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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الذي تم تجاهله

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THE OVERLOOKED ONE

An Analytical Research Project

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Submitted to
The School of Biological Sciences

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2026

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The Basmallah

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

سَمِعَ رَسُولَ اللَّهِ صلى الله عليه وسلم يَقُولُ

"فِي الْحَبَّةِ السَّوْدَاءِ شِفَاءٌ مِنْ كُلِّ دَاءٍ إِلاَّ السَّامَ"

-٥٦٨٨ رواه البخاري

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

- Bukhari 5688

The Book of Medicine

(Sunnah.com, 2026).

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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.

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 IC_{50} 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*

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1. Introduction

1.1 The Background of The Analytical Research Project

Nigella 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).

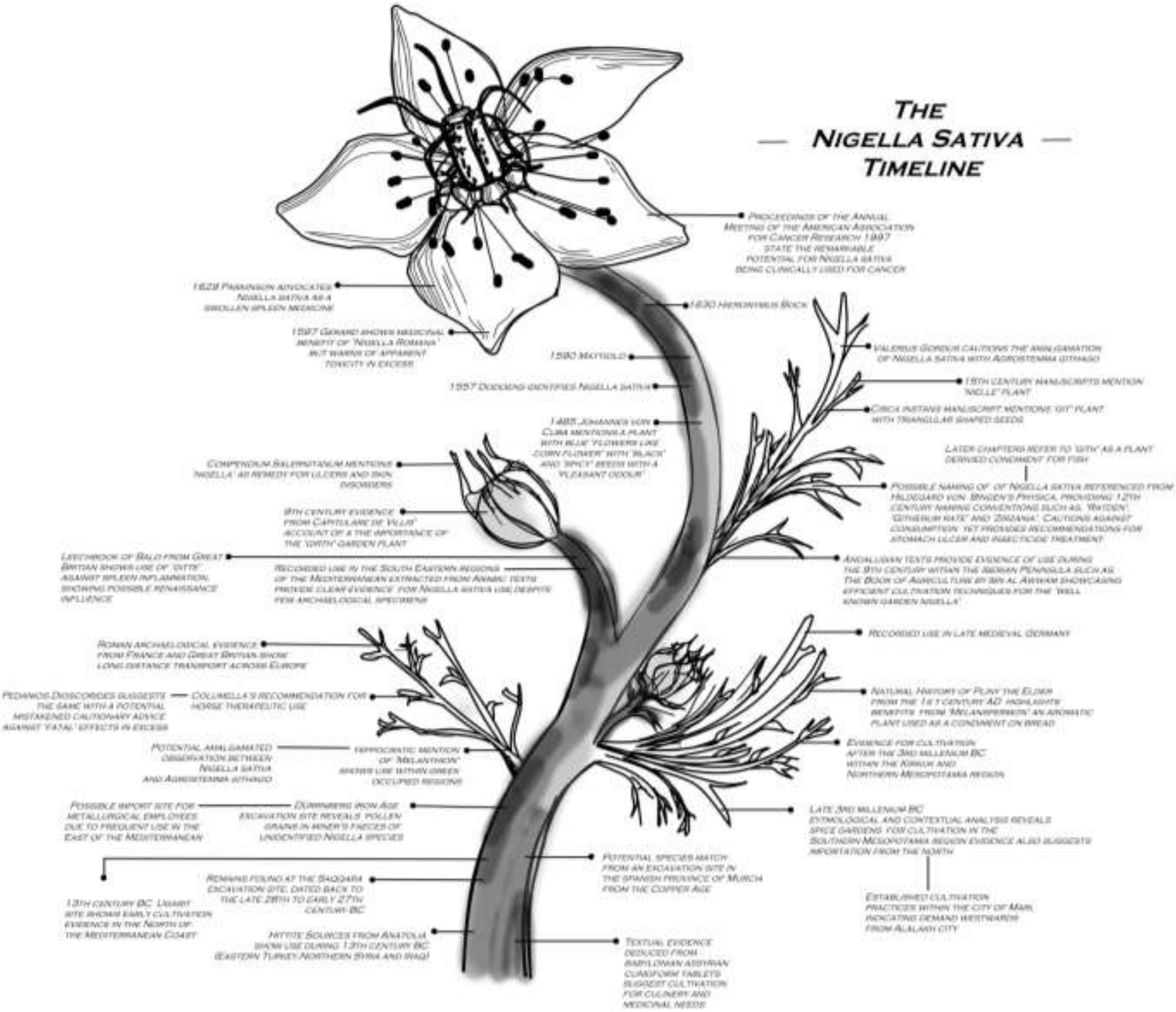


Figure 1: A *Nigella sativa* historical timeline composed from available etymological, archaeological, textual, and illustrative primary resources.

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 Commo Era (Gregorian calendar system) (Adapted from: 109; 216; 263; 313).

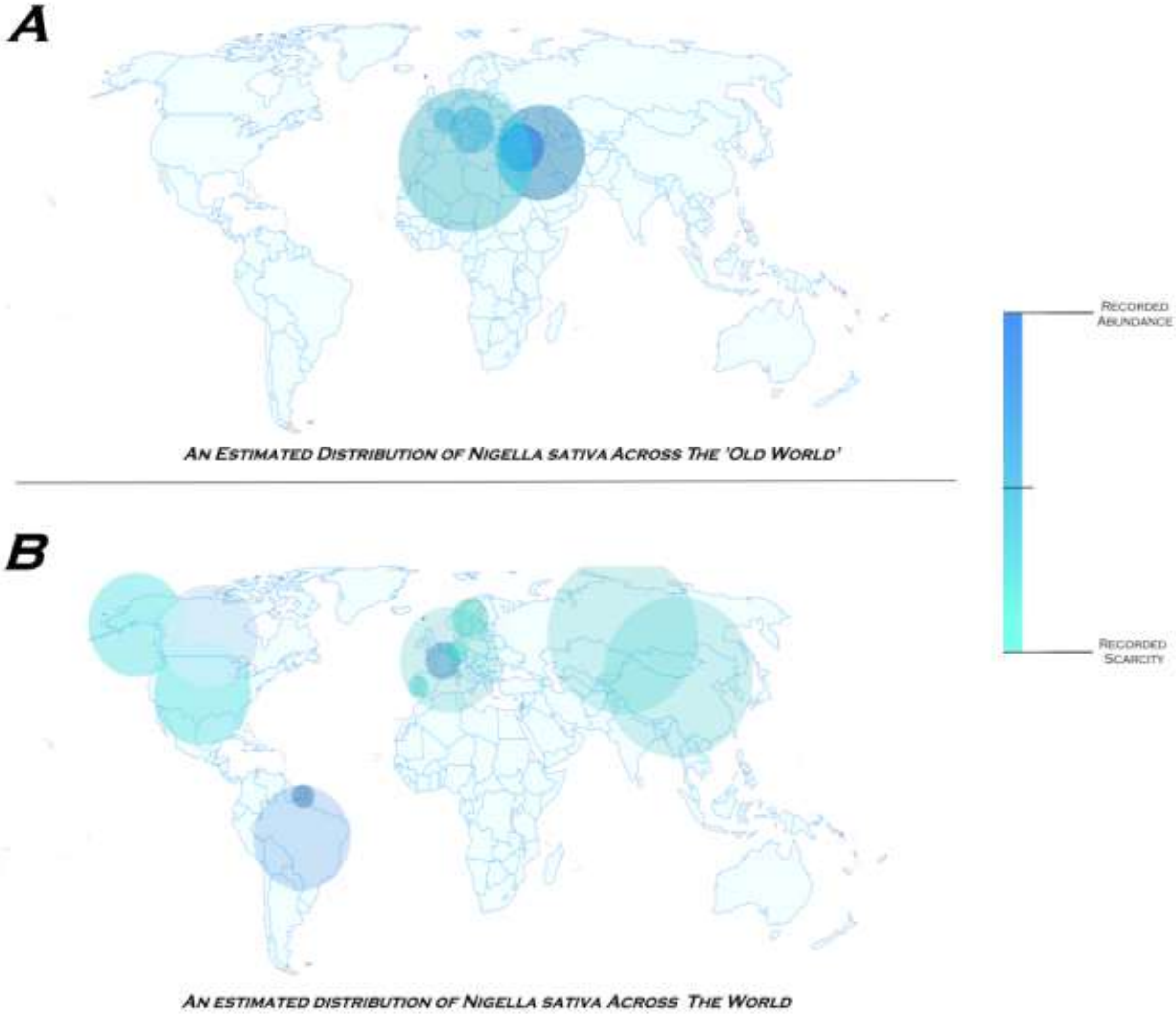


Figure 2: Two heat-maps highlighting the quantitative difference in distribution patterns of *Nigella sativa* at significantly different time-points, across the globe.

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.

