties of blackseed (*Nigella sativa* L.) extracts. *Brazilian Journal of Medical and Biological Research*. 2007 Apr 12;40(6):839–47.
- 27 McCormick Science Institute. Black Seed | McCormick Science Institute [Internet]. McCormick Science Institute. 2022. Available from: <https://www.mccormickscienceinstitute.com/resources/culinary-spices/herbs-spices/black-seed>
- 28 MedChemExpress. alpha-Hederin (Standard) [Internet]. MedchemExpress.com. 2026 [cited 2026 June 5]. Available from: <https://www.medchemexpress.com/alpha-hederin->

standard.html?srsId=AfmBOorweTwtril4db9Flcnbx10nM67st3t0ZhmXXRyYd1lv5glumZ
NQ

- 28 Melnyk H, Stalyus Vasyl L, Precarpathian S, Kozak Vasyl T, Melnyk H, Stalyus L, et al. The
1. Pharma Innovation Journal 2015; 4(3): 24-26 The perspectives of *Nigella sativa* L. growing
in the climatic conditions of the Precarpathian Region [Internet]. 2015 Apr [cited 2026 June
9]. Available from:
<https://www.thepharmajournal.com/archives/2015/vol4issue3/PartA/4-3-11.pdf>
- 28 Memorial Sloan Kettering Cancer Center. *Nigella sativa* [Internet]. Memorial Sloan
2. Kettering Cancer Center. 2011. Available from: <https://www.mskcc.org/cancer-care/integrative-medicine/herbs/nigella-sativa>
- 28 Merrill MH, Fleisher MS. FACTORS INVOLVED IN THE USE OF ORGANIC SOLVENTS AS
3. PRECIPITATING AND DRYING AGENTS OF IMMUNE SERA. *Journal of General Physiology*.
1932 Nov 20;16(2):243–56.
- 28 Miah AG, Bathgate R, Hamano K, Salma U. Effects of pre-freeze *Nigella sativa* oil
4. supplementation on cryosurvival of ovine spermatozoa. *Reproduction in Domestic
Animals*. 2018 Sept 22;53(6):1424–33.
- 28 Mid Devon District Council . New Strategic Sustainability Grants to support community
5. climate and nature action [Internet]. [Middevon.gov.uk](https://www.middevon.gov.uk). MDDC; 2026 [cited 2026 June 9].
Available from: <https://www.middevon.gov.uk/new-strategic-sustainability-grants-to-support-community-climate-and-nature-action/>
- 28 Mirzayans R, Murray D. What Are the Reasons for Continuing Failures in Cancer Therapy?
6. Are Misleading/Inappropriate Preclinical Assays to Be Blamed? Might Some Modern
Therapies Cause More Harm than Benefit? *International Journal of Molecular Sciences*.
2022 Oct 30;23(21):13217.
- 28 Mohamad EL DHAYBİ, Deniz MESUTOĞLU, Dilara Rana BİÇER. Çörek Otu Yağının Bilimsel
7. ve Sağlıklı Yaşam Üzerine Tıbbi ve Terapötik Etkileri. *Phoenix Medical Journal* [Internet].
2023 Mar 1 [cited 2024 Dec 4];5(1):6–9. Available from:
<https://dergipark.org.tr/en/pub/phnx/issue/75186/1134490>
- 28 Mohammadabadi MR, Mozafari MR. Enhanced efficacy and bioavailability of
8. thymoquinone using nanoliposomal dosage form. *Journal of Drug Delivery Science and
Technology* [Internet]. 2018 Oct 1 [cited 2022 May 17];47:445–53. Available from:
<https://www.sciencedirect.com/science/article/abs/pii/S1773224718307950>

- 28 Mohammed NK, Abd Manap MY, Tan CP, Muhialdin BJ, Alhelli AM, Meor Hussin AS. The Effects of Different Extraction Methods on Antioxidant Properties, Chemical Composition, and Thermal Behavior of Black Seed (*Nigella sativa* L.) Oil. Evidence-based Complementary and Alternative Medicine : eCAM [Internet]. 2016;2016. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5015008/>
- 29 Mohammed NK, Meor Hussin AS, Tan CP, Abdul Manap MY, Alhelli AM. Quality changes of microencapsulated *Nigella sativa* oil upon accelerated storage. International Journal of Food Properties. 2017 Dec 31;20(sup3):S2395–408.
- 29 Monteiro MM, Amorim J, Barbosa VP, De G, Squarize CH, Coletta R D, et al. <sc>PETRICCS</sc> Guideline Protocol: A Call to Improve Reporting Standards in Cell Culture Research. Journal of Oral Pathology and Medicine. 2025 Jan 27;54(2):112–9.
- 29 Moradzadeh S, Moghaddam SS, Rahimi A, Pourakbar L, El A, Sayyed RZ. Bio-Chemical Fertilizer Improves the Oil Yield, Fatty Acid Compositions, and Macro-Nutrient Contents in *Nigella sativa* L. Horticulturae. 2021 Sept 27;7(10):345–5.
- 29 Morgan Library & Museum . De materia medica [Internet]. The Morgan Library & Museum. 2017. Available from: <https://www.themorgan.org/manuscript/143825>
- 29 Mousavi SH, Tayarani-Najaran Z, Asghari M, Sadeghnia HR. Protective Effect of *Nigella sativa* Extract and Thymoquinone on Serum/Glucose Deprivation-Induced PC12 Cells Death. Cellular and Molecular Neurobiology. 2010 Jan 7;30(4):591–8.
- 29 MPS Society. Barriers and facilitators to clinical trial participation: Improving accessibility, logistics and awareness [Internet]. MPS Society. 2024 [cited 2026 Jan 16]. Available from: <https://mpssociety.org.uk/resources/barriers-and-facilitators-to-clinical-trial-participation-improving-accessibility-logistics-and-awareness>
- 29 Muir W. The city of Baghdad between 150 and 300 AH(767 and 912 AD) [Internet]. Wikimedia.org. 1883 [cited 2026 June 9]. Available from: https://upload.wikimedia.org/wikipedia/commons/0/07/Baghdad_150_to_300_AH.png
- 29 Mulani S. How to Normalize data in R [3 easy methods] | DigitalOcean [Internet]. www.digitalocean.com. 2022. Available from: <https://www.digitalocean.com/community/tutorials/normalize-data-in-r>

- 29 Mumtaz A. A. View of NIGELLA SATIVA: A BIBLIOMETRIC STUDY OF THE LITERATURE ON
8. HABBAT AL-BARAKAH [Internet]. Um.edu.my. 2005 [cited 2026 Jan 9]. Available from: <https://ajba.um.edu.my/index.php/MJLIS/article/view/8474/5899>
- 29 Naghsh N, Moridpour AH, Kavyani Z, Musazadeh V, afarzadeh JJ, Safaei E, et al. The effect
9. of Nigella sativa (black seed) supplementation on body weight and body composition: A GRADE-assessed systematic review and dose-response meta-analysis of randomized controlled trials. *Journal of Functional Foods*. 2023 June 1;105:105565–5.
- 30 Namazi N, Larijani B, Ayati MH, Abdollahi M. The effects of Nigella sativa L. on obesity: A
0. systematic review and meta-analysis. *Journal of Ethnopharmacology* [Internet]. 2018 June 12;219:173–81. Available from: <https://www.sciencedirect.com/science/article/pii/S0378874117336371?via%3Dihub>
- 30 National Cancer Institute. Oral Contraceptives and Cancer Risk [Internet]. National Cancer
1. Institute. Cancer.gov; 2018. Available from: <https://www.cancer.gov/about-cancer/causes-prevention/risk/hormones/oral-contraceptives-fact-sheet>
- 30 National Cancer Institute. Targeted Therapy to Treat Cancer [Internet]. National Cancer
2. Institute. Cancer.gov; 2022. Available from: <https://www.cancer.gov/about-cancer/treatment/types/targeted-therapies>
- 30 National Library of Medicine. PubMed [Internet]. National library of medicine. 2025.
3. Available from: <https://pubmed.ncbi.nlm.nih.gov/>
- 30 Native Plant Trust - GO BOTANY, Andrea M. Stems: Nigella sativa. [Internet].
4. gobotany.nativeplanttrust.org. [cited 2026 Jan 13]. Available from: <https://gobotany.nativeplanttrust.org/species/nigella/sativa/>
- 30 Natural Resources Conservation Service. USDA Plants Database [Internet]. Usda.gov.
5. 2026 [cited 2026 Jan 13]. Available from: <https://plants.usda.gov/plant-profile/NISA2>
- 30 nature index . Pharmacological Applications of Nigella Sativa and Thymoquinone | Clinical
6. Pharmacology and Therapeutics | Pharmacology and Pharmaceutical Sciences | Health sciences | Topics | Nature Index [Internet]. Nature.com. Nature Index; 2026 [cited 2026 June 11]. Available from: <https://www.nature.com/nature-index/topics/l4/pharmacological-applications-of-nigella-sativa-and-thymoquinone>

- 30 Nawaz A, Arif A, Jamal A, Shahid MN, Nomani I, Bahwerth FS. Medicinal plants show
7. remarkable antiproliferative potential in human cancer cell lines. *Bioscience, Biotechnology, and Biochemistry*. 2022 Jan 4;86(3):362–7.
- 30 NCI. Types of Cancer Treatment [Internet]. National Cancer Institute. 2025. Available from:
8. <https://www.cancer.gov/about-cancer/treatment/types>
- 30 Ndirangu E, Opiyo S, Ng'ang'a M. Repellent Properties of Compounds and
9. Blends from *Nigella sativa* Seeds Against *Anopheles gambiae*. *Basic Sciences of Medicine*. 2020(1):1–7.
- 31 Nepomuceno L, Carvalho LS de, Soares NP, Cruz V de S, Arnhold E, Ferreira JL, et al. In
0. vitro methodological standardization of MTT colorimetric assay for evaluation of formazan activity and drug dosage. *Research Square* [Internet]. 2020 Jan 14 [cited 2026 June 10];. Available from: <https://www.researchsquare.com/article/rs-11376/v1.pdf>
- 31 NEWCASTLE - OTTAWA, Ottawa Hospital Research Institute. NEWCASTLE -OTTAWA
1. QUALITY ASSESSMENT SCALE CASE CONTROL STUDIES [Internet]. Available from: <https://ohri.ca/sites/ohri/files/2025-09/nosgen.pdf>
- 31 News Medical. News-Medical [Internet]. News-Medical. 2025 [cited 2026 Jan 1].
2. Available from: <https://www.news-medical.net/news/20251118/Daily-black-cumin-intake-improves-blood-lipids-and-reduces-obesity-risk.aspx>
- 31 Nicholson G. The Illustrated Dictionary of Gardening, Div. VI (London, England: L. Upcott
3. Gill, 1884) [Internet]. 1884 [cited 2026 Jan 11]. Available from: https://etc.usf.edu/clipart/82700/82778/82778_nigella_2.htm
- 31 Noman AM, Sultan MT, Zafar S, Maaz M, Mazhar A, Hussain M, et al. Thymol and Carvacrol:
4. Molecular Mechanisms, Therapeutic Potential, and Synergy With Conventional Therapies in Cancer Management. *Food Science & Nutrition* [Internet]. 2025 Sept 1 [cited 2025 Oct 18];13(9). Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12436185/>
- 31 Nutsinbulk. SPECIFICATIONS OF ORGANIC BLACK CUMIN SEED [Internet]. 2021 Apr
5. [cited 2026 June 12]. Available from: <https://www.nutsinbulk.co.uk/document/preview/49/mB4dZyqOObYITRFyUrVpPgLCzmwss2NguxKjC8b.pdf>
- 31 Oakeley H. *Nigella sativa* L. Ranunculaceae Loveinthemist, Black Cumin, Nutmeg flower,
6. Roman Coriander. Distribution: SW Asia. Culpeper (1650) writes: 'Nigella seeds, boyled in oil, and the forehead anointed with it, ease pains in the head, take away leprosie, itch,

scurf, and helps scaldheads, inwardly taken they expel worms, they provoke urine and the terms, help difficulty of breathing: the smoke of them (being burned) drives away serpents and venomous beasts.' The seeds are used as a spice, but as might be expected as a member of the family Ranunculaceae, the buttercups, the plant contains a highly poisonous glycoside, in this case called melanthin. The amount of toxicity present in spices is clearly insufficient to cause problems when used as such. Photographed in the Medicinal Garden of the Royal College of Physicians, London. JSTOR [Internet]. Available from: <https://jstor.org/stable/community.24716542>

- 31 Ojueromi OO, Oboh G, Ademosun AO. Black Seed (*Nigella sativa*): A Favourable Alternative Therapy for Inflammatory and Immune System Disorders. *Inflammopharmacology*. 2022 Aug 16;30(5):1623–43.
- 31 Omid Mohammad Tavakoli-Rouzbehani, Maleki V, Mahdi Shadnough, Taheri E, Alizadeh M. A comprehensive insight into potential roles of *Nigella sativa* on diseases by targeting AMP-activated protein kinase: a review. *Daru* [Internet]. 2020 Nov 2 [cited 2024 May 12];28(2):779–87. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7704809/>
- 31 open access text. Overview of the Human Investigations of *Nigella sativa* (Black Seeds): A Complementary Drug with Historical and Clinical Significance [Internet]. Oatext.com. 2018 [cited 2026 Jan 15]. Available from: <https://www.oatext.com/overview-of-the-human-investigations-of-nigella-sativa-black-seeds-a-complementary-drug-with-historical-and-clinical-significance.php>
- 32 Orhan N. ABC Herbalgram Website [Internet]. Doi.org. 2026 [cited 2026 June 10]. Available from: <https://doi.org/10.59520/BAPP.BAPB/VPNM5432>
- 32 Ottawa Hospital Research Institute. Newcastle-Ottawa Quality Assessment Form for Cohort Studies.
- 32 Ozel A, Demirel U, Güler İ, Erden K. Effect of Different Row Spacing and Seeding Rates on Black Cumin (*Nigella Sativa* L.) Yields and Some... *Harran Tarım ve Gıda Bilimleri Dergisi* [Internet]. 2009 [cited 2026 June 9];13(1):17–26. Available from: https://www.researchgate.net/publication/301694855_Effect_of_Different_Row_Spacing_and_Seeding_Rates_on_Black_Cumin_Nigella_Sativa_L_Yields_and_Some_Agricultural_Characters
- 32 Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow C D, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *British*