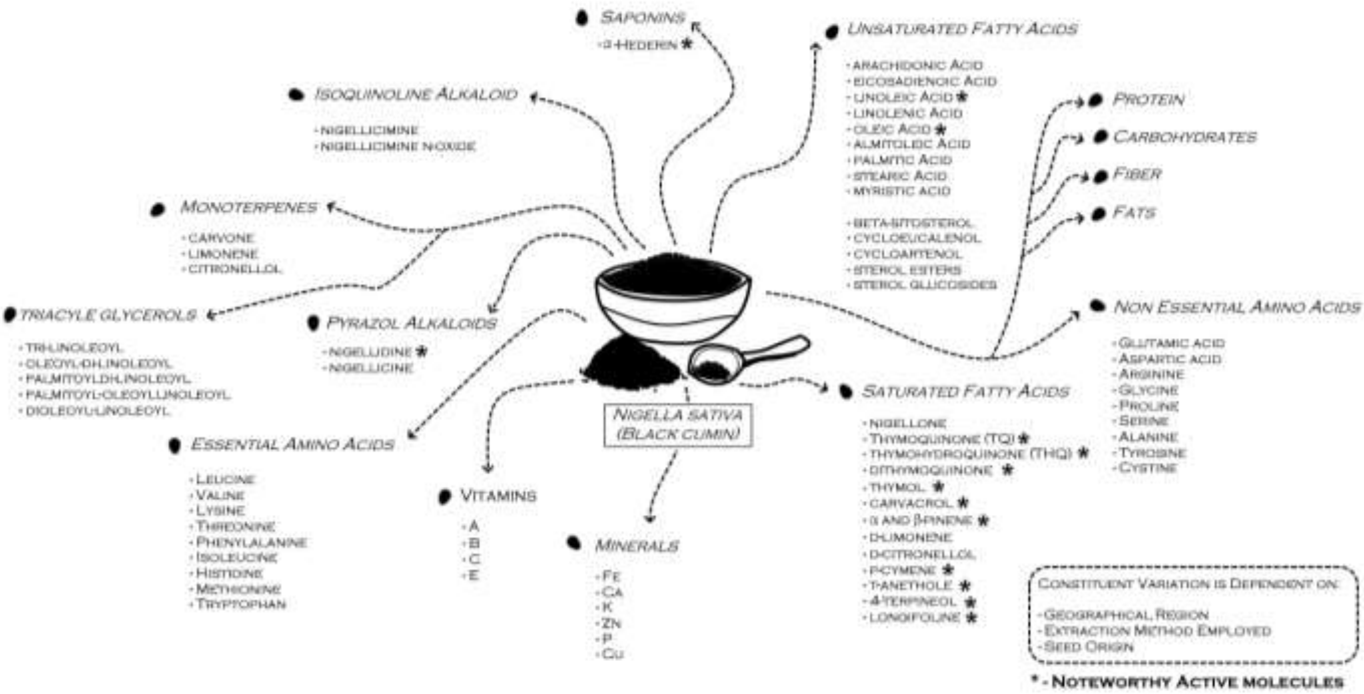


Figure 3: A summary of bio-constituents typically found in *Nigella sativa*.

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	کلونجی	*Kalonji*
Hindi	कलोजेरा	*Kalojera*
Tibetan	ཟི་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	ဆမွန်-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).

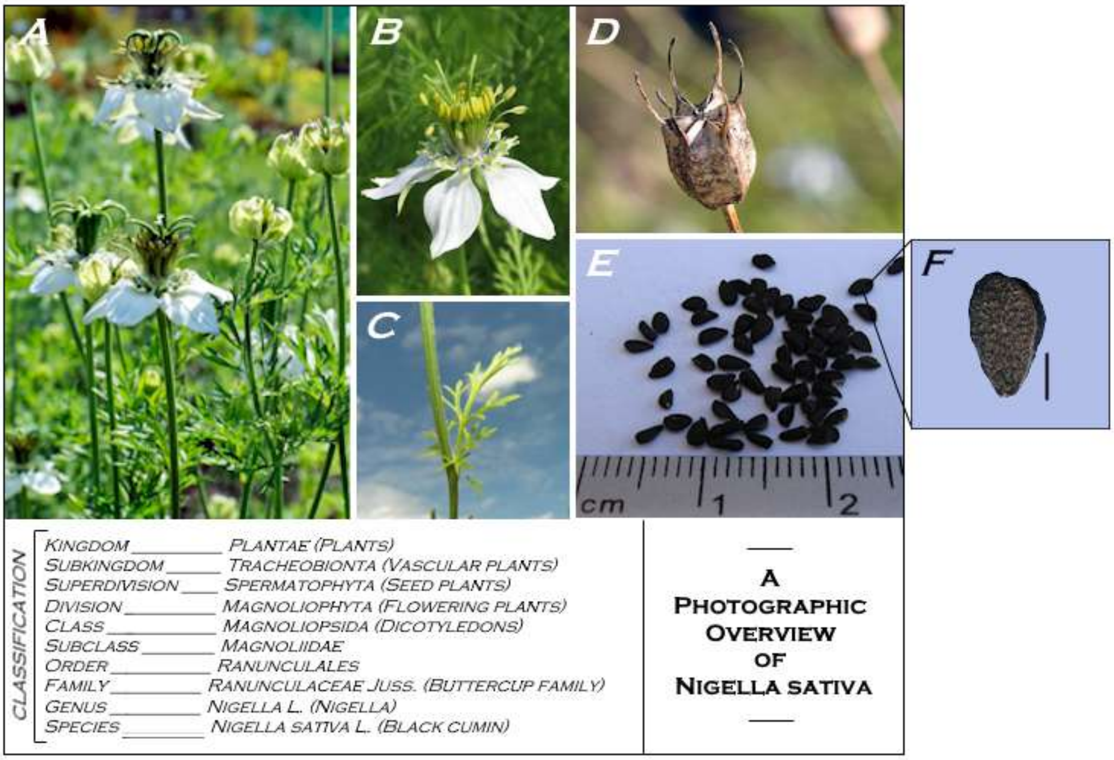


Figure 4: A Photographic Overview of *Nigella sativa* and Taxonomic Classification Profile (Source: 305).

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 (¼) 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 (Morrocco) – 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-1β/-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.

Leaf miner (Tuta absoluta)	❖ *Nigella sativa* oil exhibited strong *in-vitro* repellent activity and insecticidal character against larvae. ❖ In a dose and time dependent manner concentrations of 0.039, 0.079 and 0.157 µl/cm^2 caused 71.25 82.5 and 91.25% repulsion, respectively. ❖ Concentrations of 0.236 µl/cm^2 caused total mortality (100%) ❖ The reported LD_{50} value of 0.105 µl/cm^2, from a four-hour exposure period, highlights its strong pesticide effect	(8)
Malaria Mosquito (Anopheles gambiae)	❖ *Nigella sativa* 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 (RD_{50}) 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 *Nigella sativa* due to the recorded 99.38% repellence at 0.1g/ml	(309)
Yellow-Fever Mosquito (Aedes agyptii)	❖ Results from dual choice bioassay observations showed 64% of females significantly avoided areas concentrated with *Nigella sativa* 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)
Headlice (Pediculus humanus capitis)	❖ Assessment of Nigella *sativa*'s anti-pediculicidal activity with the filter paper diffusion method showed 100% mortality after 1 hour of exposure. ❖ Results confirmed the proposition of *Nigella sativa* 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)
Red Flour Beetles (Tribolium castaneum)	❖ *Nigella sativa* oil produced median lethal concentrations (LC_{50}) 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)
Lone-star tick (Amblyomma americanum (L.)) *American dog tick (Dermacentor variabilis)* *Deer tick (Ixodes scapularis)* *Brown dog tick (Rhipicephalus sanguineus)*	❖ All four species were repelled by *Nigella sativa* extracts. ❖ *I. scapularis* was 100% repelled by extract 1 and 4 at a concentration of 0.206 mg extract/cm^2 filter paper. ❖ *D.variabilis* was repelled at 100%, 90% with a concentration of 0.827 mg extract/cm^2 using extract 1 and 4, respectively. ❖ More than 87% of nymphs from *A. americanum* and *R. sanguineus* were repelled with a concentration of 0.413 mg extract/cm^2 filter paper of extract 1 and 4. ❖ Repellent longevity was observed against *A. americanum* was observed at 66.7% at 192 hours	(97)

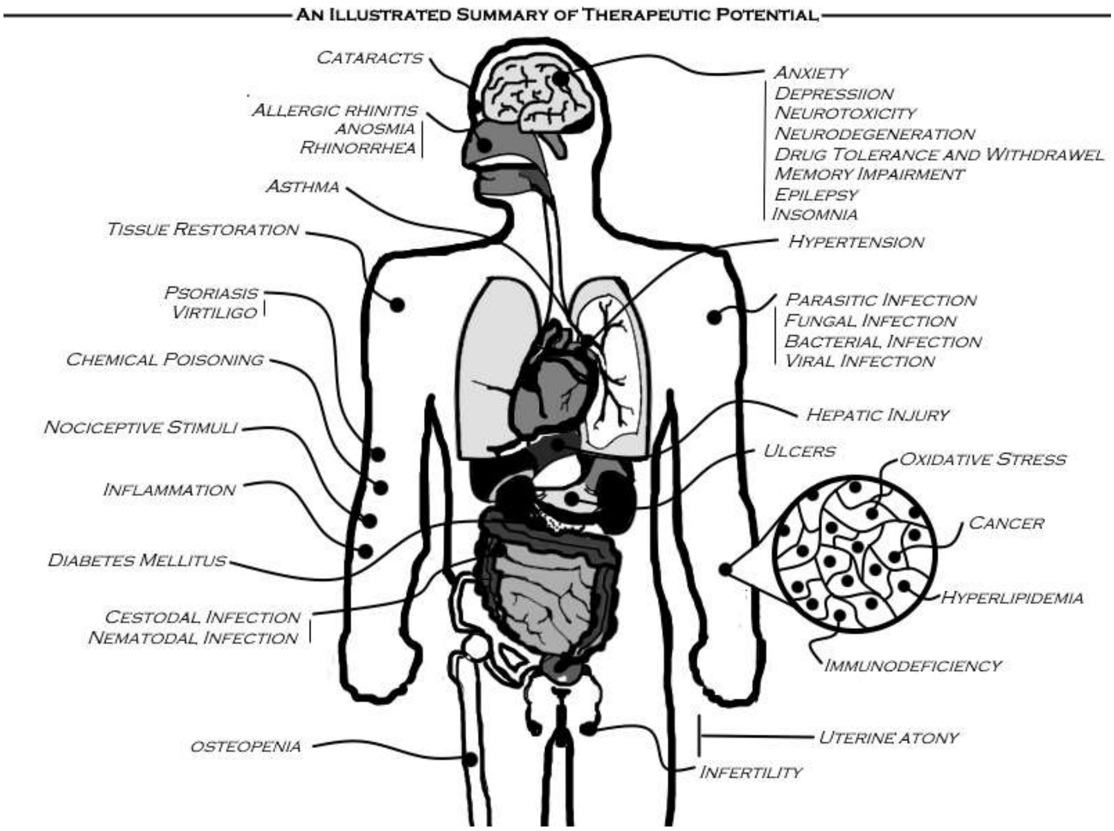


Figure 5: An anatomical diagram highlighting potential therapeutic targets of *Nigella sativa* (Adapted from: 197).

Nigella sativa been reported to exhibit anti-inflammatory, hypotensive, hepatoprotective, anti-oxidant, anti-cancer, hypoglycaemic, aphrodisiac anti-tussive, uricosuric, choleretic, 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

Anxiety	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)
Depression	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)
Neurotoxicity	Extract	❖ Reduction in SGD-initiated cytotoxicity through possible anti-oxidant pathways reducing heightened ROS production	(294)
Neurodegeneration	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)

Drug Dependency and Associated Tolerance	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)

Memory Impairment	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)
Epilepsy	Oil	❖ Reduction in oxidative stress within 'pilocarpinised' rat's hippocampus ❖ Modulation of nitric oxide, glutathione concentration, and catalase activity (back to control conditions)	(5)
Asthma	Extract	❖ Observable increase within all pulmonary functional assessments ❖ 30-minute brocho-dilatory onset duration ❖ Potent anti-asthmatic effect duration lasted for 105 minutes	(82)
Allergic rhinitis	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)
Acne vulgaris	Hydroge l	❖ 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)
Cataracts (Glucose-induced)	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)
Vitiligo	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***

Psoriasis	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)
Wound (Burn)	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. ❖ Statistical analysis showed significant shortening of the healing process	(452)
Parasitic Infection (Plasmodium yoelii)	Extract	❖ Both oral and intraperitoneal administration methods suppressed parasitic activity independent of extract type. ❖ Statistically significant results further highlight the ability to reduce parasitaemia and increase an infected hosts survival-time	(4)
Fungal Infection (Bipolaris sorokiniana)	Defen-sins	❖ 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. ❖ 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	(360)
Bacterial Infection (Streptococcus mutans)	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 ❖ The minimum inhibition concentration assay was 380mg/mL	(406)
Viral Infection (SARS-CoV-2)	Oil	❖ TSB was reduced at a considerably faster rate from the recorder 14th day mark. ❖ Lowered the rate of hospitalisation. ❖ Significant effect upon anosmia and rhinorrhoea resolution	(241)
Oxidative stress	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)
Hepatic injury	Oil	❖ Ischemia and reperfusion injured hepatic tissue analysis obtained from the treated group highlighted significant reductions in liver enzymes, TOS, OSI and MPO. ❖ 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	(455)
Cancer	Extract	❖ Within *in-vivo* 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. ❖ Observable cellular modulation of thymidine incorporation ❖ High potency with similar concentration amounts exerting 50% inhibitory effect across the *in-vivo* study. ❖ Within *in-vitro* cell culture analysis oral carcinoma cells underwent growth inhibition whereas myelogenous leukaemia cell-growth was temporarily halted.	(371)
Diabetes Mellitus	Oil	❖ The seed oil within the alloxa induced diabetic rabbit study induced a significant hypoglycaemic effect in a time dependent manner. ❖ No observable changes to basal insulin levels were found suggesting a possible non-insulin mediated hypoglycaemic mechanism being responsible for the results	(22)

Table 3: *Continued*

Cestodal Infection (Schistosoma mansoni)	Oil	❖ 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)
Nematodal Infection (Aspiculuris tetraptera)	Oil	❖ 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)
Inflammation	Oil	❖ 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)
Nociceptive Stimuli	Oil	❖ 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)
Ulcers	Extract	❖ 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)
Hypertension	Extract	❖ 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)
Hyperlipidaemia	Powder	❖ 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)
Chemical Poisoning	Extract	❖ 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)
Immunodeficiency	Extract	❖ 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)
Uterine atony	Oil	❖ 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)
Infertility	Oil	❖ 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: ❖ 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

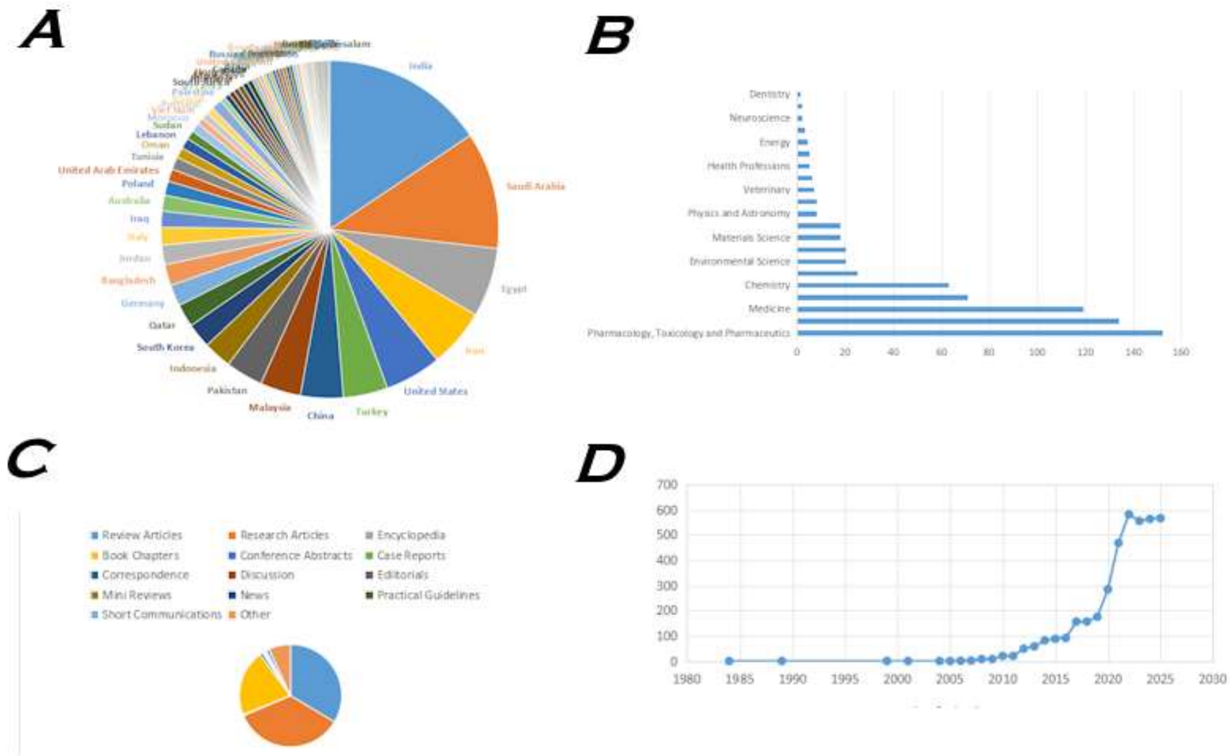


Figure 6: A contemporary summary of available literature regarding *Nigella sativa* and its derivatives.

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 (IC_{50}) value, untreated culture IC_{50} 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 IC_{50} 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 IC_{50} 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 (I^2) index. I^2 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 form 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).