Medical Journal [Internet]. 2021;372(71). Available from:
<https://www.bmj.com/content/372/bmj.n71>

- 32 Parate MA, Bajpai ND, Wasule D D. Nigella Sativa Seed: A Cosmetic Herb [Internet]. 2021 Apr. Available from: <https://ijcrt.org/papers/IJCRT2104548.pdf>
- 32 Paszkiewicz M, Godlewska K, Lis H, Caban M, Białk-Bielińska A, Stepnowski P. Advances in suspect screening and non-target analysis of polar emerging contaminants in the environmental monitoring. *TrAC Trends in Analytical Chemistry*. 2022 Sept;154:116671.
- 32 Pedrosa LM, Rafael B, Martins CC. Optimization of Harvesting and Drying Techniques for Quality Seed Production in Specialty Crops: A Systematic Review and Meta-Analysis. *Agronomy*. 2024 Aug 2;14(8):1705–5.
- 32 Periasamy VS, Athinarayanan J, Alshatwi AA. Anticancer activity of an ultrasonic nanoemulsion formulation of Nigella sativa L. essential oil on human breast cancer cells. *Ultrasonics Sonochemistry*. 2016 July;31:449–55.
- 32 Persson B, Hansson U. Hypoglycaemia in pregnancy. *Baillière's Clinical Endocrinology and Metabolism*. 1993 July;7(3):731–9.
- 32 Perveen T, Abdullah A, Haider S, Haleem D. Long-term administration of N. sativa effects on nociception and improves learning and memory in rats. *ResearchGate [Internet]*. 2008 [cited 2026 Jan 6];41(3):141–3. Available from:
https://www.researchgate.net/publication/285499631_Long-term_administration_of_N_sativa_effects_nociception_and_improves_learning_and_memory_in_rats
- 33 Pidugu VK, Pidugu HB, Wu MM, Liu CJ, Lee TC. Emerging Functions of Human IFIT Proteins in Cancer. *Frontiers in Molecular Biosciences*. 2019 Dec 19;6.
- 33 Pittino OM, Semprini MS, Ruzzi F, Angelicola S, Cappello C, Scalambra L, et al. Alternatives for fetal bovine serum (FBS) and sustainability of milk derivatives in cell culture. *Biological Research*. 2026 Mar 28;59(1).
- 33 Pollard DA, Pollard TD, Pollard KS. Empowering statistical methods for cellular and molecular biologists. Drubin DG, editor. *Molecular Biology of the Cell*. 2019 June;30(12):1359–68.

- 33 Prakhova TYa. Ecological Aspects of the Productivity of *Nigella* Varieties under the
3. Conditions of the Middle Volga Region. Russian Agricultural Sciences. 2022
June;48(3):169–73.
- 33 Precedence Research. Oncology Combination Therapy Market Size, Report by 2034
4. [Internet]. Precedenceresearch.com. Precedence Research ; 2025 [cited 2026 Jan 17].
Available from: [https://www.precedenceresearch.com/oncology-combination-therapy-](https://www.precedenceresearch.com/oncology-combination-therapy-market)
market
- 33 Pro-Mix Gardening. Sowing annuals: *Nigella damascena* Love-in-a-Mist [Internet]. PRO-
5. MIX Gardening. 2026 [cited 2026 Jan 25]. Available from:
<https://www.promixgardening.com/en/tips/nigella-damascena>
- 33 PubChem. PubChem [Internet]. Nih.gov. National Library of Medicine; 2004. Available
6. from: <https://pubchem.ncbi.nlm.nih.gov/>
- 33 Quanticate. Nutraceutical trials: demand, design and challenges [Internet].
7. Quanticate.com. 2018. Available from: [https://www.quanticate.com/blog/nutraceutical-](https://www.quanticate.com/blog/nutraceutical-trials-design)
trials-design
- 33 Queiroz A, Wada MT, Perillo C, Pelissari C, Trierveiler M. Effects of serum-free culture
8. media on human apical papilla cells properties. Archives of Oral Biology. 2020 Nov
2;121:104962–2.
- 33 Quran.com. Quran [Internet]. Al-Qur'an al-Kareem - القرآن الكريم. Al-Quran - القرآن الكريم;
9. 2025. Available from: <https://quran.com/>
- 34 Rahim MA, Shoukat A, Khalid W, Ejaz A, Itrat N, Majeed I, et al. A Narrative Review on
0. Various Oil Extraction Methods, Encapsulation Processes, Fatty Acid Profiles, Oxidative
Stability, and Medicinal Properties of Black Seed (*Nigella sativa*). Foods [Internet]. 2022
Sept 13;11(18):2826. Available from:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9498113/>
- 34 Rahman A. Studies in Natural Products Chemistry. Elsevier; 2025.
1.
- 34 Rahman A, Ilhan S, Oguz F, Atmaca H. Studies in Natural Products Chemistry. Elsevier;
2. 2025.

- 34 Rahman M, Yang DK, Kim GB, Lee SJ, Kim SJ. Nigella sativa seed extract attenuates the
3. fatigue induced by exhaustive swimming in rats. Biomedical Reports. 2017 Feb
24;6(4):468–74.
- 34 Rahmani AH, Alzohairy MA, Khan MA, Aly SM. Therapeutic Implications of Black Seed and
4. Its Constituent Thymoquinone in the Prevention of Cancer through Inactivation and
Activation of Molecular Pathways. Evidence-Based Complementary and Alternative
Medicine [Internet]. 2014 [cited 2019 Sept 26];2014:1–13. Available from:
<https://www.hindawi.com/journals/ecam/2014/724658/>
- 34 Rai Y, Pathak R, Kumari N, Sah DK, Pandey S, Kalra N, et al. Mitochondrial biogenesis and
5. metabolic hyperactivation limits the application of MTT assay in the estimation of radiation
induced growth inhibition. Scientific Reports. 2018 Jan 24;8(1).
- 34 Rajkapoor B, Anandan R, Jayakar B. Anti-ulcer effect of Nigella sativa Linn. against gastric
6. ulcers in rats. CURRENT SCIENCE. 2002;82(2).
- 34 Rathi B, Devanesan S, AlSalhi MS, Singh R. In-vitro free radical scavenging effect and
7. cytotoxic analysis of Black Cummins and Honey formulation. Saudi Journal of Biological
Sciences [Internet]. 2021 Jan 9 [cited 2025 Feb 27];28(3):1576–81. Available from:
<https://pmc.ncbi.nlm.nih.gov/articles/PMC7938148/>
- 34 Rathod ML, Angadi BM, Bhavi IG. A review on optimization of process parameters of cold
8. pressed oil extraction for high output and for enhanced quality and retained nutrients. Food
and Humanity [Internet]. 2025 July 3;5:100698. Available from:
<https://www.sciencedirect.com/science/article/abs/pii/S2949824425002022>
- 34 Razmpoosh E, Safi S, Mazaheri M, Khalesi S, Nazari M, Mirmiran P, et al. A crossover
9. randomized controlled trial examining the effects of black seed (Nigella sativa)
supplementation on IL-1 β , IL-6 and leptin, and insulin parameters in overweight and obese
women. BMC Complementary Medicine and Therapies. 2024 Jan 5;24(1).
- 35 Rchid H, Nmila R, Bessière JM, Sauvaire Y, Chokaïri M. Volatile Components of *Nigella*
0. *damascena* L. and *Nigella sativa* L. Seeds. Journal of Essential Oil Research. 2004
Nov;16(6):585–7.
- 35 Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies
1. revisited. The FASEB Journal. 2007 Oct 17;22(3):659–61.

- 35 RHS. *Nigella damascena* ‘Mulberry Rose’ | love-in-a-mist ‘Mulberry Rose’ Annual
2. Biennial/RHS [Internet]. Rhs.org.uk. Royal Horticultural Society; 2025 [cited 2026 Jan 5]. Available from: <https://www.rhs.org.uk/plants/139239/nigella-damascena-mulberry-rose/details>
- 35 RHS. *Nigella damascena* Persian Jewels Group | love-in-a-mist Persian Jewels Group
3. Annual Biennial/RHS [Internet]. Rhs.org.uk. Royal Horticultural Society; 2026 [cited 2026 June 12]. Available from: <https://www.rhs.org.uk/plants/280968/nigella-damascena-persian-jewels-group/details>
- 35 RHS. *Nigella sativa* | black cumin Annual Biennial/RHS Gardening [Internet].
4. www.rhs.org.uk. Available from: <https://www.rhs.org.uk/plants/136295/nigella-sativa/details>
- 35 Riss T, Moravec R, Niles AL, Hélène Benink. Cell viability assays [Internet]. ResearchGate.
5. unknown; 2013. Available from: https://www.researchgate.net/publication/303085850_Cell_viability_assays
- 35 Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, et al. Cell Viability
6. Assays [Internet]. Nih.gov. Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2016. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK144065/>
- 35 Rixon C, Adorada D, Umansky F, Chauhan B, Bhattarai S, Biggs I, et al. Kalonji Grower
7. Guide [Internet]. 2025. Available from: <https://crcna.com.au/wp-content/uploads/2025/03/Kalonji-Grower-Guide-Jan-2025.pdf>
- 35 Rizwana Batool, Ramzan R, Raza A, Aziz M, Madiha Rohi, Naeem A, et al. Dietary
8. supplementation of black cumin (*Nigella sativa*) meal in the formulation of protein-enriched cookies, further in vivo evaluation of protein quality with physicochemical and organoleptic characterization. Food Science & Nutrition. 2024 July 23;12(10):7405–16.
- 35 Roepke M, Diestel A, Bajbouj K, Walluscheck D, Schonfeld P, Roessner A, et al. Lack of p53
9. augments thymoquinone-induced apoptosis and caspase activation in human osteosarcoma cells. Cancer Biology & Therapy. 2007 Feb;6(2):160–9.
- 36 Rogozhin EA, Oshchepkova YI, Odintsova TI, Khadeeva NV, Veshkurova ON, Egorov TA, et
0. al. Novel antifungal defensins from *Nigella sativa* L. seeds. Plant Physiology and Biochemistry. 2010 Nov 20;49(2):131–7.

- 36 Roszkowski S. Application of Polyphenols and Flavonoids in Oncological Therapy.
 1. Molecules. 2023 May 13;28(10):4080–0.
- 36 RStudio. RStudio Desktop [Internet]. Posit. 2023. Available from:
 2. <https://posit.co/download/rstudio-desktop/>
- 36 S. VT, S. F, B. HM, D. MS. A review on therapeutic potential of *Nigella sativa* (kalonji) seeds.
 3. Journal of Medicinal Plants Research. 2014 Jan 17;8(3):167–77.
- 36 S.A TE, S.A ES. Potential Assessment Multi-Repeating Abiotic/Biotic Motivation Coincide
 4. Biofertilizers to Optimize Black Cumin (<i>Nigella sativa</i> L.) Seed Yield Production and Quality. Agricultural Sciences. 2021;12(02):69–83.
- 36 Sadeghi E, Imenshahidi M, Hosseinzadeh H. Molecular mechanisms and signaling
 5. pathways of black cumin (*Nigella sativa*) and its active constituent, thymoquinone: a review. Molecular Biology Reports. 2023 May 8;50.
- 36 Safwan Ahamad Bustamam M, Hadithon KA, Mediani A, Abas F, Rukayadi Y, Lajis N, et al.
 6. Stability Study of Algerian *Nigella sativa* Seeds Stored under Different Conditions. Journal of Analytical Methods in Chemistry. 2017 Jan 1;2017:1–12.
- 36 SALAHELDIN S, HENDAWY SF, HUSSEIN MS, SOLIMAN WS. ASSESSMENT THE YIELD
 7. AND QUALITY OF NIGELLA SATIVA UNDER DIFFERENT ENVIRONMENTAL CONDITIONS. International Journal of Pharmacy and Pharmaceutical Sciences. 2020 Sept 2;29–33.
- 36 Salama SM. Black cumin seeds as animal feed. Elsevier eBooks. 2022 Nov 11;119–26.
 - 8.
- 36 Salih B, Sipahi T, Dönmez EO. Ancient nigella seeds from Boyalı Höyük in north-central
 9. Turkey. Journal of Ethnopharmacology. 2009 July;124(3):416–20.
- 37 SALIM EI. Cancer chemopreventive potential of volatile oil from black cumin seeds, *Nigella*
 0. *sativa* L., in a rat multi-organ carcinogenesis bioassay. Oncology Letters. 2010 Sept;1(5):913–24.
- 37 Salomi NJ, Nair SC, Jayawardhanan KK, Varghese CD, Panikkar KR. Antitumour principles
 1. from *Nigella sativa* seeds. Cancer Letters. 1992 Mar 31;63(1):41–6.

- 37 Samak YO, Santhanes D, El-Massik MA, Coombes AGA. Formulation strategies for
2. achieving high delivery efficiency of thymoquinone-containing *Nigella sativa* extract to the colon based on oral alginate microcapsules for treatment of inflammatory bowel disease. *Journal of Microencapsulation* [Internet]. 2019 Mar 1 [cited 2022 Feb 20];36(2):204–14. Available from: <https://pubmed.ncbi.nlm.nih.gov/31164027/>
- 37 Sammar M, Abu-Farich B, Rayan I, Falah M, Rayan A. Correlation between cytotoxicity in
3. cancer cells and free radical-scavenging activity: In vitro evaluation of 57 medicinal and edible plant extracts. *Oncology Letters*. 2019 Nov 5;.
- 37 Santos L, Francisco P, DINIZ EA, Rahhal JG, Sipert CR. Different concentrations of fetal
4. bovine serum affect cytokine modulation in Lipopolysaccharide-activated apical papilla cells in vitro. *Journal of Applied Oral Science*. 2023 Jan 1;31.
- 37 Sarah Raven. *Nigella damascena* ‘Deep Blue’ [Internet]. Sarah Raven. 2020 [cited 2026 Jan
5. 25]. Available from: <https://www.sarahraven.com/products/nigella-damascena-deep-blue>
- 37 Schneider-Stock R, Fakhoury IH, Zaki AM, El-Baba CO, Gali-Muhtasib HU. Thymoquinone:
6. fifty years of success in the battle against cancer models. *Drug Discovery Today* [Internet]. 2014 Jan 1;19(1):18–30. Available from: <https://www.sciencedirect.com/science/article/pii/S1359644613002882>
- 37 Schumm WR, Higgins M, Lockett L, Huang S, Abdullah N, Asiri A, et al. Does Dividing the
7. Range by Four Provide an Accurate Estimate of a Standard Deviation in Family Science Research? A Teaching Editorial. *Marriage & Family Review*. 2016 June 8;53(1):1–23.
- 37 Schumm WR, Higgins M, Lockett L, Huang S, Abdullah N, Asiri A, et al. Does Dividing the
8. Range by Four Provide an Accurate Estimate of a Standard Deviation in Family Science Research? A Teaching Editorial. *Marriage & Family Review*. 2016 June 8;53(1):1–23.
- 37 ScienceDirect. Elsevier B.V. [Internet]. [id.elsevier.com](https://www.sciencedirect.com). 2026. Available from:
9. <https://www.sciencedirect.com/search/entry>
- 38 Scopus . Scopus search | Elsevier [Internet]. www.elsevier.com. 2026. Available from:
0. <https://www.elsevier.com/en-gb/products/scopus/search>
- 38 Seed Ville USA. 150 NIGELLA Sativa BLACK CUMIN Spice Black Caraway Fennel Flower
1. Herb Seeds [Internet]. Seedville USA. 2026 [cited 2026 June 12]. Available from:

<https://seedvilleusa.com/products/150-nigella-sativa-black-cumin-spice-black-caraway-fennel-flower-herb-seeds-320140346>

38 selleck. Thymoquinone | $\geq 99\%$ (HPLC) | Selleck | Immunology & Inflammation related 阻害

2. 劑 [Internet]. Selleck.co.jp. 2026 [cited 2026 June 5]. Available from:

<https://www.selleck.co.jp/products/thymoquinone.html>

38 Shaddel R, Rashidinejad A, Karimkhani MM, Tarhan O, Jafari SM. Nanodelivery systems of

3. thymoquinone for improving its bioavailability and efficiency in the food and biomedical applications. Industrial Crops and Products [Internet]. 2025 July 24;234:121555. Available from: <https://www.sciencedirect.com/science/article/pii/S092666902501101X>

38 Shady, Abeer M, Nooh, Hanaa Z. Effect of Black Seed (Nigella Sativa) on Compact Bone of

4. Streptozotocin Induced Diabetic Rats [Internet]. Egyptian Journal of Histology. 2010 [cited 2026 Jan 9]. Available from:

https://journals.lww.com/ejhistology/fulltext/2010/03000/Effect_of_Black_Seed__Nigella__Sativa__on_Compact.16.aspx

38 Shah SH. Physiological effects of pre-sowing seed treatment with gibberellic acid on

5. Nigella sativa L. Acta Botanica Croatica [Internet]. 2007 Apr 16 [cited 2026 June 10];66(1):67–73. Available from: <https://hrcak.srce.hr/clanak/18110>

38 SHAH SH. Photosynthetic and Yield Responses of Nigella sativa L. to Pre-sowing Seed

6. Treatment with GA3 [Internet]. TÜBİTAK Academic Journals. 2022 [cited 2026 June 10]. Available from: <https://journals.tubitak.gov.tr/biology/vol31/iss2/7/>

38 Sharma N, Ahirwar D, Jhade D, Gupta S. Medicinal and Phamacological Potential of Nigella

7. sativa: A Review. Ethnobotanical Review. 2009;13:946–55.

38 Sharma P, Roy M, Roy B, Deka SD. Post Harvest Management Strategies and Storage

8. Approaches for Quality Seed Production. Emerging Issues in Agricultural Sciences. 2023 Mar 25;2:110–29.

38 Sherwood SC. Methane Mitigation and Its Effects on Energy Utilization in Lactating Dairy

9. Cattle [Internet]. DigitalCommons@University of Nebraska - Lincoln. 2025 [cited 2026 June 9]. Available from: <https://digitalcommons.unl.edu/animalscidiss/285/>

39 shutterstock, Dionisvera. Black cumin seeds with nigella sativa flower on grey background

0. [Internet]. [cited 2024 Nov 12]. Available from: <https://www.shutterstock.com/image-photo/black-cumin-seeds-nigella-sativa-flower-2544390783>

- 39 Siebenthaler's Plant Care Poison Plant List. Poison Plant List | Siebenthaler's [Internet].
 1. Siebenthaler.com. 2020 [cited 2026 Jan 16]. Available from:
<https://www.siebenthaler.com/plant-care-guides-and-handouts/poison-plant-list/>
- 39 Sileem TM, Mehany AL, Hassan RS. Fumigant toxicity of some essential oils against Red
2. Flour Beetles, *Tribolium castaneum* (Herbst) and its safety to mammals. Brazilian Journal of Biology. 2019 Dec 3;80(4):769–76.
- 39 simplemaps. Free World SVG Map | Resources [Internet]. simplemaps.com. Available
3. from: <https://simplemaps.com/resources/svg-world>
- 39 Sina H ibn, Hamdard University. Canon of Medicine Book II Materia Medica English
4. translation of the critical Arabic text With Index to Latin and English plant names [Internet]. Department of Islamic Studies Hamdard University; 1998 [cited 2026 Jan 21] p. 306–7. Available from: <https://www.rjwhelan.co.nz/articles/pdf/CANON-Book-II-Hamdard.pdf>

Book II Materia Medica
- 39 SINGH KK, GOSWAMI TK. STUDIES ON CRYOGENIC GRINDING OF CUMIN SEED. Journal
5. of Food Process Engineering. 1999 Aug 1;22(3):175–90.
- 39 Soleymani S, Zargaran A, Farzaei MH, Iranpanah A, Heydarpour F, Najafi F, et al. The effect
6. of a hydrogel made by *Nigella sativa* L. on acne vulgaris: A randomized double-blind clinical trial. Phytotherapy Research. 2020 June 17;34(11):3052–62.
- 39 Soleymani S, Zargaran A, Farzaei MH, Iranpanah A, Heydarpour F, Najafi F, et al. The effect
7. of a hydrogel made by *Nigella sativa* L. on acne vulgaris: A randomized double-blind clinical trial. Phytotherapy Research. 2020 June 17;34(11):3052–62.
- 39 South Downs National Park Trust. Beelines Fund - South Downs Trust [Internet]. South
8. Downs Trust. 2025. Available from: <https://southdownstrust.org.uk/beelines-fund/>
- 39 Spherical Insights LLP. Global Black Seed Oil Market Size To Worth USD 41.56 Billion By
9. 2033 | CAGR Of 7.76% [Internet]. Yahoo Finance. 2024 [cited 2026 Jan 17]. Available from:
<https://finance.yahoo.com/news/global-black-seed-oil-market-060000372.html?guccounter=1>
- 40 Squires P, Cook EE, Song Y, Wang CY, Jiang AX, Zhang A, et al. Real-world economic burden
0. of disease recurrence in patients with muscle-invasive bladder cancer: A population-level

claims-based analysis. *Journal of Managed Care & Specialty Pharmacy*. 2025 Sept 17;31(12):1248–58.

- 40 Stevens Barron JC, de la Rosa LA, Alvarez-Parrilla E, Wall-Medrano A, Chapa González C.
1. Antiproliferative Activity of α -Tocopherol, γ -Tocopherol and Tocotrienols and Their Drug Interactions Evaluated Using Loewe and Chou–Talalay Models in HeLa and MCF-7 Cancer Cell Lines. *Biomedicines*. 2026 Feb 18;14(2):458.
- 40 Sun S, Yu Y, Jo Y, Han JH, Xue Y, Cho M, et al. Impact of extraction techniques on
2. phytochemical composition and bioactivity of natural product mixtures. *Frontiers in Pharmacology*. 2025 July 30;16.
- 40 Sunnah.com. Sahih al-Bukhari 5688 - Medicine - كتاب الطب - Sunnah.com - Sayings and
3. Teachings of Prophet Muhammad (صلى الله عليه وسلم) [Internet]. Sunnah.com. 2026 [cited 2026 Jan 6]. Available from: <https://sunnah.com/bukhari:5688>
- 40 SURI S, KUMAR V, TANWAR B, GOYAL A, GAT Y. Impact of Soaking and Germination Time
4. on Nutritional Composition and Antioxidant Activity of *Nigella Sativa*. *Current Research in Nutrition and Food Science Journal*. 2019 Mar 8;7(1):142–9.
- 40 Swamy SMK, Tan BKH. Cytotoxic and immunopotentiating effects of ethanolic extract of
5. *Nigella sativa* L. seeds. *Journal of Ethnopharmacology*. 2000 Apr;70(1):1–7.
- 40 T Rostinawati, S Karipaya, Iskandar Y. Antibacterial Activity of Ethanol Extract of *Nigella*
6. *Sativa* L. Seed Against *Streptococcus mutans*. *IOP Conference Series Earth and Environmental Science*. 2019 Oct 1;334(1):012050–0.
- 40 Tackenberg O, iFlora. *Nigella sativa* L. Black-Cumin [Internet]. [cited 2026 Jan 13].
7. Available from: <https://www.i-flora.com/en/the-smartphone-apps/iflora-deutschland/species/art/show/nigella-sativa-1.html>
- 40 Tahraoui A, El-Hilaly J, Israili ZH, Lyoussi B. Ethnopharmacological survey of plants used in
8. the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). *Journal of Ethnopharmacology*. 2007 Mar;110(1):105–17.
- 40 Talorete TPN, Bouaziz M, Sayadi S, Isoda H. Influence of medium type and serum on MTT
9. reduction by flavonoids in the absence of cells. *Cytotechnology* [Internet]. 2007 Feb 24 [cited 2019 Apr 23];52(3):189–98. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3449413/>

- 41 Thabede PM, Shooto ND. Application of black cumin (*Nigella sativa* L.) seeds for the
0. removal of metal ions and methylene blue from aqueous solutions. Cogent Engineering. 2021 Dec 27;9(1).
- 41 Thabrew MI, Mitry RR, Morsy MA, Hughes RD. Cytotoxic effects of a decoction of *Nigella*
1. *sativa*, *Hemidesmus indicus* and *Smilax glabra* on human hepatoma HepG2 cells. Life Sciences. 2005 Aug;77(12):1319–30.
- 41 Thakur V, Nawalagatti CM, Chetti MB, Hilli JS, Patil RV. Changes in Blackgram Seed Quality
2. during Storage as Influenced by Various Packaging Materials and Storage Conditions. Indian Journal of Ecology. 2022 Dec 30;.
- 41 Thani PR, Johnson JB, Bhattarai S, Trotter T, Walsh K, Broszczak D, et al. Optimizing *Nigella*
3. Oil Extraction Temperature for Sustainable Production. Applied Sciences [Internet]. 2024 Sept 18;14(18):8377–7. Available from: <https://www.mdpi.com/2076-3417/14/18/8377>
- 41 The Editors of Encyclopedia Britannica. Ebers papyrus | Egyptian texts. In: Encyclopædia
4. Britannica [Internet]. 2018. Available from: <https://www.britannica.com/topic/Ebers-papyrus>
- 41 The International Chamber of Commerce United Kingdom. Why patents are essential for
5. innovation and growth [Internet]. 2025. Available from: <https://iccwbo.uk/wp-content/uploads/2025/06/Report-on-Patents-Innovation-and-Growth-ICC-UK-CIPA-IP-Federation.pdf>
- 41 The metafor Package. Bootstrapping with Meta-Analytic Models [The metafor Package]
6. [Internet]. Metafor-project.org. 2022 [cited 2026 June 12]. Available from: https://www.metafor-project.org/doku.php/tips:bootstrapping_with_ma
- 41 The metafor Package. Funnel Plot with Trim and Fill [The metafor Package] [Internet].
7. Metafor-project.org. 2023. Available from: https://www.metafor-project.org/doku.php/plots:funnel_plot_with_trim_and_fill
- 41 The Pennsylvania State University. 2.3 - Tukey Test for Pairwise Mean Comparisons
8. [Internet]. Psu.edu. 2026. Available from: <https://online.stat.psu.edu/stat502/book/export/html/777>
- 41 The Prince Ghazi Trust for Qur'anic Thought. Basmallah - Adham Fadi Al-Ja'fari [Internet].
9. [cited 2026 Jan 6]. Available from: <https://freeislamiccalligraphy.com/?portfolio=basmallah-11>