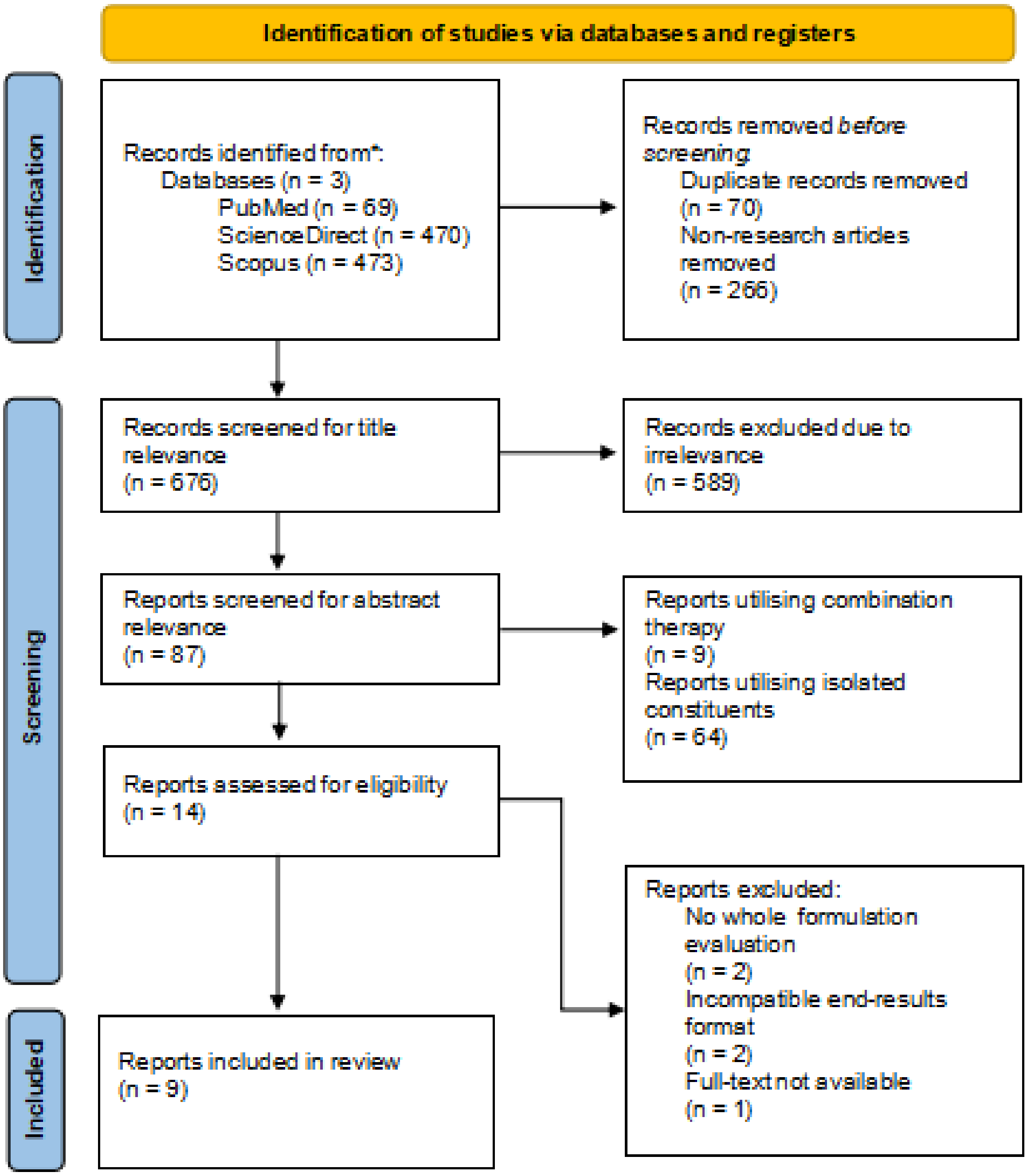


Figure 7: PRISMA flow-diagram of the literature search and study selection-process.

(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 *et al.* (26)	A-549 (H.Lung)	10000	96	Extract	500.00
Das *et al.* (121)	MCF-7 (H.Breast)	231	96	Oil	25.00
Al-Oqail *et al.* (23)	HepG2 (H.Liver)	10000	96	Oil	44.60
Ali *et al.* (34)	HCT (H.Colon)	*NOT REPORTED*	96*	Oil	4.24
Hago *et al.* (179)	MCF-7 (H.Breast)	*NOT REPORTED*	96	Extract	10.07
Nawaz *et al.* (307)	HepG2 (H.Liver)	*NOT REPORTED*	96	Extract	25.91
Toy *et al.* (423)	PC3 (H.Prostate)	6000	96	Extract	12.16
Maqbool *et al.* (271)	HeLa (H.Cervical)	*NOT REPORTED*	96	Extract	495.21
Banerjee *et al.* (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 *et al.* (26)		✸	✸	✸	✸✸		✸	✸	✸✸✸✸✸✸✸ (7)	**Good**
Das *et al.* (121)		✸	✸	✸	✸✸		✸	✸	✸✸✸✸✸✸✸ (7)	**Good**
Al-Oqail *et al.* (23)	✸	✸	✸	✸	✸✸		✸	✸	✸✸✸✸✸✸✸✸ (8)	**Good**
Ali *et al.* (34)	✸	✸	✸	✸	✸✸		✸	✸	✸✸✸✸✸✸✸✸ (8)	**Good**
Hago *et al.* (179)		✸	✸	✸	✸✸		✸	✸	✸✸✸✸✸✸✸ (7)	**Good**

Table 5: ***Continued***

Nawaz ***et al.*** **(307)**	✸	✸	✸	✸	✸✸		✸	✸	✸✸✸ ✸✸✸ ✸✸ **(8)**	**Good**
Toy ***et al.*** **(423)**		✸	✸	✸	✸✸		✸	✸	✸✸✸ ✸✸✸ ✸ **(7)**	**Good**
Maqbool ***et al.*** **(271)**	✸	✸	✸	✸	✸✸		✸	✸	✸✸✸ ✸✸✸ ✸✸ **(8)**	**Good**
Banerjee ***et al.*** **(61)**	✸	✸	✸	✸	✸✸		✸	✸	✸✸✸ ✸✸✸ ✸✸ **(8)**	**Good**

(Adapted from: 321)

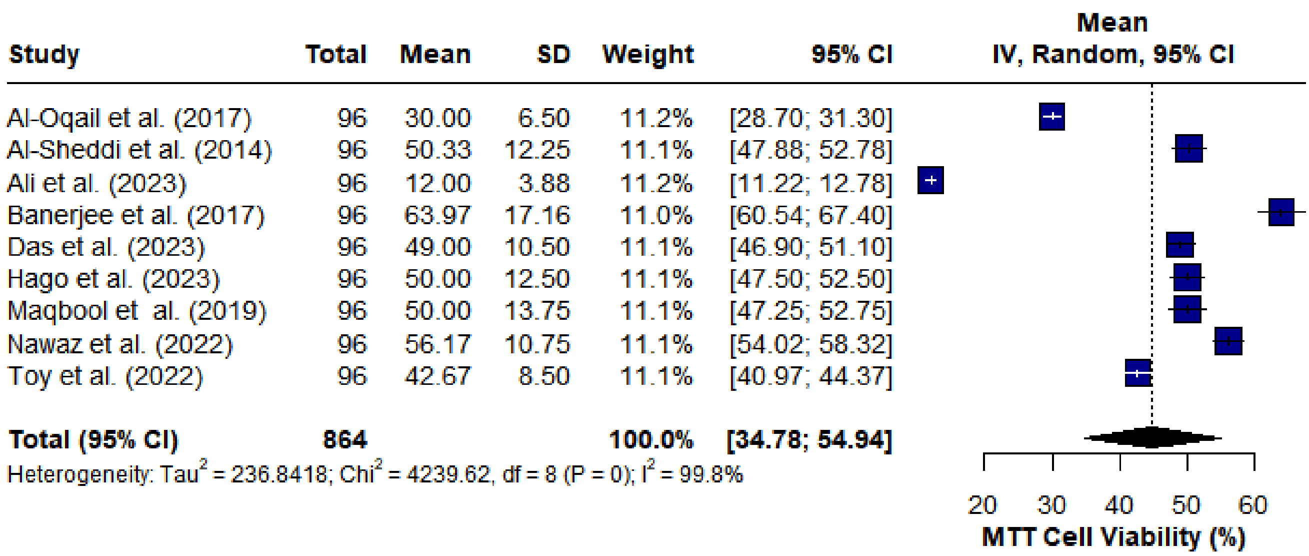


Figure 8: A forest-plot of the cytotoxic effects of *Nigella sativa* upon various cancer cell-lines using mean-values and 95% confidence intervals (CIs).

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.

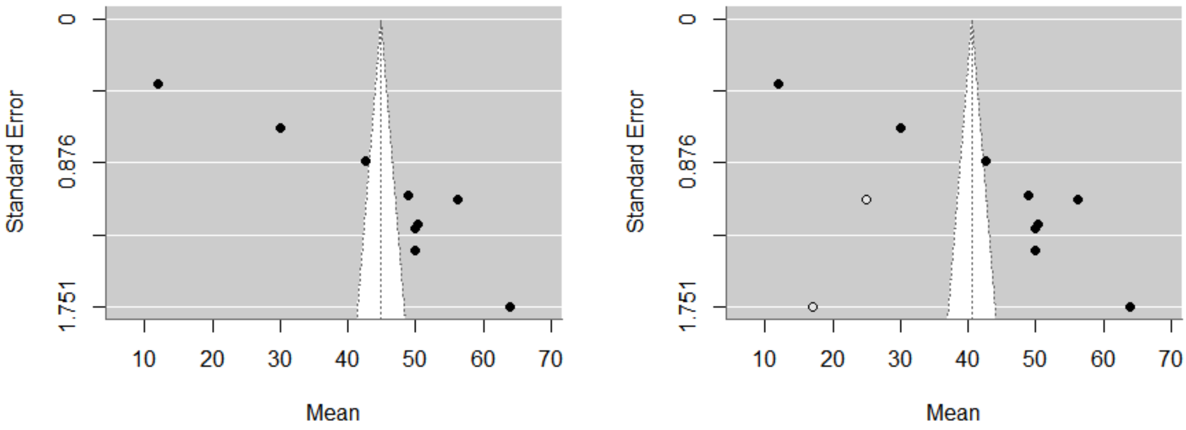


Figure 9: A funnel-plot and trim-and-fill funnel plot.