- 42 Thermo Fisher Scientific. L-Glutamine & GlutaMAX Supplement - US [Internet].
 0. www.thermofisher.com. Available from: <https://www.thermofisher.com/uk/en/home/life-science/cell-culture/mammalian-cell-culture/media-supplements/glutamax-media.html>
- 42 Tiwari RS, Chandra KK, Dubey S, Tripathi S. Influence of Packaging Materials and Storage
 1. Conditions on Seed Germination Ability and Biochemical Changes in Some Medicinal Plants of Indian Forests. *Frontiers in Forests and Global Change*. 2022 June 10;5.
- 42 Toy Y, Kivrik M, Alkhafaji MH, Faraj AM, Gecibesler IH, Ağca CA. Nigella sativa Methanol
 2. Extract Inhibits PC-3 Cell Line Colonization, Induced Apoptosis, and Modulated LC3-based Autophagy. *Karbala International Journal of Modern Science*. 2022 May 5;8(2):44–51.
- 42 TOY Y, KIVRIK M, Alkhafaji MH, FARAJ AM, GECIBESLER IH, AĞCA CA. Nigella sativa
 3. Methanol Extract Inhibits PC-3 Cell Line Colonization, Induced Apoptosis, and Modulated LC3-based Autophagy. *Karbala International Journal of Modern Science*. 2022 May 5;8(2):44–51.
- 42 Trisilowati, Mallet DG. In Silico Experimental Modeling of Cancer Treatment. ISRN
 4. *Oncology* [Internet]. 2012 Feb 1;2012. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3302021/>
- 42 Tsz J, Leng Y, Chow KL, Ren F, Ge X, Wang K, et al. Cell culture medium as an alternative to
 5. conventional simulated body fluid. *Acta Biomaterialia*. 2011 June 1;7(6):2615–22.
- 42 Twisk JW. Refrain From Statistical Testing in Medical Research; It Does More Harm Than
 6. Good. *Annals of Public Health & Epidemiology*. 2024 Mar 21;2(3).
- 42 Uju M, Garba DE, Elechenu EC, Okeke ES. Assessment of Acute Toxicity, LD50 and
 7. Histopathological Evaluation of Nigella sativa and Moringa oleifera Seed Extracts in Wistar Rats. *ResearchGate* [Internet]. 2022 Nov [cited 2026 Jan 20];6(11):1840–3. Available from: https://www.researchgate.net/publication/365996228_Assessment_of_Acute_Toxicity_LD_50_and_Histopathological_Evaluation_of_Nigella_sativa_and_Moringa_oleifera_Seed_Extracts_in_Wistar_Rats
- 42 UK Research and Innovation (UKRI). ARIA: Programmable Plants - opportunity seeds -
 8. Innovate UK Business Connect [Internet]. Innovate UK Business Connect. 2025 [cited 2026 June 9]. Available from: <https://iuk-business-connect.org.uk/opportunities/aria-programmable-plants-opportunity-seeds/>

- 42 Ukwubile CA, Jr OO, Gangpete SI. Development of *Nigella sativa* (black seed) extract-loaded chitosan nanoparticles for targeting *Klebsiella pneumoniae*-induced metastatic colon cancer. *Nano and Medical Materials* [Internet]. 2025 Jan 10 [cited 2026 June 11];5(1):2119. Available from: <https://ojs.acad-pub.com/index.php/NMM/article/view/2119>
- 43 Ullah A, Iqbal S, Luqman M, Jahangeer A, Akhtar N, Saqib M. STANDARDIZATION OF PLANTING TIME AND SEED RATE OF BLACK CUMIN (*NIGELLA SATIVA* L.) IN SOUTH PUNJAB, PAKISTAN. *Journal of Agricultural Research (JAR)* [Internet]. 2024 [cited 2026 June 9];62(1):27–35. Available from: <https://jaragri.com/jar/index.php/jar/article/view/46>
- 43 Unicode CLDR Project. Unicode Transliteration Guidelines [Internet]. Unicode.org. 2024. Available from: <https://cldr.unicode.org/index/cldr-spec/transliteration-guidelines>
- 43 University of Bath. Think tanks, advocacy groups, business and charities [Internet]. Bath.ac.uk. 2024. Available from: <https://www.bath.ac.uk/guides/think-tanks-advocacy-groups-business-and-charities/>
- 43 University of Virginia. Understanding Dunnett’s Test | UVA Library [Internet]. Virginia.edu. 2018. Available from: <https://library.virginia.edu/data/articles/understanding-dunnetts-test>
- 43 U.S. Food and Drug Administration. Substances Added to Food (formerly EAFUS) [Internet]. Fda.gov. 2025 [cited 2026 Jan 5]. Available from: <https://hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances&id=CARAWAYBLACK>
- 43 Valizadeh N, Zakeri HR, Shafiee A, Sarkheil P, Heshmat R, Larijani B. The effect of *Nigella sativa* extract on biochemical bone markers in osteopenic postmenopausal women. [Internet]. CABI Digital Library. *Iranian Journal of Endocrinology and Metabolism*; 2009 [cited 2026 Jan 19]. Available from: <https://www.cabidigitallibrary.org/doi/full/10.5555/20093149960>
- 43 Varastegani B, Lubowa M, Teixeira JA, Easa AM. Storage Stability of Spray Dried *Nigella sativa* (Ranunculaceae Family) Instant Beverage Powder: Effect of Carrier Agents on the Physicochemical, Phenolic Compounds and Antioxidant Properties. *Current Research in Nutrition and Food Science Journal*. 2019 Dec 9;7(3):648–61.
- 43 Ver Sagun J, Yadav UP, Alonso AP. Progress in understanding and improving oil content and quality in seeds. *Frontiers in Plant Science*. 2023 Jan 26;14.

- 43 volkova. Diagonal lines background. straight stripes texture background. simple seamless
8. pattern. line pattern. Geometric background [Internet]. Vecteezy. 2025. Available from:
<https://www.vecteezy.com/free-vector/line-pattern>
- 43 WAKANA H, KONO H, FUKUSHIMA H, NAKATA Y, AKAZAWA Y, MARUYAMA S, et al.
9. Effects of Medium-chain Triglycerides Administration in Chemically-induced
Carcinogenesis in Mice. *Anticancer Research*. 2019 Dec;39(12):6653–60.
- 44 Waliullah Md, Mokter Hossain Md, Habibur Rahman Md. Influence of Sowing Dates and
0. Sowing Methods on Growth and Seed Yield of Black Cumin (*Nigella sativa* L.). *Journal of
Tropical Crop Science*. 2021 July 4;8(02):124–33.
- 44 Wei C, Cheng J, Zou H, Zheng M, Liu X, Du J, et al. TQFL13, a novel synthetic derivative of
1. thymoquinone (TQ), is more capable of inhibiting breast cancer progression than TQ.
Bioorganic Chemistry. 2025 Sept;164:108837.
- 44 Wei Keat Ng, Latifah Saiful Yazan, Ismail M. Thymoquinone from *Nigella sativa* was more
2. potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-
2 protein. *Toxicology in Vitro*. 2011 Oct 1;25(7):1392–8.
- 44 Wells A. Which Countries Produce the Most Clinical Trials? | Arkivum [Internet]. Arkivum.
3. 2025. Available from: <https://arkivum.com/blog/which-countries-produce-the-most-clinical-trials/>
- 44 West SL, Gartlehner G, Mansfield AJ, Poole C, Tant E, Lenfestey N, et al. Table 7, Summary
4. of common statistical approaches to test for heterogeneity [Internet].
www.ncbi.nlm.nih.gov. 2010. Available from:
<https://www.ncbi.nlm.nih.gov/books/NBK53317/table/ch3.t2/>
- 44 WHO. Global Cancer Burden growing, Amidst Mounting Need for Services [Internet].
5. World Health Organization. 2024. Available from: <https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing--amidst-mounting-need-for-services>
- 44 Wijayanti F, Naim M, Sina I. Analysis of Black Seed Effect on *Aedes aegyptii*. *International
6. Journal of Zoological Research* [Internet]. 2019 May 31 [cited 2024 June 23];15(1):13–20.
Available from: <https://scialert.net/fulltext/?doi=ijzr.2019.13.20>
- 44 Wikimedia Commons. *Nigella Sativa* Seed [Internet]. 2006 [cited 2026 Jan 13]. Available
7. from: https://commons.wikimedia.org/wiki/File:Nigella_Sativa_Seed.jpg

- 44 Wikimedia Commons. File:Jungfer im Grünen.JPG - Wikimedia Commons [Internet].
8. Wikimedia.org. 2010 [cited 2026 Jan 25]. Available from:
https://commons.wikimedia.org/wiki/File:Jungfer_im_Gr%C3%BCnen.JPG
- 44 Wild Flowers Provence. Nigella damascena - wild in Provence [Internet]. Wild Flowers
9. Provence. 2022. Available from: <https://www.wildflowersprovence.fr/plant/nigella-damascena/>
- 45 Wisner R. Potential Benefits and Risks for Farmers and Rural Communities The Economics
0. of Pharmaceutical Crops [Internet]. 2005. Available from:
<https://www.ucs.org/sites/default/files/2019-10/ucs-economics-pharma-crops.pdf>
- 45 World Health Organization. Global traditional medicine strategy 2025–2034. World Health
1. Organization; 2025.
- 45 Yaman I, Durmus A, Ceribasi S, Yaman M. Effects of Nigella sativa and silver sulfadiazine
2. on burn wound healing in rats. Veterinární Medicína. 2010 Dec 20;55(No. 12):619–24.
- 45 Yanardağ AB. Effect of *Nigella sativa* Residue and Its Biochar on Soil
3. Microbial Activity, Carbon Sequestration and Nitrogen Cycling Across Different Soil Types. Soil Use and Management. 2025 Apr;41(2).
- 45 Yi T, Cho SG, Yi Z, Pang X, Rodriguez M, Wang Y, et al. Thymoquinone inhibits tumor
4. angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. Molecular Cancer Therapeutics [Internet]. 2008 July 1 [cited 2021 Feb 19];7(7):1789–96. Available from:
<https://mct.aacrjournals.org/content/7/7/1789.figures-only>
- 45 Yildiz F, Coban S, Terzi A, Ates M, Aksoy N, Cakir H, et al. Nigella sativa relieves the
5. deleterious effects of ischemia reperfusion injury on liver. World Journal of Gastroenterology. 2008;14(33):5204.
- 45 Yin R, Zhang K, Li Y, Tang Z, Zheng R, Ma Y, et al. Lipopolysaccharide-induced depression-
6. like model in mice: meta-analysis and systematic evaluation. Frontiers in Immunology [Internet]. 2023;14:1181973. Available from: <https://pubmed.ncbi.nlm.nih.gov/37359525/>
- 45 Yu C, Liu Z, Su T, Li Z, Jiang Z, Zhong W, et al. The effect of anxiety on sleep disorders in
7. medical students: a moderated mediation model. Frontiers in Psychology. 2024 Mar 11;15.

- 45 Yuan Y, Li X, Yao X, Fu X, Cheng J, Shan H, et al. Mechanisms underlying the formation of
8. complex color patterns on *Nigella orientalis* (Ranunculaceae) petals. *New Phytologist*. 2023 Jan 9;237(6):2450–66.
- 45 Zafar A, Khatoon S, Khan MJ, Abu J, Naeem A. Advancements and limitations in traditional
9. anti-cancer therapies: a comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy. *Discover Oncology*. 2025 Apr 24;16(1).
- 46 Zafar I, Safder A, Afridi HI, Riaz S, ur-Rehman R, Unar A, et al. In silico and in vitro study of
0. bioactive compounds of *Nigella sativa* for targeting neuropilins in breast cancer. *Frontiers in chemistry*. 2023 Oct 11;11.
- 46 Zaitoun S, Al-Ghzawi AAM, Samarah N, Alqudah A. Comparative Study in Seed Yield and
1. Flowers Attractivity to Bee Visitors Between *Nigella Sativa* L. and *Nigella Damascena* L. (Ranunculaceae) Grown Under Semiarid Conditions. *Italian Journal of Agronomy*. 2008 June 30;3(2):125.
- 46 Zaoui A, Cherrah Y, Lacaille-Dubois M, Settaf A, Amarouch H, Hassar M. Diuretic and
2. hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat [Internet]. *Europepmc.org*. 2016 [cited 2026 Jan 8]. Available from: <https://europepmc.org/article/med/10967716>
- 46 Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M. Acute and chronic
3. toxicity of *Nigella sativa* fixed oil. *Phytomedicine*. 2002 Jan;9(1):69–74.
- 46 Zitter R, Chugh RM, Saha S. Patient Derived Ex-Vivo Cancer Models in Drug Development,
4. Personalized Medicine, and Radiotherapy. *Cancers*. 2022 June 18;14(12):3006.
- 46 Çakaloğlu Ebcim B, Hazal Ozyurt V, Otles S. Cold press in oil extraction. A review [Internet].
5. *ResearchGate*. 2018. Available from: https://www.researchgate.net/publication/329993761_Cold_press_in_oil_extraction_A_review
- 46 Özcan MM, Ghafoor K, Al Juhaimi F, Ahmed IAM, Babiker EE. Effect of cold-press and
6. soxhlet extraction on fatty acids, tocopherols and sterol contents of the *Moringa* seed oils. *South African Journal of Botany*. 2019 Aug;124:333–7.
- 46 Üstün K, Taysı S, Sezer U, Demir E, Baysal E, Demir T, et al. Radio-protective effects
7. of *Nigella sativa* oil on oxidative stress in tongue tissue of rats. *Oral Diseases*. 2013 Feb 28;20(1):109–13.

46 Şenyiğit U, Arslan M. Effects of Irrigation Programs Formed by Different Approaches on the
8. Yield and Water Consumption of Black Cumin (*Nigella sativa* L.) under Transition Zone in
the West Anatolia Conditions. *Tarim Bilimleri Dergisi-journal of Agricultural Sciences*. 2018
Apr 5;22–32.

46 مؤسسة الشيخ محمد بن [Internet]. القول المفيد على كتاب التوحيد. العثيمين الخيرية.

9. 2026 [cited 2026 June 3]. Available from:

<https://ibnothaimeen.net/products/%D8%A7%D9%84%D9%82%D9%88%D9%84-%D8%A7%D9%84%D9%85%D9%81%D9%8A%D8%AF-%D8%B9%D9%84%D9%89-%D9%83%D8%AA%D8%A7%D8%A8-%D8%A7%D9%84%D8%AA%D9%88%D8%AD%D9%8A%D8%AF>

470. Khanna T, Zaidi FA, Dandiya PC. CNS and analgesic studies on *Nigella sativa*.
1993;64:407–10.

471. Google Trends. Google trends [Internet]. Google Trends. 2025 [cited 2026 Aug 2].
Available from: <https://trends.google.com/trends/>

Appendix A. Supplementary Material

Table S1. PICO elements curated for this research project.

Element	Description
Problem	Uncontrolled cancer cell proliferation
Intervention	<i>Nigella sativa</i> formulations (oil/extract)
Comparison	Cell viability and selective cytotoxicity (<i>in-vitro</i> MTT assay studies)
Outcome	(selective) anti-cancer activity through cel-viability modulation

All planning levels may be compatible with the AND Boolean command. PICO= P, Patient, problem, or population; I, Intervention; C, Comparison/control/comparator; O, Outcome(s)

The PICO protocol (table S1) was used to structure and address the research question: **Can *Nigella sativa* exert an anti-cancer effect cancer cell-proliferation and induce apoptosis, *in-vitro*?**

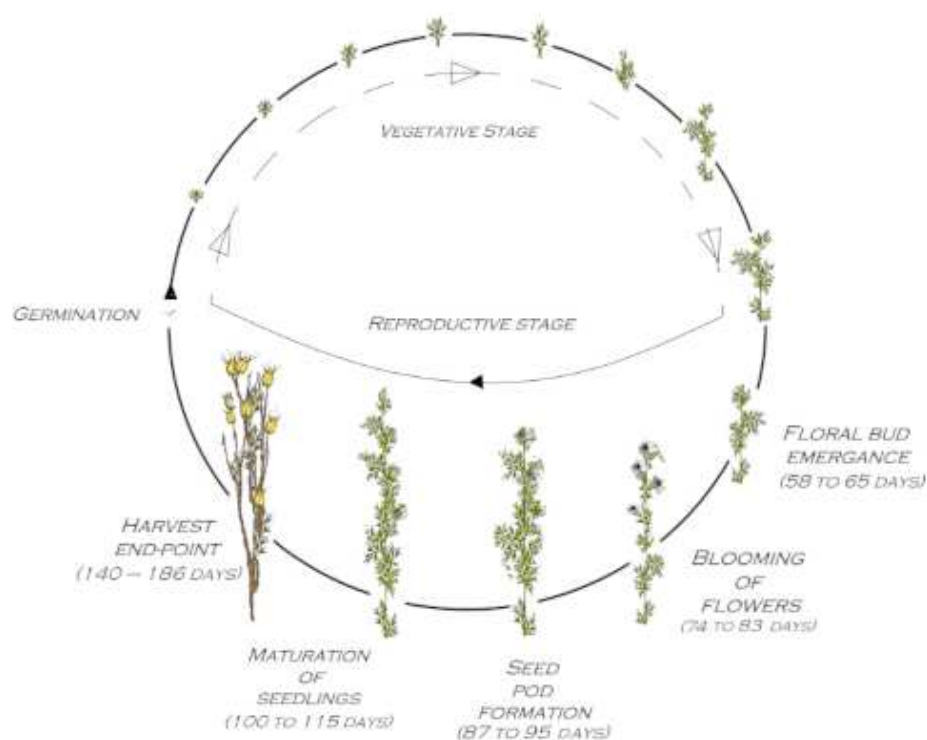


Figure S1: Potential agricultural targets for therapeutic gain. A phytological visual diagram of the stages that may improve yield and constituent 'make-up' within *Nigella sativa* if modulated appropriately through techniques possibly derived from further research. (274)

Preliminary iterations of search queries used across known databases:

((("Thymoquinone" OR "alpha-hederin" OR "α-Hederin" OR "terpins" OR "oloeresin" OR "alkaloids" OR "nigellidine" OR "nigellimine" OR "nigellicine" OR "saponins" OR "thymol" OR "essential oil*" OR "?-cymene" OR "α-thujen" OR "thymohydroquinone" OR "carvacrol" OR "linoleic acid" OR "oleic acid" OR "eicosadienoic acid" OR "unsaturated fatty acid*" OR "fatty acid*" OR "phenolic compounds" OR "flavonoids" OR "tannins" OR "Herbal-Melanin" OR "Nigella sativa" OR "TQ" OR "NS" OR "Black Cumin" OR "Black Caraway" OR "Black Seed") AND ("Cancer* Cell Line" AND ("MTT")))

(((((("Nigella sativa"[Mesh] AND "Nigella sativa oil" [Supplementary Concept])) OR "Antineoplastic Agents"[Mesh]) OR "antiproliferative factor APF, human" [Supplementary Concept]) OR ("Apoptotic Protease-Activating Factor 1"[Mesh] OR "Apoptosis Regulatory Proteins"[Mesh] OR "AP24 apoptotic protease" [Supplementary Concept] OR "DNA Fragmentation"[Mesh] OR "Apoptosomes"[Mesh] OR "Caspase 12"[Mesh] OR "Receptors, Death Domain"[Mesh] OR "Apoptosis Inducing Factor"[Mesh] OR "3-(4, 5-diMethylThiazol-2-yl)-2,5-diphenyltetrazolium bromide" [Supplementary Concept])

((("Thymoquinone" OR "alpha-hederin" OR "α-Hederin" OR "oloeresin" OR "nigellidine" OR "nigellimine" OR "nigellicine" OR "thymol" OR "?-cymene" OR "α-thujen" OR "thymohydroquinone" OR "carvacrol" OR "linoleic acid" OR "oleic acid" OR "eicosadienoic acid" OR "Herbal-Melanin" OR "Nigella sativa" OR "TQ" OR "NS" OR "Black Cumin" OR "Black Caraway" OR "Black Seed") AND ("Cancer* Cell Line" AND ("MTT"))))

((("Thymoquinone" OR "alpha-hederin" OR "α-Hederin" OR "oloeresin" OR "Nigella sativa" OR "Black Seed") AND ("Cancer Cell Line" AND ("MTT"))))

ALL((("Thymoquinone" OR "alpha-hederin" OR "α-Hederin" OR "α-thujen" OR "oloeresin" OR "nigellidine" OR "nigellimine" OR "nigellicine" OR "p-cymene" OR "r-cymene" OR "thymohydroquinone" OR "carvacrol" OR "linoleic acid" OR "oleic acid" OR "eicosadienoic acid" OR "Herbal-Melanin" OR "Nigella sativa" OR "TQ" OR "NS" OR "Black Cumin" OR "Black Caraway" OR "Black Seed") AND ("Cancer Cell Line" AND ("MTT"))) NOT "review") OR (((("Nigella sativa"[Mesh] OR "Nigella sativa oil" [Supplementary Concept])) OR "Antineoplastic Agents"[Mesh]) OR "antiproliferative factor APF, human" [Supplementary Concept]) OR ("Apoptotic Protease-Activating Factor 1"[Mesh] OR "Apoptosis Regulatory Proteins"[Mesh] OR "AP24 apoptotic protease" [Supplementary Concept] OR "DNA Fragmentation"[Mesh] OR "Apoptosomes"[Mesh] OR "Caspase 12"[Mesh] OR "Receptors, Death Domain"[Mesh] OR "Apoptosis Inducing Factor"[Mesh] OR "3-(4, 5-diMethylThiazol-2-yl)-2,5-diphenyltetrazolium bromide" [Supplementary Concept])

("Thymoquinone" OR "alpha-hederin" OR "α-Hederin" OR "α-thujen" OR "oloeresin" OR "nigellidine" OR "nigellimine" OR "nigellicine" OR "p-cymene" OR "r-cymene" OR "thymohydroquinone" OR "carvacrol" OR "linoleic acid" OR "oleic acid" OR "eicosadienoic acid" OR "Herbal-Melanin" OR "Nigella sativa" OR "TQ" OR "NS" OR "Black

Cumin" OR "Black Caraway" OR "Black Seed" OR "Nigella sativa"[Mesh] OR "Nigella sativa oil" [Supplementary Concept]) AND ("Cancer Cell Line" AND ("MTT" OR "3-(4, 5-diMethylThiazol-2-yl)-2,5-diphenyltetrazolium bromide" [Supplementary Concept]))

("Thymoquinone" OR "alpha-hederin" OR "α-Hederin" OR "α-thujen" OR "oloeresin" OR "nigellidine" OR "nigellimine" OR "nigellicine" OR "p-cymene" OR "r-cymene" OR "thymohydroquinone" OR "carvacrol" OR "linoleic acid" OR "oleic acid" OR "eicosadienoic acid" OR "Herbal-Melanin" OR "Nigella sativa" OR "TQ" OR "NS" OR "Black Cumin" OR "black pearl" OR "kalonji" OR "Kalojira" OR "Hasb as-sauda" OR "Zi ra nag po" OR "Hei Zhong Cao Zi" OR "Melanthion" OR "Habbat-ul-barakah" OR "Black Caraway" OR "Black Seed" OR "Nigella sativa"[Mesh] OR "Nigella sativa oil" [Supplementary Concept]) AND ("Cancer* Cell Line" AND ("MTT" OR "3-(4, 5-diMethylThiazol-2-yl)-2,5-diphenyltetrazolium bromide" [Supplementary Concept]))

Modified Newcastle-Ottawa Quality Assessment Scale:

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

- 1) Representativeness of the exposed cohort
 - a) truly representative of the average cancer types in the community *
 - b) somewhat representative of the average cancer types in the community *
 - c) selected type of cancer
 - d) no description of the derivation of the cohort
- 2) Selection of the non exposed cohort
 - a) drawn from the same origin as the exposed cohort *
 - b) drawn from a different source
 - c) no description of the derivation of the non exposed cohort
- 3) Ascertainment of exposure
 - a) secure record (eg quantitative percentage) *
 - b) structured interview *
 - c) written self report
 - d) no description
- 4) Demonstration that outcome of interest was not present at start of study
 - a) yes *
 - b) no

Comparability

- 1) Comparability of cohorts on the basis of the design or analysis
 - a) study controls for original cell viability *
 - b) study controls for any additional factor * (The formulation type administered across cohort)

Outcome

- 1) Assessment of outcome
 - a) independent blind assessment *
 - b) record linkage *
 - c) self-administered quantitative report
 - d) no description
- 2) Was follow-up long enough for outcomes to occur
 - a) yes (at least 24 hours of exposure) *
 - b) no
- 3) Adequacy of follow up of cohorts
 - a) complete follow up - all tested components of cohort accounted for *
 - b) subjects lost to follow up unlikely to introduce bias - small number lost - > 1 well plate examined (follow up, or description provided of those lost) *
 - c) follow up rate < 95 well plates examined (no description of those lost)
 - d) no statement

Table S2: The 'Variable-Table' used for data collation for a random-effects model meta-analysis evaluation:

Variable	Coding Scheme
Study	First author's lastname followed by et al. (year)
Cancer Cell Line Type	Manual input of cell-line identifier followed by (H(uman).cancer type)
Administered Cell Line Number	Manual input of a general numerical value
Well Culture Plate Number	Manual input of a general numerical value
Type of <i>N.sativa</i> Product Administered	If the derived product is of an oil base the identifier ' Oil ' is used, if a product of an extraction process ' Extract ' is used
Therapy IC ₅₀ (µg/mL) 2 d.p.	The concentration value for potency of the therapeutic administered to produce 50% cell viability within the cell-line, to two decimal places
Therapy MTT Assay Mean (%) 2 d.p.	The mean percentage derived from the range of reported administered therapeutic concentrations, as a measure of overall cell viability (post-effect), to two decimal places
Control Type	Categories: Untreated (utilisation of same cell-line type), Normal (cell-line identifier) (non-cancerous cell-line type), Untreated/Normal (combination of both usually by independent experimentation)
Control IC ₅₀ (µg/mL) 2 d.p.	The concentration value for potency of the therapeutic administered to produce 50% cell viability within the cell-line, to two decimal places
Control MTT Assay Mean (%) 2 d.p.	The mean percentage derived from the range of reported administered therapeutic concentrations, as a measure of overall cell viability (post-effect), to two decimal places
Additional Notes	Context for results derived along with any transformations or inference executed

*'**Not Reported**' is an applicable category for all variables listed.

Additional estimated variables are not included that were concatenated during the meta-analysis code construction.

Table S3: Statistics for current estimates of *Nigella sativa* plant-specimen distributions around the world.

COUNTRY	COUNT
FRANCE	282
GERMANY	61
PORTUGAL	48
UNITED STATES OF AMERICA	33
SWEDEN	29
NETHERLANDS (KINGDOM OF THE)	16
UNKNOWN COUNTRY	13
CHINA	12
POLAND	12
AUSTRIA	10
BELGIUM	10
PALESTINE	10
NORWAY	9
RUSSIAN FEDERATION	9
SPAIN	5
SWITZERLAND	4
CZECHIA	4
ITALY	4
UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELA...	3
CANADA	2
ESTONIA	2
NEW ZEALAND	2
BRAZIL	1
FINLAND	1
NIGERIA	1
PAKISTAN	1
SOUTH AFRICA	1

(Source: 160)

Table S4: The current clinical-trial characteristics.