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; tau^2 estimator: REML), tau^2 (estimated amount of total heterogeneity): 284.5633 (SE = 127.9175), tau (square root of estimated tau^2 value): 16.8690, I^2 (total heterogeneity / total variability): 99.70%, H^2 (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; tau^2 estimator: REML). tau^2 (estimated amount of residual heterogeneity): 134.0003 (SE = 72.3597). tau (square root of estimated tau^2 value): 11.5758. I^2 (residual heterogeneity / unaccounted variability): 99.35%. H^2 (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; tau^2 estimator: REML), tau^2 (estimated amount of residual heterogeneity): 134.0003 (SE = 72.3597), tau (square root of estimated tau^2 value):11.5758, I^2 (residual heterogeneity / unaccounted variability): 99.35%, H^2 (unaccounted variability / sampling variability): 154.61, R^2 (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.

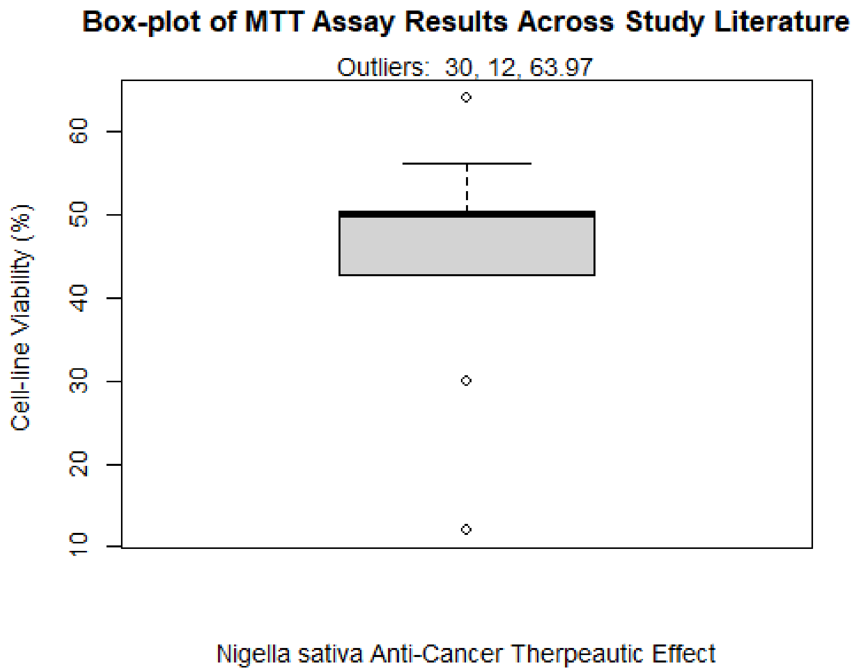


Figure 10: A Box plot utilising the mean cell-viability percentages across the collated studies.

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$therapy_mtt_assay_mean_percentage_, G = 1.23766, U = 0.78459, p-value = 0.9369, alternative hypothesis: highest value 63.97 is an outlier. (Results for Banerjee *et al.,* 2017) data: cbind_df$therapy_mtt_assay_mean_percentage_, G = 2.13603, U = 0.35838, p-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-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 ug/ml, respectively; with doxorubicin showing consistent reductions in potency, in comparison to NS oil, with IC_{50} values of 101, 188 and 244 ug/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 IC_{50} 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 there 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 IC_{50} 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 tocols 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 zeaxanfluin 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 IC_{50} 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 IC_{50} 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).

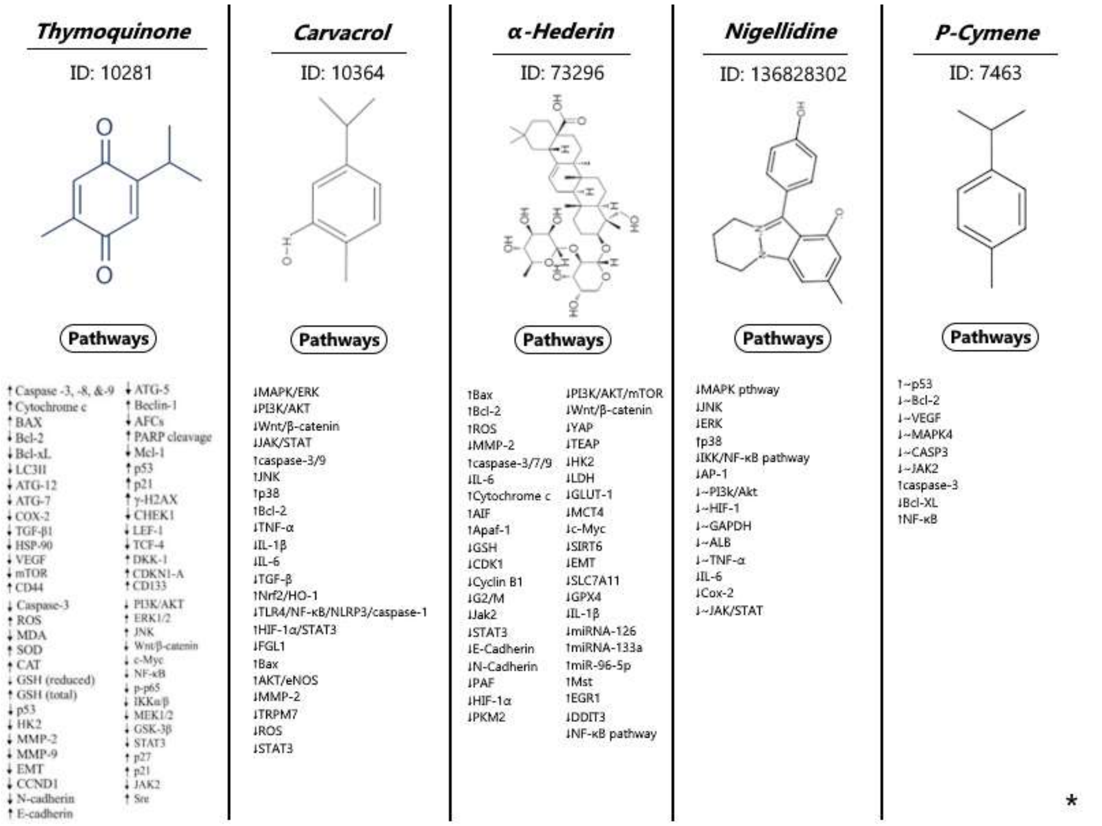


Figure 11: Key thermal-sensitive bio-active constituents of *Nigella sativa* and their molecular impacts.

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).

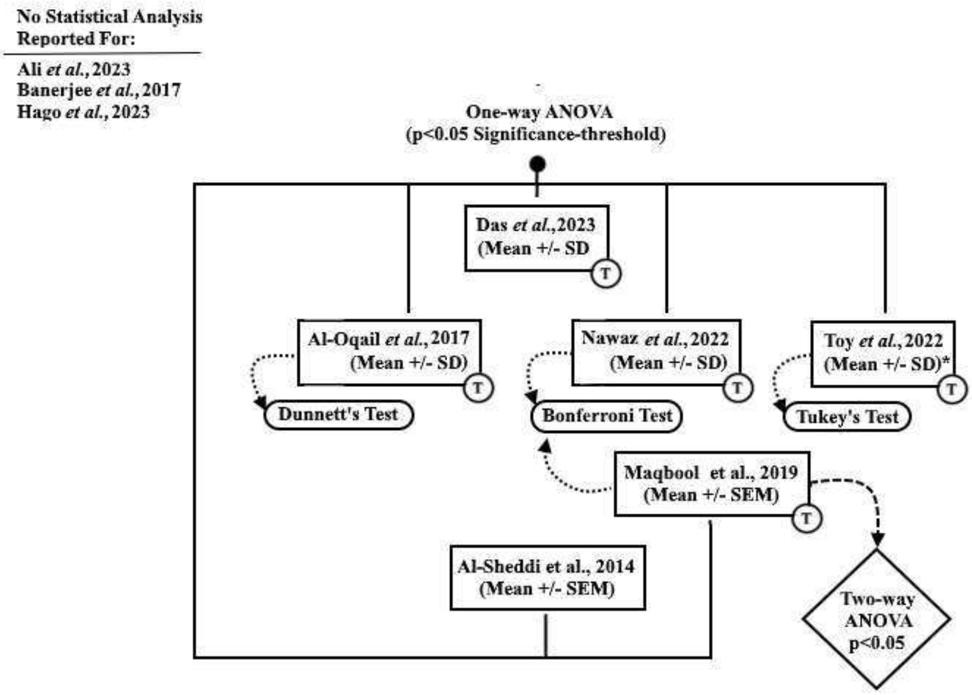
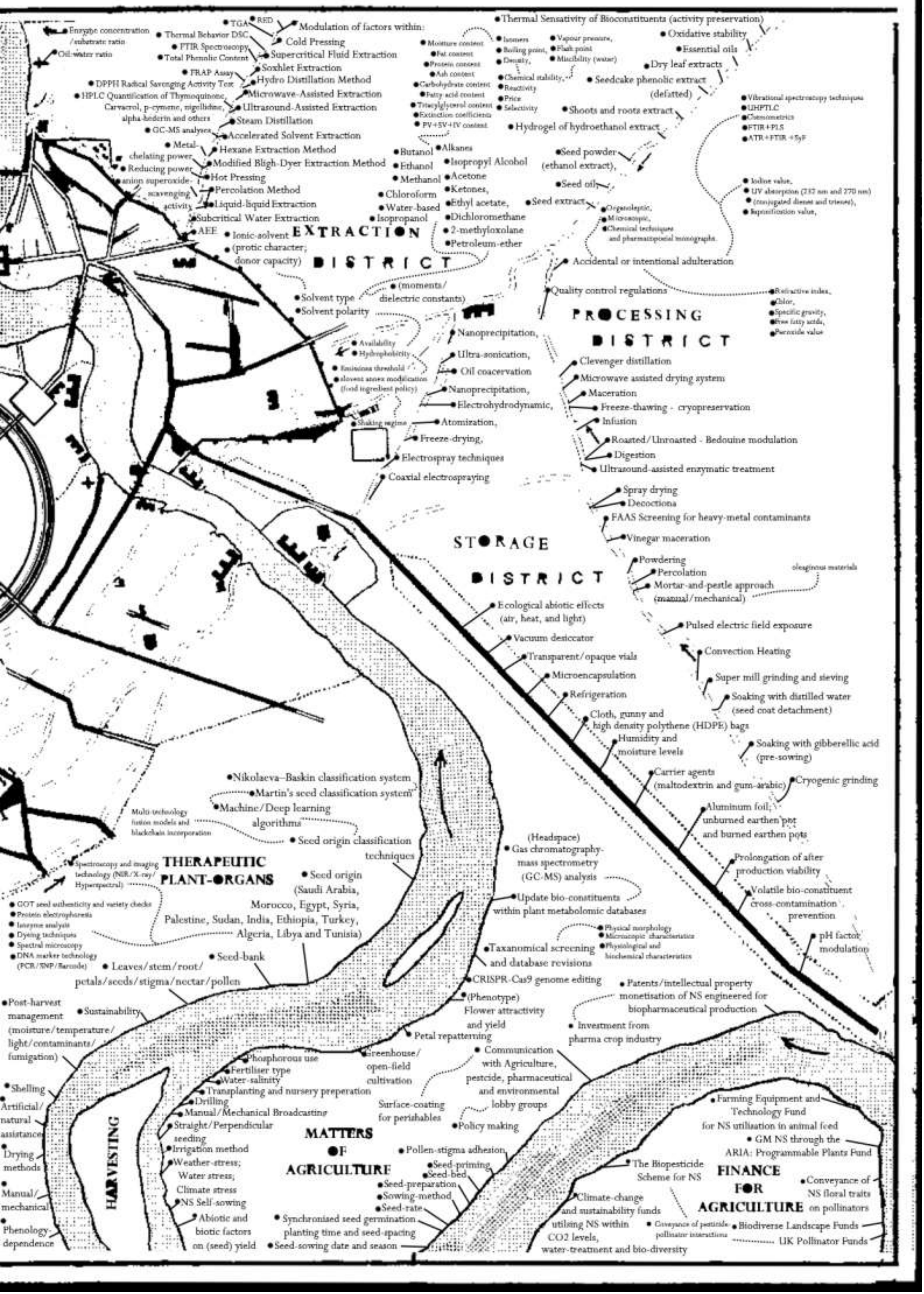
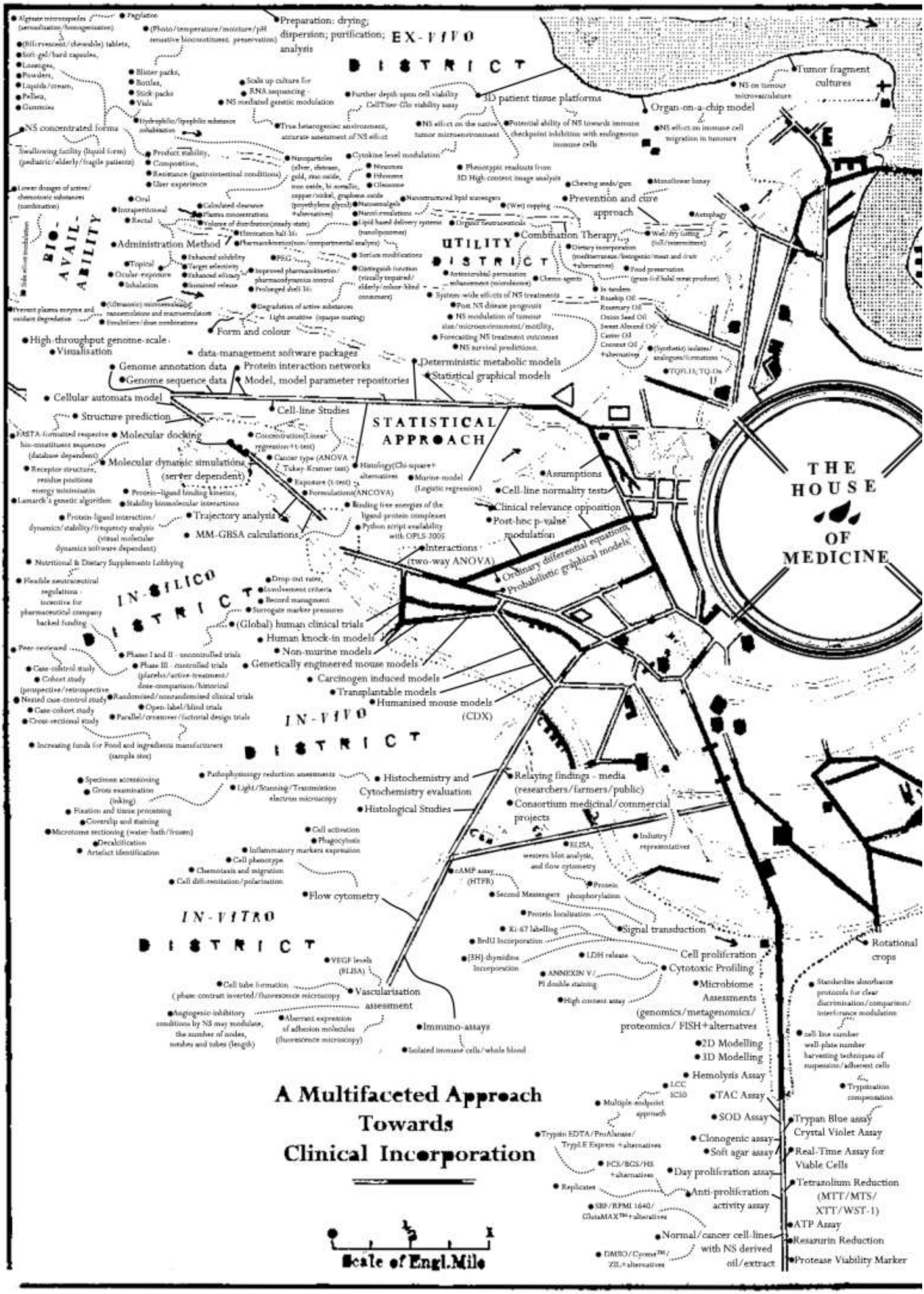


Figure 12: A visual overview of the statistical-analysis and post-hoc analytical approaches employed across the research-papers.

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).