Study Title	Study Status	Conditions	Interventions	Locations
Effect Of Nigella Sativa Oil for the Treatment of Hypertension	ACTIVE_NOT_RECRUITING	Hypertension	COMBINATION_PRODUCT: Nigella sativa (black seed)	Pakistan
Efficacy and Safety of Black Seed Oil With Vonoprazan Based Triple Therapy in Treatment of Helicobacter Pylori	ACTIVE_NOT_RECRUITING	Helicobacter Pylori Infection	DRUG: Black Seed Oil Cap/Tab DRUG: Vonoprazan, Amoxicillin and Clarithromycin	Egypt
Nigella Sativa in COVID-19	COMPLETED	COVID-19 SARS-CoV-2	DIETARY_SUPPLEMENT: Nigella sativa oil	Saudi Arabia
Nigella Sativa Supplementation in Hyperlipidemia Treatment	COMPLETED	Hyperlipidaemia Hyperlipidemia	DIETARY_SUPPLEMENT: Nigella Sativa Oil capsule BEHAVIORAL: Diet	Turkiye
Honey & Nigella Sativa Trial Against COVID-19	COMPLETED	Coronavirus Infection Sars-CoV2	DRUG: Honey DRUG: Nigella Sativa / Black Cumin DRUG: Placebos	Pakistan
Nigella Sativa for Selected Outcomes in Chronic Rhinosinusitis	COMPLETED	Chronic Rhinosinusitis	OTHER: Nigella sativa nasal oil drops DRUG: standard treatment	Egypt
Effectiveness of Nigella Sativa (Kalonji) Seed in Dyslipidemia	COMPLETED	Hypercholesterolemia Hypertension Diabetes Mellitus Metabolic Syndrome X	DRUG: Nigella sativa seed	Pakistan
Effect of Nigella Sativa on Lipid Profiles in Elderly	COMPLETED	Dyslipidemia	DRUG: Nigella sativa DIETARY_SUPPLEMENT: black sticky rice	Indonesia
Comparative Effect of Nigella Sativa and Conventional Management for OSMF	COMPLETED	Oral Submucous Fibrosis	DIETARY_SUPPLEMENT: Nigella sativa oil DRUG: Betamethasone Valerate	Pakistan
Investigation of the Effect of Nigella Sativa Oil and Sesame Oil in Preventing Phlebitis	COMPLETED	Phlebitis Peripheral Venous Catheterization	OTHER: sesame oil application OTHER: nigella sativa oil application	Turkiye
Effect of Nigella Sativa in the Treatment of Palmer Arsenical Keratosis	COMPLETED	Arsenical Keratosis	DIETARY_SUPPLEMENT: Vitamin E DIETARY_SUPPLEMENT: Nigella sativa DIETARY_SUPPLEMENT: Placebo	Bangladesh
Nigella 5 in the Treatment of SARS COV2 (COVID-19)	COMPLETED	SARS-CoV2 Infection	DRUG: Nigella DRUG: Placebo	Tunisia

Evaluation of the Effect of Nigella Sativa for the Prophylaxis to Radiation Induced Dermatitis in Breast Cancer Patients	COMPLETED	Radiation Dermatitis	DRUG: Imtenan Oil DRUG: Baraka Capsules	Egypt
Effects of Nigella Sativa on T Cells and Cytokine Profile in Pediatric SLE Patients	COMPLETED	SLE (Systemic Lupus)	COMBINATION_PRODUCT: Standard Treatment for SLE	Indonesia
Effects of Nigella Sativa Oil on Pain Intensity and Physical Functions in Patients With Knee Osteoarthritis	COMPLETED	Knee Osteoarthritis	BIOLOGICAL: Nigella sativa oil BIOLOGICAL: Naproxen and lidocaine gel BIOLOGICAL: Massage	Turkiye
Evaluation of a Nigella Sativa Extract (10% Thymoquinone, Nisatol?) in Perimenopausal Women With Metabolic Syndrome	COMPLETED	Metabolic Syndrome Elevated Blood Pressure	DIETARY_SUPPLEMENT: Nigella sativa extract (Nisatol?) BEHAVIORAL: Mediterranean Diet	Italy
Hydroxy Urea, Omega 3, Nigella Sativa, Honey on Oxidative Stress and Iron Chelation in Pediatric Major Thalassemia	COMPLETED	Iron Overload Oxidative Stress Thalassemia Major	DRUG: Omega 3 DRUG: Nigella Sativa Oil DRUG: Hydroxyurea DRUG: Honey DRUG: Deferoxamine PROCEDURE: blood transfusion session	Saudi Arabia and Egypt
Clinical and Radiographic Evaluation of Turmeric, Thymus Vulgaris, Nigella Sativa and Aloe Vera as Pulpotomy Medicaments in Primary Teeth	COMPLETED	Pulpitis - Irreversible	PROCEDURE: Pulpotomy	Egypt
Effect of a Food Supplement Based on Nigella Sativa Seeds Oil and Vitamin E on Glyco-lipid Parameters in Dysglycemic and Dyslipidemic Perimenopausal Women	COMPLETED	Perimenopausal Disorder Impaired Glucose Tolerance Impaired Fasting Glycemia	DIETARY_SUPPLEMENT: Nisatol OTHER: Placebo	Italy

The Clinical Assessment of Nigella Sativa Oil vs Chlorohexidine as a Therapeutic Aid for Gingivitis, Effect on Gingival IL-6 and IL-18 and Antimicrobial Efficacy.	COMPLETED	Gingivitis	OTHER: Nigella Sativa oil DRUG: Chlorhexidine mouthwash	Saudi Arabia
Omega-3, Nigella Sativa, Indian Costus, Quinine, Anise Seed, Deglycyrrhizinated Licorice, Artemisinin, Febrifugine on Immunity of Patients With (COVID-19)	COMPLETED	Covid19 Immunodeficiency	DRUG: Omega 3/ Nigella Sativa Oil DRUG: Omega 3/ Nigella Sativa Oil/ Indian Costus DRUG: Omega 3/ Nigella Sativa Oil/ Quinine pills DRUG: Omega 3/ Nigella Sativa Oil/ Anise seed capsule DRUG: Omega 3/ Nigella Sativa Oil/ Deglycyrrhizinated Licorice DRUG: Active Comparator	Saudi Arabia
Black Seed Oil in Treatment of Osteoarthritis	COMPLETED	Osteo Arthritis Knee	DIETARY_SUPPLEMENT: Black seed oil capsule DIETARY_SUPPLEMENT: Placebo Black seed oil capsule	Iran
Potential Benefits of Black Seed Oil Supplementation on Asthma Inflammation and Outcomes	COMPLETED	Asthma	DIETARY_SUPPLEMENT: Black seed oil OTHER: Placebo	Saudi Arabia
Golden Black Seed: Support of Metabolic Health and General Wellness.	COMPLETED	Hyperlipidemias Hyperglycemia	DIETARY_SUPPLEMENT: Golden Black Seed	United States
The Effect of Reflexology and Knee Massage With Black Cumin Oil on Pain and Fatigue	COMPLETED	Knee Osteoarthritis	COMBINATION_PRODUCT: Knee Massage With Black Cumin Oil Group: COMBINATION_PRODUCT: Foot Reflexology Group With Black Cumin Oil COMBINATION_PRODUCT: placebo group	Turkiye
Efficacy of Oral Nigella Sativa as Adjuvant Therapy in Children With Moderate Persistent Asthma	NOT_YET_RECRUITING	Community Acquired Pneumonia	DIETARY_SUPPLEMENT: Nigella sativa Oil Capsules (100 mg/kg/day) DIETARY_SUPPLEMENT: Nigella sativa Oil Capsules (50 mg/kg/day) DRUG: Fluticasone / Long-Acting Beta-2 Agonist Combination	

The Clinical Assessment of Nigella Sativa Oil vs Chlorohexidine as a Therapeutic Aid for Gingivitis, Effect on Gingival IL-6 and IL-18 and Antimicrobial Efficacy.	COMPLETED	Gingivitis	OTHER: Nigella Sativa oil DRUG: Chlorohexidine mouthwash	Saudi Arabia
Omega-3, Nigella Sativa, Indian Costus, Quinine, Anise Seed, Deglycyrrhizinated Licorice, Artemisinin, Febrifugine on Immunity of Patients With (COVID-19)	COMPLETED	Covid19 Immunodeficiency	DRUG: Omega 3/ Nigella Sativa Oil DRUG: Omega 3/ Nigella Sativa Oil/ Indian Costus DRUG: Omega 3/ Nigella Sativa Oil/ Quinine pills DRUG: Omega 3/ Nigella Sativa Oil/ Anise seed capsule DRUG: Omega 3/ Nigella Sativa Oil/ Deglycyrrhizinated Licorice DRUG: Active Comparator	Saudi Arabia
Black Seed Oil in Treatment of Osteoarthritis	COMPLETED	Osteo Arthritis Knee	DIETARY_SUPPLEMENT: Black seed oil capsule DIETARY_SUPPLEMENT: Placebo Black seed oil capsule	Iran
Potential Benefits of Black Seed Oil Supplementation on Asthma Inflammation and Outcomes	COMPLETED	Asthma	DIETARY_SUPPLEMENT: Black seed oil OTHER: Placebo	Saudi Arabia
Golden Black Seed: Support of Metabolic Health and General Wellness.	COMPLETED	Hyperlipidemias Hyperglycemia	DIETARY_SUPPLEMENT: Golden Black Seed	United States
The Effect of Reflexology and Knee Massage With Black Cumin Oil on Pain and Fatigue	COMPLETED	Knee Osteoarthritis	COMBINATION_PRODUCT: Knee Massage With Black Cumin Oil Group: COMBINATION_PRODUCT: Foot Reflexology Group With Black Cumin Oil COMBINATION_PRODUCT: placebo group	Turkiye
Efficacy of Oral Nigella Sativa as Adjuvant Therapy in Children With Moderate Persistent Asthma	NOT_YET_RECRUITING	Community Acquired Pneumonia	DIETARY_SUPPLEMENT: Nigella sativa Oil Capsules (100 mg/kg/day) DIETARY_SUPPLEMENT: Nigella sativa Oil Capsules (50 mg/kg/day) DRUG: Fluticasone / Long-Acting Beta-2 Agonist Combination	

Black Seed Oil in ADHD	NOT_YET_RECRUITING	ADHD	DRUG: Atomoxetine DRUG: Black Seed Oil Cap/Tab	
A Comparative Single Blind Clinical Study on the Effect of Alvogyl, Eugenol, and Nigella Sativa (Black Seed Oil) for Alveolar Osteitis.	NOT_YET_RECRUITING	Alveolar Osteitis	DRUG: Alvogyl DRUG: Eugenol DRUG: Nigella Sativa Oil	Pakistan
Nigella Sativa as an Adjuvant Therapy in the Treatment of Pediatric Pneumonia	RECRUITING	Nigella Sativa Oil as an Adjuvant Therapy in the Treatment of Pneumonia	DRUG: Nigella Sativa Oil capsule	Egypt
Effect of Nigella Sativa in Atorvastatin Treated Hyperlipidaemia	RECRUITING	Hyperlipidemia	DRUG: Nigella Sativa capsule 500mg DRUG: Placebo	Bangladesh
Effect of Consumption of Black Cumin (Nigella Sativa L.) Water Extract on Weight Loss in Overweight Women	WITHDRAWN	Obesity	DIETARY_SUPPLEMENT: Black cumin water extract as a traditional medicine	

(Source: 108)

Table S5: Country statistics for *Nigella sativa* related literature within the Scopus database.

India	92
Saudi Arabia	66
Egypt	39
Iran	33
United States	32
Turkey	24
China	24
Malaysia	23
Pakistan	21
Indonesia	16
South Korea	14
Qatar	13
Germany	12
Bangladesh	12
Jordan	11
Italy	10
Iraq	9
Australia	9
Poland	8
United Arab Emirates	7
Tunisia	7
Oman	6
Lebanon	6
Sudan	5
Morocco	5
Viet Nam	4
Portugal	4
Kuwait	4
Palestine	6
Sri Lanka	3
South Africa	3
Romania	3

Mauritius 3
Hong Kong 3
Canada 3
Brazil 3
Yemen 2
United Kingdom 2
Taiwan 2
Spain 2
Slovakia 2
Singapore 2
Russian Federation 2
Mexico 2
Kazakhstan 2
Japan 2
France 2
Ecuador 2
Czech Republic 2
Bosnia and Herzegovina 2
Undefined 1
Türkiye 1
Thailand 1
Sweden 1
Serbia 1
Nigeria 1
New Zealand 1
Netherlands 1
Macao 1
Libya 1
Hungary 1
Finland 1
Ethiopia 1
Djibouti 1
Cyprus 1

Chile 1
Brunei Darussalam 1
Belarus 1

Table S6: Publication type distributio data for a '*Nigella sativa* Anticancer' search query within the ScienceDirect databse.

SEARCH QUERY	NIGELLA SATIVA ANTICANCER
TOTAL	1,745
REVIEW ARTICLES	586
RESEARCH ARTICLES	611
ENCYCLOPEDIA	7
BOOK CHAPTERS	363
CONFERENCE ABSTRACTS	17
CASE REPORTS	3
CORRESPONDENCE	3
DISCUSSION	3
EDIITORIALS	3
MINI REVIEWS	7
NEWS	1
PRACTICAL GUIDELINES	1
SHORT COMMUNICATIONS	21
OTHER	119

(Source: 379)

Table S7: Annual results for the search-query '*Nigella sativa* Anticancer' within the PubMed database.