EXTRACTION
DISTRICT
Enzyme concentration /substrate ratio
Oil-water ratio
TGA
RED
Thermal Behavior DSC
FTIR Spectroscopy
Total Phenolic Content
FRAP Assay
DPPH Radical Savenging Activity Test
HPLC Quantification of Thymoquinone, Carvacrol, p-cymene, nigellidine, alpha-hederin and others
GC-MS analyses
Metal-chelating power
Reducing power
anion superoxide-scavenging activity
Modulation of factors within:
Cold Pressing
Supercritical Fluid Extraction
Soxhlet Extraction
Hydro Distillation Method
Microwave-Assisted Extraction
Ultrasound-Assisted Extraction
Steam Distillation
Accelerated Solvent Extraction
Hexane Extraction Method
Modified Bligh-Dyer Extraction Method
Hot Pressing
Percolation Method
Liquid-liquid Extraction
Subcritical Water Extraction
AEE
Ionic-solvent
(protic character; donor capacity)
Moisture content
Fat content
Protein content
Ash content
Carbohydrate content
Fatty acid content
Triacylglycerol content
Extinction coefficients
PV+SV+IV content
Isomers
Boiling point,
Density,
Vapour pressure,
Flash point
Miscibility (water)
Chemical stability,
Reactivity
Price
Selectivity
Butanol
Ethanol
Methanol
Chloroform
Water-based
Isopropanol
Alkanes
Isopropyl Alcohol
Acetone
Ketones,
Ethyl acetate,
Dichloromethane
2-methyloxolane
Petroleum-ether
Solvent type
Solvent polarity
(moments/ dielectric constants)
Availability
Hydrophobicity
Emissions threshold
solvent annex modification (food ingredient policy)
Shaking regime
Thermal Sensativity of Bioconstituents (activity preservation)
Oxidative stability
Essential oils
Dry leaf extracts
Seedcake phenolic extract (defatted)
Shoots and roots extract
Hydrogel of hydroethanol extract
Seed powder (ethanol extract),
Seed oil
Seed extract
Vibrational spectroscopy techniques
UHPTLC
Chemometrics
FTIR+PLS
ATR+FTIR+SyF
Iodine value,
UV absorption (232 nm and 270 nm)
(conjugated dienes and trienes),
Saponification value,
Organoleptic,
Microscopic,
Chemical techniques and pharmacopoeial monographs
Accidental or intentional adulteration
Quality control regulations
Refractive index,
Color,
Specific gravity,
Free fatty acids,
Peroxide value
PROCESSING
DISTRICT
Nanoprecipitation,
Ultra-sonication,
Oil coacervation
Nanoprecipitation,
Electrohydrodynamic,
Atomization,
Freeze-drying,
Electrospray techniques
Coaxial electrospraying
Clevenger distillation
Microwave assisted drying system
Maceration
Freeze-thawing - cryopreservation
Infusion
Roasted/Unroasted - Bedouine modulation
Digestion
Ultrasound-assisted enzymatic treatment
Spray drying
Decoctions
FAAS Screening for heavy-metal contaminants
Vinegar maceration
Powdering
Percolation
Mortar-and-pestle approach (manual/mechanical)
oleaginous materials
Pulsed electric field exposure
Convection Heating
Super mill grinding and sieving
Soaking with distilled water (seed coat detachment)
Soaking with gibberellic acid (pre-sowing)
Cryogenic grinding
STORAGE
DISTRICT
Ecological abiotic effects (air, heat, and light)
Vacuum desiccator
Transparent/opaque vials
Microencapsulation
Refrigeration
Cloth, gunny and high density polythene (HDPE) bags
Humidity and moisture levels
Carrier agents (maltodextrin and gum-arabic)
Aluminum foil; unburned earthen pot and burned earthen pots
Prolongation of after production viability
Volatile bio-constituent cross-contamination prevention
pH factor modulation
Nikolaeva–Baskin classification system
Martin's seed classification system
Machine/Deep learning algorithms
Multi-technology fusion models and blockchain incorporation
Seed origin classification techniques
Spectroscopy and imaging technology (NIR/X-ray/ Hyperspectral)
THERAPEUTIC
PLANT-ORGANS
GOT seed authenticity and variety checks
Protein electrophoresis
Isozyme analysis
Dyeing techniques
Spectral microscopy
DNA marker technology (PCR/SNP/Barcode)
Seed origin (Saudi Arabia, Morocco, Egypt, Syria, Palestine, Sudan, India, Ethiopia, Turkey, Algeria, Libya and Tunisia)
Seed-bank
Leaves/stem/root/ petals/seeds/stigma/nectar/pollen
(Headspace) Gas chromatography-mass spectrometry (GC-MS) analysis
Update bio-constituents within plant metabolomic databases
Physical morphology
Microscopic characteristics
Physiological and biochemical characteristics
Taxanomical screening and database revisions
CRISPR-Cas9 genome editing
(Phenotype) Flower attractivity and yield
Petal repatterning
Patents/intellectual property monetisation of NS engineered for biopharmaceutical production
Investment from pharma crop industry
Post-harvest management (moisture/temperature/ light/contaminants/ fumigation)
Sustainability
Shelling
Artificial/ natural assistance
Drying methods
Manual/ mechanical
Phenology-dependence
HARVESTING
Phosphorous use
Fertiliser type
Water-salinity
Transplanting and nursery preperation
Drilling
Manual/Mechanical Broadcasting
Straight/Perpendicular seeding
Irrigation method
Weather-stress; Water stress; Climate stress
NS Self-sowing
Abiotic and biotic factors on (seed) yield
Greenhouse/ open-field cultivation
Surface-coating for perishables
MATTERS
OF
AGRICULTURE
Pollen-stigma adhesion
Seed-priming
Seed-bed
Seed-preparation
Sowing-method
Seed-rate
Synchronised seed germination, planting time and seed-spacing
Seed-sowing date and season
Communication with Agriculture, pestcide, pharmaceutical and environmental lobby groups
Policy making
Farming Equipment and Technology Fund for NS utilisation in animal feed
GM NS through the ARIA: Programmable Plants Fund
The Biopesticide Scheme for NS
FINANCE
FOR
AGRICULTURE
Conveyance of NS floral traits on pollinators
Climate-change and sustainability funds utilising NS within CO2 levels, water-treatment and bio-diversity
Conveyance of pesticide-pollinator interactions
Biodiverse Landscape Funds
UK Pollinator Funds