YEAR	COUNT
2025	566
2024	563
2023	555
2022	581
2021	468
2020	285
2019	176
2018	157
2017	157
2016	92
2015	89
2014	83
2013	59
2012	50
2011	21
2010	21
2009	9
2008	9
2007	3
2006	2
2005	1
2004	1
2001	1
1999	1
1989	1
1984	1

(Source: 303)

Table S8: '*Nigella sativa* Anticancer' related literature distributed across different fields of research, within the Scopus database.

FIELD	PUBLICATION NUMBER
PHARMACOLOGY, TOXICOLOGY AND PHARMACEUTICS	152
BIOCHEMISTRY, GENETICS AND MOLECULAR BIOLOGY	134
MEDICINE	119
AGRICULTURAL AND BIOLOGICAL SCIENCES	71
CHEMISTRY	63
CHEMICAL ENGINEERING	25
ENVIRONMENTAL SCIENCE	20
ENGINEERING	20
MATERIALS SCIENCE	18
IMMUNOLOGY AND MICROBIOLOGY	18
PHYSICS AND ASTRONOMY	8
MULTIDISCIPLINARY	8
VETERINARY	7
NURSING	6
HEALTH PROFESSIONS	5
COMPUTER SCIENCE	5
ENERGY	4
EARTH AND PLANETARY SCIENCES	3
NEUROSCIENCE	2
MATHEMATICS	2
DENTISTRY	1

(Source: 380)

Table S9: Global trends for '*Nigella sativa*' across all categories.

Month	Nigella sativa: (Worldwide)
2004-01	26
2004-02	25
2004-03	29
2004-04	33
2004-05	32
2004-06	20
2004-07	25
2004-08	18
2004-09	18
2004-10	0
2004-11	16
2004-12	24
2005-01	25
2005-02	23
2005-03	28
2005-04	22
2005-05	19
2005-06	28
2005-07	18
2005-08	17
2005-09	20
2005-10	15
2005-11	17
2005-12	17
2006-01	13
2006-02	19
2006-03	21
2006-04	21
2006-05	13
2006-06	12
2006-07	13
2006-08	12
2006-09	14
2006-10	15
2006-11	18
2006-12	13
2007-01	12
2007-02	20
2007-03	18
2007-04	12
2007-05	19
2007-06	16

2007-07	12
2007-08	12
2007-09	18
2007-10	13
2007-11	16
2007-12	8
2008-01	20
2008-02	15
2008-03	15
2008-04	19
2008-05	18
2008-06	23
2008-07	12
2008-08	15
2008-09	14
2008-10	17
2008-11	17
2008-12	16
2009-01	17
2009-02	18
2009-03	17
2009-04	26
2009-05	17
2009-06	18
2009-07	18
2009-08	18
2009-09	18
2009-10	19
2009-11	20
2009-12	20
2010-01	19
2010-02	20
2010-03	19
2010-04	22
2010-05	19
2010-06	21
2010-07	16
2010-08	17
2010-09	19
2010-10	18
2010-11	17
2010-12	17
2011-01	17
2011-02	18
2011-03	21
2011-04	21

2011-05	21
2011-06	16
2011-07	18
2011-08	17
2011-09	17
2011-10	21
2011-11	18
2011-12	21
2012-01	21
2012-02	21
2012-03	23
2012-04	23
2012-05	21
2012-06	20
2012-07	18
2012-08	18
2012-09	20
2012-10	21
2012-11	21
2012-12	20
2013-01	30
2013-02	28
2013-03	26
2013-04	35
2013-05	29
2013-06	31
2013-07	25
2013-08	28
2013-09	35
2013-10	37
2013-11	53
2013-12	49
2014-01	37
2014-02	33
2014-03	35
2014-04	38
2014-05	36
2014-06	33
2014-07	30
2014-08	28
2014-09	32
2014-10	32
2014-11	31
2014-12	32
2015-01	43
2015-02	42

2015-03	33
2015-04	31
2015-05	30
2015-06	28
2015-07	25
2015-08	27
2015-09	25
2015-10	31
2015-11	33
2015-12	34
2016-01	34
2016-02	36
2016-03	32
2016-04	33
2016-05	34
2016-06	28
2016-07	25
2016-08	24
2016-09	27
2016-10	29
2016-11	29
2016-12	29
2017-01	32
2017-02	33
2017-03	39
2017-04	33
2017-05	30
2017-06	33
2017-07	28
2017-08	28
2017-09	34
2017-10	30
2017-11	30
2017-12	30
2018-01	32
2018-02	31
2018-03	33
2018-04	31
2018-05	29
2018-06	30
2018-07	29
2018-08	30
2018-09	29
2018-10	30
2018-11	30
2018-12	31

2019-01	29
2019-02	32
2019-03	33
2019-04	33
2019-05	31
2019-06	31
2019-07	33
2019-08	28
2019-09	29
2019-10	29
2019-11	32
2019-12	33
2020-01	32
2020-02	35
2020-03	84
2020-04	70
2020-05	43
2020-06	41
2020-07	40
2020-08	32
2020-09	35
2020-10	30
2020-11	33
2020-12	35
2021-01	31
2021-02	30
2021-03	31
2021-04	32
2021-05	32
2021-06	34
2021-07	30
2021-08	42
2021-09	39
2021-10	39
2021-11	46
2021-12	46
2022-01	59
2022-02	46
2022-03	37
2022-04	35
2022-05	33
2022-06	34
2022-07	32
2022-08	30
2022-09	30
2022-10	29

2022-11	30
2022-12	29
2023-01	33
2023-02	32
2023-03	30
2023-04	29
2023-05	32
2023-06	30
2023-07	42
2023-08	41
2023-09	33
2023-10	41
2023-11	35
2023-12	34
2024-01	36
2024-02	35
2024-03	32
2024-04	33
2024-05	35
2024-06	34
2024-07	33
2024-08	32
2024-09	29
2024-10	31
2024-11	30
2024-12	29
2025-01	34
2025-02	31
2025-03	31
2025-04	35
2025-05	38
2025-06	37
2025-07	42
2025-08	89
2025-09	85
2025-10	94
2025-11	100
2025-12	79
2026-01	37

(Source: 471)

Table S10 : A comprehensive list of known historical findings across the world for *Nigella sativa* or *Nigella* species.

TYPE	BC E/ CE	DATE /PERI OD	SOURCE	OBSERVATION S	NIGEL LA SATIV A	NIGEL LA SPECI ES
ARCH AELOG ICAL	CE	1900– 1600	Libya: Wadi el-Agial	unreported number of desiccated seeds	X	
		16th cent.	France: Rigny-ussé, rectory	1 mineralised seed from a cesspit	X	
		1530– 1580	Germany: Göttingen, Johannisstraße	20 mineralised seeds from a cesspit	X	
		14th/1 3th cent.	Germany: Cottbus	7 waterlogged seeds from a well	X	
		14th/1 3th cent.	Germany: Höxter	mineralised seeds from a cesspit	X	
		12th– 8th cent.	Spain: Lleida, Costa de Magdalena (Islamic)	3 mineralised seeds		X
		th–7th cent.	Syria: Qasr al-Hayr al-Sharqi	unreported number of mineralised seeds		
		600	Tunisia: Carthage (Late Antiquity)	pollen grains in the harbour sediments	X	
		3rd– 1st cent.	UK: Carlisle, Annetwell Street	1 waterlogged seed	X	
		3rd– 1st cent.	Egypt: Dakleh oasis	unreported number of seeds	X	
		2nd cent.	France: Biesheim/Kunheim	2 mineralised seeds in a pit	X	
		2nd/1s t cent.	Egypt: Mons Claudianus (Roman)	62 desiccated + 2 charred seeds	X	
		2nd/1s t cent.	Egypt: Mons Porphyrites (Roman)	31 desiccated + 12 charred seeds	X	
	BC E	330– 578 BCE	Austria: Dürrnberg mine (La Tène)	pollen grains in human faeces		X
		7th– 11th cent.	Egypt: Gebel Roma (Intermediate Per. 3)	1 desiccated grain from a midden layer	X	
		7th– 16th cent.	Egypt: Gebel Qarn el-Gir (New Kingdom/Intermediate Per. 3)	4 desiccated seeds	X	
		7th– 16th cent.	Egypt: Wadi el-Huôl (New Kingdom/Intermediate Per. 3)	1 desiccated seed	X	
		810	Bosnia-Herzegovina: Klisura Kadića Brdo	1 waterlogged seed		X?
		800	Jordan: Tell Deir ‘Allā	9 desiccated seeds	X	

		11th– 16th cent.	Egypt: Thebes (New Kingdom? Passalacqua Collect., Berlin, Inv.-No. 6997)	unreported number of desiccated seeds	X
		11th– 16th cent.	Egypt: Gebel Roma (New Kingdom)	2 desiccated grains from a midden layer	X
		9250– 10450	Syria: Tell Qaramel	1 charred grain	X
		1323	Egypt: Tutankhamun tomb (New Kingdom, 18th dynasty)	unreported number of desiccated seeds in a pot	X
		late 14th cent.	Turkey: ulu Burun shipwreck	unreported number of waterlogged seeds	X
		15th– 19th cent.	Serbia: Feudvar	1 charred seed	X
		1650	Turkey: Boyalı Höyük (Old Hittite)	180,000 charred seeds in a flask	X
		1771– 1991	Egypt: Kahun (Middle Kingdom, 12th dynasty)	unreported number of seeds	X
		2216– 2707	Egypt: Saqqara (Old Kingdom, 4th–6th dynasty)	unknown number of charred seeds	X
		2300– 2900	Spain: El Prado (Jumilla, Murcia)	unreported number of seeds	X
TEXTU AL	CE	15th cent.	liber de simplici medicina / circa instans (by Matthaeus Platearius?): p. 96r; p. 232v; p. 205v;	Git; nielle (FR)	X?
		14th cent.	Nihayal al-Arab fi funun al-adab (by Al-Nuwayri)	? (AR)	X
		14th cent.	Fasl fi ma'rifat al-matānim wa-al asiqā (royal crop register of Rasulid yemen, by Al-Afdal)	? (al-ḥabba al- sawdā') (AR)	X
		1350	compendium Salernitanum (by Bartholomaeus Copho?)	Nigella	X
		13th– 10th cent.	medical prescriptions from the Cairo Genizah Taylor-Schechter Collection	? (AD)	X
		12th cent.	liber simplicis medicinae / “physica” (by Hildegard von Bingen)	book 5, cap. 24: gith	X
		12th cent.	al-Aqrābādīn (The Dispensatory by Ibn at-Tilmīd)		X
		12th cent.	Kitāb al-Filāha (The Book of Agriculture, by Ibn al-‘Awwam): ch. 26, art. 3		X
		12th cent.	Kitābu'l Kulliyāt fi al-Tibb (General Rules of Medicine, by Ibn Rushd / Averroës): p. 5		X
		11th cent.	Diwan al-Filāha (Treatise on Agriculture, by Ibn Bassal)		X
		11th/1 0th cent.	Mishnaic glossary Hebrew-Greek: No. 73	MEΛANΘH (melanthe) (GR); מֶלַנְתָּהוּ (HE)	X

10th/9th cent.	Kitāb al-Qanūn fī al-Tibb (The Canon of Medicine, by Ibn Sina / Avicenna): book 2, ch. 21	(Habbet as-suda) (AR)	X
9th cent.	Bald's Leechbook: f. 93r	gitte(r) (GR/EN); sutherne wyrt (EN)	X?
9th cent.	capitulare de villis vel curtis imperii (by Alcuin of York? Ansegisus of Saint-Wandrille? Louis the Pious?)	git (GR)	X?
9th cent.	Kitāb al-ʿSumūm (Book on Poisons, by Ibn Wahshīya)	(Ḥabba sawdāʿ) (AR)	X
1st cent.	de medicina (On Medicine, by Aulus Cornelius Celsus): lib. 4	gith	X?
1st cent.	de re rustica (On Agriculture, by Lucius Iunius Moderatus Columella), lib. 6, 23	git	X?
1st cent.	de materia medica (On Medicaments, by Pedanios Dioscorides)	μελάνθιον (melanthion) (GR)	X?
1st cent.	naturalis historia (Natural History, by Plinius Maior / Pliny the Elder): lib. 20, 71	git; μελάνθιον (melanthion), ? (melanospermon) (GR)	X?
4th cent.	“corpus Hippocratis” (by Hippocrates of Kos?)	μελάνθιον (melanthion) (GR)	X?
7th cent. or older	various medical prescriptions on Assyrian cuneiform tablet	? (zibūm) (AS)	X?
8th cent.	description of the contents of the garden of Mesopotamian king Merodach Balada	? (AS)	X?
20th cent.	Hurrian text from Nuzi (Nuzu): tab. HSS 13,353	? (AS)	X?
19th–16th cent.	Iatrosophikon	? (mavrokokkon, melanthion) (GR)	X?
1688 AD	The English Gardener (by Leonard Meager)	nigella; fennel flower (EN)	X
17th/16th cent.	medical manuscripts (by Hayyim ben Joseph Vital)		X
1660	1660 Rate Book	Nigella	X?
1630	Kräutterbuch (by Hieronymus Bock/Tragus); p. 92 f.	Nigella; Schwarz Coriander (DE)	X
1629	Paradisi in paradiso terrestris (by John Parkinson); p. 287 f	Romane Nigella (EN)	X
1625	Neuw vollkommentlich Kreuterbuch (by Jacob Theodor / Tabernaemontanus): lib. 1, 172–176	Melanthium sativum; Zamenisamen (DE)	X

1613	Hortvs Eystettensis (by Basilius Besler): plates 174; 180	Nigella peregrina flore multiplici	X
1597	The Herball or Generall Historie of Plantes (by John Gerarde): p. 924 f.	Melanthium, Nigella Romana; Garden Nigella (EN)	X
1590	Kreutterbuch (by Pietro Andrea Mattioli / Matthiolus): p. 276 f.	Nigella sativa; schwartzzer Coriander (DE)	X
1572	Compendio della faculta de' semplici (...) (by Philippus Florentinus): p. 38–39	Melantio (IT)	X
1570?	The names of all sortes of sedes (...)	nigella Romana, Fennel Flower (EN)	X?
1561	In Pedacii Disocoridis Anazarbei primum de medica materia librum, Annotationes (by Valerius Cordus) Lib. III, Cap. 93	Nigella; μελάνθιον (melanthion) (GR); Schwartzzer Kümmel (DE); warns of confusion with Raden (Agrostemma)	X
1561	De plantis (by Valerius Cordus), lib. II, Cap. 23	Nigella	X
1561	Horti Germaniae (Konrad Gesner): p. 268	Gith vulgaris	X
1557	Histoire des plantes (by Rembert Dodoens): p. 195 f.	Melanthium sativum, Nigella domestica; Nielle domestique (FR); Schwartz kumich, Schwartz kumel (DE)	X
1543	New Kreüterbuch (by Leonhart Fuchs): Cap. 192	Schwartz Kümich (DE) vs. geeler Kümmich (DE)	X
1541 AD	Historia plantarum (by Konrad Gesner): p. 118	gith, nigella	X
1537	Examen omnium simplicium medicamentorum (...) (by Antonio Musa Brasavola) p. 174	nigella nigra, papaver nigrum; nelle noyre (FR)	

Abbreviations: cent. = century, Collect. = collection, Inv.= inventory, No.= number, CE = Common Era, BCE = Before Common Era, Per. = period, tab. = tablet, lib.= library, ? = uncertainty, p = page, ch. = chapter, art. =article, cap. = caption, f. = following page, possible german convention, AR = Arabic, AS = Assyrian, DE = German (Deutsch), EN = English, FR = French, GR = Greek, HE = Hebrew, IT = Italian, AD = Arabic-derivative. (Adapted from: 192 (p158-164)).