EX-VIVO DISTRICT
Preparation: drying; dispersion; purification; analysis
3D patient tissue platforms
Organ-on-a-chip model
Tumor fragment cultures
NS on tumour microvasculature
Further depth upon cell viability CellTiter-Glo viability assay
Scale up culture for RNA sequencing
NS mediated genetic modulation
True heterogenic environment, accurate assessment of NS effect
NS effect on the native tumor microenvironment
Potential ability of NS towards immune checkpoint inhibition with endogenous immune cells
NS effect on immune cell migration in tumours
Cytokine level modulation
Phenotypic readouts from 3D High content image analysis
Alginate microcapsules (aerosolisation/homogenisation)
Pegylation
(Photo/temperature/moisture/pH sensitive bioconstituent preservation)
(Effervescent/chewable) tablets, Soft-gel/hard capsules, Lozenges, Powders, Liquids/cream, Pellets, Gummies
Blister packs, Bottles, Stick-packs, Vials
NS concentrated forms
Hydrophilic/lipophilic substance cohabitation
Swallowing facility (liquid form) (pediatric/elderly/fragile patients)
Product stability, Composition, Resistance (gastrointestinal conditions), User experience
Nanoparticles (silver, chitosan, gold, zinc oxide, iron oxide, bi-metallic, copper/nickel, graphene oxide (polyethylene glycol) +alternatives)
Niosomes, Ethosomes, Oleosome
Nanoemulgels, Nanoformulations
Nanostructured lipid scavengers
Lipid based delivery systems
Organic Neutraceuticals
Chewing seeds/gum
Monoflower honey
(Wet) cupping
Prevention and cure approach
Autophagy
Lower dosages of active/chemotoxic substances (combination)
BIO-AVAILABILITY
Oral, Intraperitoneal, Rectal
Calculated clearance, Plasma concentrations, Volume of distribution (steady state), Elimination half-life, Pharmacokinetics (non/compartmental analysis)
Administration Method
Topical, Ocular-exposure, Inhalation
Enhanced solubility, Target selectivity, Enhanced efficacy, Sustained release
PEG
Improved pharmacokinetics/pharmacodynamics control
Prolonged shelf-life
Surface modifications
Distinguish function (visually impaired/elderly/colour-blind consumers)
UTILITY DISTRICT
Combination Therapy
Wet/dry cutting (toll/intermittent)
Dietary incorporation (mediterranean/ketogenic/meat and fruit +alternatives)
Food preservation (grass-fed halal meat produce)
Antimicrobial permeation enhancement (microbiome)
Chemo-agents
In-tandem: Rosehip Oil, Rosemary Oil, Onion Seed Oil, Sweet Almond Oil, Castor Oil, Coconut Oil +alternatives
(Synthetic) isolates/analogues/formations
TQFL15; TQ-Os
System-wide effects of NS treatments
Post NS disease prognosis
NS modulation of tumour size/microenvironment/motility,
Forecasting NS treatment outcomes
NS survival predictions.
Prevent plasma enzyme and oxidant degradation
(Ultrasonic) microencapsulation, nanoemulsions and macroemulsions
Emulsifiers/dose combinations
Degradation of active substances
Light sensitive (opaque coating)
Form and colour
High-throughput genome-scale
Visualisation
data-management software packages
Genome annotation data
Genome sequence data
Protein interaction networks
Model, model parameter repositories
Deterministic metabolic models
Statistical graphical models
Cellular automata model
Structure prediction
Cell-line Studies
STATISTICAL APPROACH
FASTA-formatted respective bio-constituent sequences (database dependent)
Molecular docking
Receptor structure, residue positions, energy minimisation
Lamarck's genetic algorithm
Molecular dynamic simulations (server dependent)
Protein-ligand binding kinetics, Stability biomolecular interactions
Concentration (Linear regression + t-test)
Cancer type (ANOVA + Tukey-Kramer test)
Exposure (t-test)
Formulations (ANCOVA)
Histology (Chi-square + alternatives)
Murine-model (Logistic regression)
Assumptions
Cell-line normality tests
Clinical relevance opposition
Post-hoc p-value modulation
Protein-ligand interaction/dynamics/stability/frequency analysis (visual molecular dynamics software dependent)
Trajectory analysis
MM-GBSA calculations
Binding free energies of the ligand-protein complexes
Python script availability with OPLS-2005
Interactions (two-way ANOVA)
Ordinary differential equations
Probabilistic graphical models
THE HOUSE OF MEDICINE
Nutritional & Dietary Supplements Lobbying
Flexible neutraceutical regulations - incentive for pharmaceutical company backed funding
IN-SILICO DISTRICT
Drop-out rates, Involvement criteria, Record management, Surrogate marker pressures
(Global) human clinical trials
Human knock-in models
Non-murine models
Genetically engineered mouse models
Carcinogen induced models
Transplantable models
Humanised mouse models (CDX)
Peer-reviewed
Case-control study, Cohort study (prospective/retrospective), Nested case-control study, Case-cohort study, Cross-sectional study
Phase I and II - uncontrolled trials
Phase III - controlled trials (placebo/active-treatment/dose-comparison/historical
Randomised/nonrandomised clinical trials
Open-label/blind trials
Parallel/crossover/factorial design trials
IN-VIVO DISTRICT
Increasing funds for Food and ingredients manufacturers (sample size)
Specimen accessioning, Gross examination (inking), Fixation and tissue processing, Coverslip and staining, Microtome sectioning (water-bath/frozen), Decalcification, Artefact identification
Pathophysiology reduction assessments
Light/Scanning/Transmission electron microscopy
Histochemistry and Cytochemistry evaluation
Histological Studies
Relaying findings - media (researchers/farmers/public)
Consortium medicinal/commercial projects
Industry representatives
Cell activation, Phagocytosis, Inflammatory markers expression, Cell phenotype, Chemotaxis and migration, Cell differentiation/polarisation
Flow cytometry
ELISA, western blot analysis, and flow cytometry
cAMP assay (HTFR)
Protein phosphorylation
Second Messengers
Protein localisation
Signal transduction
IN-VITRO DISTRICT
Ki-67 labelling
BrdU Incorporation
(3H)-thymidine Incorporation
Cell proliferation
LDH release
ANNEXIN V/PI double staining
Cytotoxic Profiling
High content assay
Microbiome Assessments (genomics/metagenomics/proteomics/ FISH+alternatves
Rotational crops
VEGF levels (ELISA)
Cell tube formation (phase-contrast inverted/fluorescence microscopy
Vascularisation assessment
Angiogenic-inhibitory conditions by NS may modulate, the number of nodes, meshes and tubes (length)
Aberrant expression of adhesion molecules (fluorescence microscopy)
Immuno-assays
Isolated immune cells/whole blood
Standardise absorbance protocols for clear discrimination/comparison/interference modulation
cell-line number well-plate number harvesting techniques of suspension/adherent cells
Trypsination compensation
2D Modelling
3D Modelling
Hemolysis Assay
LC50 IC50
Multiple endpoint approach
TAC Assay
SOD Assay
Trypan Blue assay
Crystal Violet Assay
Trypsin EDTA/ProAlanase/TrypLE Express +alternatives
Clonogenic assay
Soft agar assay
Real-Time Assay for Viable Cells
FCS/BCS/HS +alternatives
Day proliferation assay
Replicates
Anti-proliferation activity assay
Tetrazolium Reduction (MTT/MTS/XTT/WST-1)
SRF/RPMI 1640/ GlutaMAX™+alteratives
ATP Assay
Normal/cancer cell-lines with NS derived oil/extract
Resazurin Reduction
DMSO/Cyrene™/ZIL+alternatives
Protease Viability Marker
A Multifaceted Approach Towards Clinical Incorporation
Scale of Engl.Mile

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 less 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=zwitterionic 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, IC_{50} 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/hexsomes/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/biofetilisers 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, Alliumcepa, 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 defendable 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 phenotypical 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.

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Appendix A. Supplementary Material

Table S1. PICO elements curated for this research project.

Element	*Description*
Problem	Uncontrolled cancer cell proliferation
Intervention	*Nigella sativa* formulations (oil/extract)
Comparison	Cell viability and selective cytotoxicity (*in-vitro* 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*?**

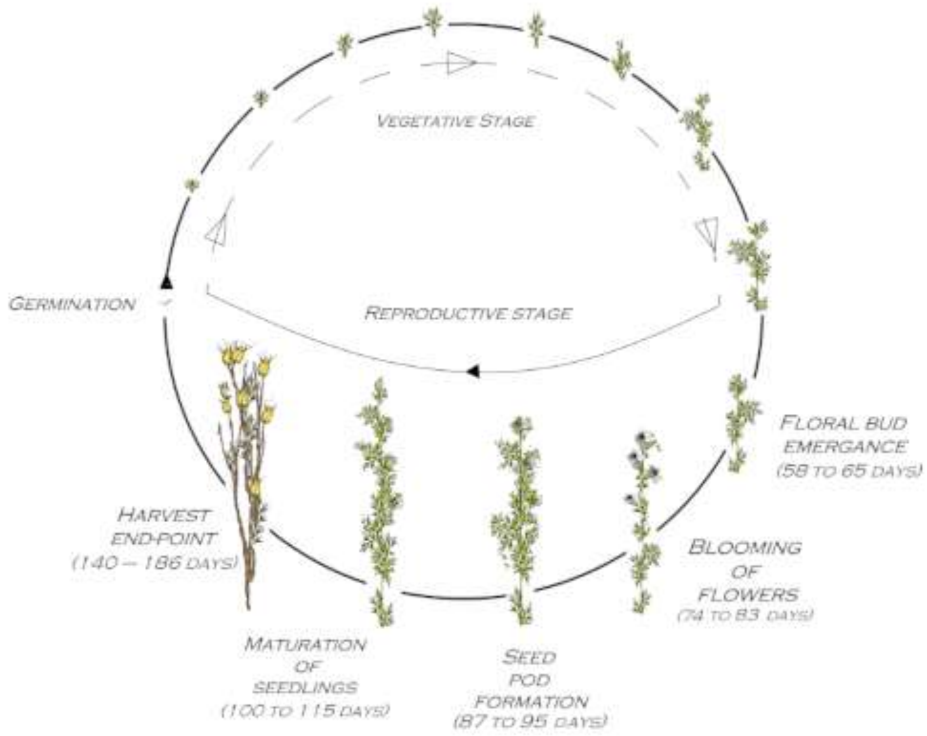

Figure S1: Potential agricultural targets for therapeutic gain. A phytological visual diagram of the stages that may improve yield and constituent 'make-up' within *Nigella sativa* if modulated appropriately through techniques possibly derived from further research. (274)

Preliminary interations 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 *N.sativa* 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/Norma**l (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: 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	

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	BCE/ CE	DATE /PERIOD	SOURCE	OBSERVATIONS	NIGELLA SATIVA	NIGELLA SPECIES
ARCHAELOGICAL	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/13th cent.	Germany: Cottbus	7 waterlogged seeds from a well	X	
		14th/13th 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/1st cent.	Egypt: Mons Claudianus (Roman)	62 desiccated + 2 charred seeds	X	
		2nd/1st cent.	Egypt: Mons Porphyrites (Roman)	31 desiccated + 12 charred seeds	X	
	BCE	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
TEXTUAL	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 fī funun al-adab (by Al-Nuwayri)	? (AR)	X	
		14th cent.	Fasl fī 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 fī 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/10th cent.	Mishnaic glossary Hebrew-Greek: No. 73	ΜΕΛΑΝΘΗ (melanthe) (GR); ךלבהז(HE)	X	

10th/9th cent.	Kitāb al-Qanûn 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‘Sumūm (Book on Poisons, by Ibn Wahshīya)	(Ḥabba sawdā‘) (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 Colu mella), 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 Hippo crati cum” (by Hippokra tes of Kos?)	μελάνθιον (melanthion) (GR)	X?
7th cent. or older	various medical prescriptions on Assyrian cuneiform tablet	? (zibûm) (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 paradisus 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; Zam Nardensamen (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; schwartzer 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); Schwartzer 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 simplicimum 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 Commo 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 15^{th} century Constantinople manuscripts such as Dioscorides' De Materia Medica (Πεδάνιος Διοσκουρίδης, Περὶ ὕλης ἰατρικῆς 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), stim-ulate 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) 12^{th} 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, nrows = 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
```



```
boxplot(cbind_df$therapy_mtt_assay_mean_percentage_,
        xlab = "Nigella sativa Anti-Cancer Therpeautic 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)

#infuence
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("model1.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 - Modereation Analysis
model <- lm(therapy_mtt_assay_mean_percentage_ ~ therapy_type_ * therapy_ic50_µgmL_,
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")
```

النَّبِيِّ 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

The Book of Medicine

(Sunnah.com, 2026).

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

[55:13]

(Quran.com, 2025)

(Amman Panorama Art Gallery, 2026)

NOTES

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الشخص الذي تم تجاهله

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THE OVERLOOKED ONE