Figure S2: *Nigella sativa*. The ancient ingredient.

(Left) Ancient Egyptian texts such as the Ebers' Papyrus (circa 1550 BCE), acquired by George Maurice Ebers (1873), references 'nigella' as a digestive aid. Thus, such an artefact could be considered one of the earliest records of *Nigella sativa* as an herbal remedy, in human history. BCE="before the common (or current) era. (Source: 414) (Adapted from: 252). (Middle) Circa 15th century Constantinople manuscripts such as Dioscorides' De Materia Medica (Πεδάνιος Διοσκουρίδης, Περί ὕλης ἱατρικῆς may refer to *Nigella sativa* through the term 'Melanthion' 'for use as food (sprinkled on bread) or as a treatment for difficult breathing (when drunk with soda), headaches (applied to forehead), toothaches (boiled with vinegar and pitch pine; used as mouth wash), imperfections of the skin, leprosy (applied with vinegar), incipient cataracts (applied in the nostril as an unguent [an ointment or lubricant] made from iris [*Iris* spp., Iridaceae]), and catarrh (as nasal inhalant). Dioscorides also described the use of melanthion to remove corns (applied with old urine to incised corns), expel roundworms (applied by smearing with water), stimulate menstruation and urination, and repel snakes (fumi-gation).' (Source: 293). (Right) Abu 'Ali Al-Husayn Ibn 'Abdullah Ibn Sina (ابن سينا; Avicenna) (D. Ah 428/1037 CE): *Al-Qanun Fi Al-Tibb* (القانون في الطب). An example of (Abbasid, Baghdad) 12th Century textual evidence for potential *Nigella sativa* uses across various maladies. CE=Common Era; Ah=After Hijra; D.=Death. (Source: 106).



Figure S3: *Nigella sativa* seeds present within amphorae artefacts.

(Left) Amphorae removed from the Uluburun archeological seabed site, in which some were found to contain NS seeds. (Right) An Amphora; a large gold chalice and a pedestal base. 'Photos ©2017 Institute of Nautical Archaeology'. (Source: 143).

Figure S4: The R(studio) code used to curate the random-effect model meta-analysis and associated statistical analyses, used within this research project.

```
#Install the packages
install.packages("estmeansd")
install.packages("meta")
install.packages("metafor")

#Load the packages
library (metafor)
library (meta)
library (estmeansd)
library (ggplot2)

#Directory set for work-flow
setwd('C:\\Users\\Z\\Desktop')

#Data-set in .csv format
data <- read.csv("Bootstrap Analysis v3.csv", fileEncoding = "Latin1", check.names =
F, nrow = 9)
summary(data)

#Normalisation of data-set
scale_data <- as.data.frame(scale(data[,c(7,8)]))
summary(scale_data)

# Column binding
cbind_df <- cbind(data, scale_data)
print(cbind_df)

#Meta-Analysis of Single (percentage) Means and estimated standard deviations
mm.mtt <- metamean(cbind_df$well_culture_plate_number_,
cbind_df$therapy_mtt_assay_mean_percentage_.1,
cbind_df$estimated_therapy_mtt_assay_sd.1,
data = cbind_df,
sm = "MRAW",
studlab = study)

summary(mm.mtt)

#Forest plot generation
forest(mm.mtt,
layout="Revman5",
sortvar=studlab,
xlab="MTT Cell Viability (%)",
ff.xlab = "bold", fs.xlab = 12,
leftcols=c("studlab", "n", "mean", "sd", "w.random", "ci"),
rightcols=FALSE,
pooled.totals = TRUE,
digits.mean = 2, digits.sd = 2,
random = TRUE, fixed = FALSE,
fs.heading = 12, fs.study = 12, fs.hetstat = 10,
colgap = "5mm", colgap.forest = "5mm",
col.square="darkblue", col.square.lines="black",
col.diamond="maroon", col.diamond.lines="black",
print.Q = TRUE, print.pval.Q = TRUE, print.tau.ci = TRUE, overall.hetstat=TRUE)

influence(mm.mtt)

library(ggplot2)

summary(cbind_df$therapy_mtt_assay_mean_percentage_)

boxplot.stats(cbind_df$therapy_mtt_assay_mean_percentage_)$out
out <- boxplot.stats(cbind_df$therapy_mtt_assay_mean_percentage_)$out
out_ind <- which(cbind_df$therapy_mtt_assay_mean_percentage_ %in% c(out))
out_ind

XXIV
boxplot(cbind_df$therapy_mtt_assay_mean_percentage_,
xlab = "Nigella sativa Anti-Cancer Therapeutic Effect",
ylab = "Cell-line Viability (%) ",
```

```

    main = "Box-plot of MTT Assay Results Across Study Literature"
)
mtext(paste("Outliers: ", paste(out, collapse = ", ")))

install.packages("outliers")
library(outliers)

# Grubbs test
test <- grubbs.test(cbind_df$therapy_mtt_assay_mean_percentage_, opposite = TRUE)
test

# Grubbs test
test <- grubbs.test(cbind_df$therapy_mtt_assay_mean_percentage_)
test

# Grubbs test
test <- grubbs.test(cbind_df$therapy_mtt_assay_mean_percentage_, type=20,
opposite=FALSE)
test

#Single Mean Meta-analysis
test <- escalc(measure="MN", mi=cbind_df$therapy_mtt_assay_mean_percentage_,
               sdi=cbind_df$estimated_therapy_mtt_assay_sd,
               ni=cbind_df$well_culture_plate_number_,
               slab=study,
               data=cbind_df)
test2 <- rma(yi, vi, data=test)
summary(test2)
forest(test2)

#Forest plot generation
forest(test2,
       layout="Revman5",
       sortvar=slab,
       xlab="MTT Cell Viability (%)",
       ff.xlab = "bold", fs.xlab = 12,
       leftcols=c("slab", "n", "mean", "sd", "w.random", "ci"),
       rightcols=FALSE,
       pooled.totals = TRUE,
       digits.mean = 2, digits.sd = 2,
       random = TRUE, fixed = FALSE,
       fs.heading = 12, fs.study = 12, fs.hetstat = 10,
       colgap = "5mm", colgap.forest = "5mm",
       col.square="darkblue", col.square.lines="black",
       col.diamond="maroon", col.diamond.lines="black",
       print.Q = TRUE, print.pval.Q = TRUE, print.tau.ci = TRUE, overall.hetstat=TRUE)

#influence
influence(test2)

#forest plot
funnel(test2)

##categorical moderator

#moderator test to calculate qbetween value for categorical moderator
mod.gradeq <- rma(yi, vi, mods = ~ factor(therapy_type_), data=test)
mod.gradeq

# Save QM Test and write it into a text file
Qgrade_collapsed_string <- paste(mod.gradeq[["QMdf"]])
Qgrade_type1 <- data.frame(CollapsedQMdf = Qgrade_collapsed_string)
Qgrade_type2 <- round(mod.gradeq$QM, 2)
Qgrade_type3 <- round(mod.gradeq$QMp, 3)

QgradeQ <- paste(
  "Qb(", Qgrade_collapsed_string, ") =",
  Qgrade_type2,
  ", p =",

```

```

Qgrade_type3,
collapse = " "
)

cat(QgradeQ, "\n")
gradeQtest <- data.frame(Text = QgradeQ)
write.table(gradeQtest, file = "QgradeQ.txt", row.names = FALSE, col.names = FALSE,
quote = FALSE)

#moderator test to get mean effect size for each group categorical moderator (removes
intercept)
mod.grade <- rma(yi, vi, mods = ~ factor(therapy_type_)-1, data=test)
#Display moderator result
mod.grade

##save moderator results to table
#Only save table results to new data item
mod.grade_table <- coef(summary(mod.grade))
#calculate participants in each group and add it to the table, then save the table.
gradeSumParticipants <- test %>%
  group_by(therapy_type_) %>%
  summarise(nexp = sum(well_culture_plate_number_, na.rm = TRUE),
            nctrl = sum(well_culture_plate_number_, na.rm = TRUE))
gradeNumComp <- test %>%
  count(therapy_type_)
gradeNumComp <- rename(gradeNumComp, kcomparisons = n)
grade_type_table.final<- cbind(gradeSumParticipants,gradeNumComp[c(2)],
mod.grade_table)
write.csv(grade_type_table.final, "Mod.gradeResult.csv")

##continuous moderator
#continuous moderator test
mod.cont <- rma(yi, vi, mods = ~ cont, data=test)
mod.cont

##save moderator results to table
install.packages("XLConnect")
library(XLConnect)
writeWorksheetToFile("modell.xlsx",
                    data = summary(mod.gradeq),
                    sheet = "summary",
                    header = TRUE,
                    clearSheets = TRUE)

# carry out trim-and-fill analysis
trimandfill2 <- trimfill(test2)
trimandfill2
funnel(trimandfill2)

#Multiple Linear Regression Model - Modereaction Analysis
model <- lm(therapy_mtt_assay_mean_percentage_ ~ therapy_type_ * therapy_ic50_ugmL_,
cbind_df)
summary(model)

#Publication Bias testing
#Funnel Plot
funnel(mm.mtt)

# carry out trim-and-fill analysis
trimandfill <- trimfill(mm.mtt)
trimandfill
funnel(trimandfill)

#Eggers regression
regtest(mm.mtt)

```

```
#Eggers regression
regtest(test2)

#Rosenthal, Orwin, & Rosenberg Fail Safe N test
fsn(yi, vi, data=test)
fsn(yi, vi, data=test, type="Orwin")
fsn(yi, vi, data=test, type="Rosenberg")
```

النَّبِيُّ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ قَالَ
"مَا أَنْزَلَ اللَّهُ دَاءً إِلَّا أَنْزَلَ لَهُ شِفَاءً"

- ٥٣٥٤ رواه البخاري

The Prophet (ﷺ) said, “Allah did not send down any disease but that he also sent down the cure.”

- Bukhari 5354



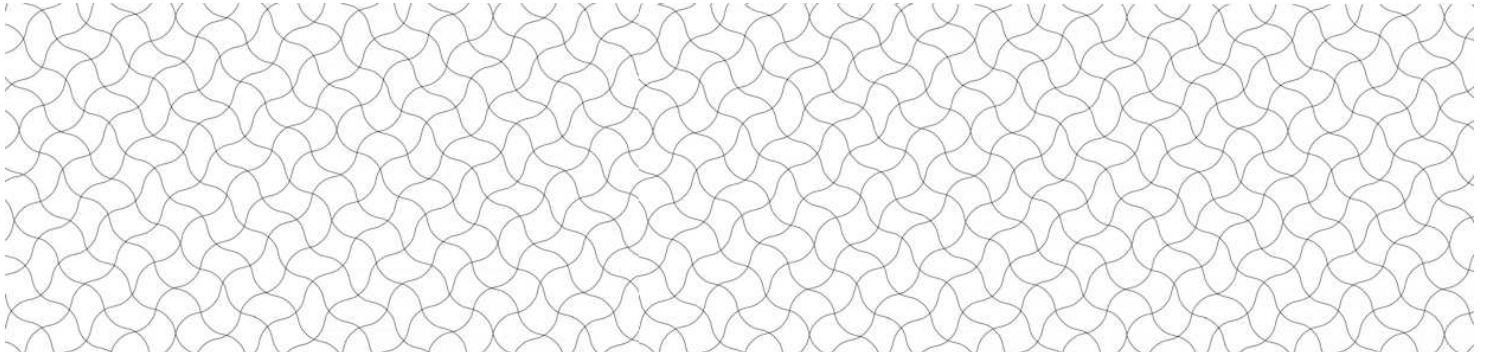
Which is it, of the favours of your Lord, that you deny?

[55:13]

(Quran.com, 2025)

(Amman Panorama Art Gallery, 2026)

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.





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2026

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الشخص الذي تم تجاهله
THE OVERLOOKED ONE