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A review of ethnobotany, phytochemistry, antimicrobial pharmacology and toxicology of *Nigella sativa* L.

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ABSTRACT

Nigella sativa L. is one of the most extensively used traditional medicinal plants. This widely studied plant is known to display diverse pharmacological actions, including antimicrobial activities. Current literature has documented its multi-target mode of antimicrobial actions. *N. sativa* or its bioactive compounds, such as thymoquinone, can induce oxidative stress, cell apoptosis (by producing reactive oxygen species), increase membrane permeability, inhibit efflux pumps, and impose strong biocidal actions. Despite its well-documented antimicrobial efficacy in the experimental model, to the best of our knowledge its antimicrobial mechanisms highlighting the multi-targeting properties have yet to be well discussed. Is *N. sativa* or thymoquinone a valuable lead compound for therapeutic development for infectious diseases? Are *N. sativa*'s bioactive compounds potential antimicrobial agents or able to overcome antimicrobial resistance? This review aims to discuss the antimicrobial pharmacology of *N. sativa*-based treatments. Additionally, it provides a holistic overview of the ethnobotany, ethnopharmacology, and phytochemistry of *N. sativa*.

1. Introduction

Infectious diseases (IDs) are among the top ten deadly diseases globally, and the menace of growing antimicrobial resistance (AMR) has challenged and reduced the effectiveness of most clinical antibiotics [1]. Irrational use of antibiotics causes an increased rate of AMR, resulting in high AMR-related deaths globally [2]. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) infected patients are 64% more likely to die than patients infected with drug-sensitive strains [1]. Additionally, host susceptibility changes and altering the genetic makeup of microorganisms themselves have caused a remarkable growth of AMR worldwide [3].

A multi-target drug approach could be a sustainable strategy to overcome AMR problems, because bacterial resistance to single-target antibiotics develops too rapidly to be sustainable, and resistance to new drugs often develops even before they reach the market [4].

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Therefore, the current drug discovery trend has changed from *"one--molecule-one-target-one-disease"* to *"one molecule-multi-targets modulation."* Naturally, plant-derived molecules possess the ability to multi-target as they fight against predators like bacteria, fungi, viruses, insects, and herbivores, from their germination to maturity stages, and undergo challenging defense mechanisms for survival [5-7]. So, instead of developing new antibiotics, exploring potential antimicrobial agents from plant sources is convenient and cost-effective. Moreover, access to plant sources is easy and reliable since about 75–80% (more than six billion) of the world's population depends on traditional medicine-based health care, especially in the less developed and developing countries of the world [8–10].

Nigella sativa L. (NS) is a potent medicinal plant used since ancient times that has been investigated widely to prove its traditional claims. The seeds or extracted oil of NS are traditionally used alone or in combination with other products like honey, lint, melted butter, and astringents in Islamic medicine, ayurvedic medicine, Unani traditional medicine, and indigenous medicine. They are used for various diseases or health conditions, including abdominal disorders, abscesses, anorexia, worm infections, asthma, bronchitis, cardiovascular disease, chronic headache, colic, coryza, coughs, dermatosis, diabetes, diarrhoea, dispiritedness, eye infections, fatigue, fever, galactopoiesis, headaches, haemorrhoids, hypertension, jaundice, liver ailments, metabolic syndrome, nasal ulcers, obesity, orchitis, pulmonosis, reproductive disorders, respiratory infections, rheumatism, sinusitis and sore eyes, and also as a stimulant for body energy [11–15].

The therapeutic effects of NS are due to its richness in several phytochemicals, nutritionally vital constituents, and polyunsaturated fatty acids (PUFA). NS oil is on the United States Food and Drug Administration's (FDA) *"Generally Recognized as Safe"* list. It has multiple bioactive molecules, including thymoquinone (TQ), *p*-cymene, α-thujene, carvacrol, β-pinene, limonene, methyl linoleate, sabinene, D-limonene, 4,5-epoxy-1-isopropyl-4- methyl-1-cyclohexene, and 4-terpineol [16–19], of which TQ exerts many pharmacological activities, including antimicrobial, anticonvulsant, anticancer, anti-histaminic, anti-diabetic, anti-inflammatory, and antioxidant [20]. It also significantly inhibits the formation of biofilm, which increases AMR in antibiotic-susceptible microorganisms [21,22]. This action probably increases the production of reactive oxygen species in bacterial cells.

Antimicrobial activities of NS or its secondary metabolites have been reported. TQ and other bioactive compounds produce ROS, which prompts oxidative stress and cell apoptosis [22] or increases bacteria membrane permeability, thereby promoting the influx of antimicrobial substances [23]. Nigellidine has a binding capacity to N-terminus protease and nucleocapsid, and inhibits viral growth [24]. Some other compounds, such as α-hederin and thymohydroquinone, bind to the ACE2 receptor and disrupt viral host interaction [25]. The essential oil of NS (1.5 mg/ml) inhibits the production of mycelia (67.4%) and aflatoxin in *Aspergillus parasiticus* and TQ generates oxidative stress in *Candida glabrata* [26,27]. Additionally, NS shows strong biocidal properties against parasites [28]. Numerous in vitro and in vivo studies, including many clinical trials, have demonstrated the therapeutic efficacy of NS against IDs [20,21,27,29–33]. Is NS or TQ a valuable lead compound for therapeutic development for IDs? Are NS's bioactive compounds potential antimicrobial agents or able to overcome AMR? This review aims to answer these questions by considering the various contributing mechanisms to the antimicrobial pharmacology of NS-based treatment. Additionally, this review provides a holistic overview of the ethnobotany, ethnopharmacology, and phytochemistry of NS, prior to exemplifying its antimicrobial properties and involved mechanisms. Key references are cited to illustrate the concept of mechanisms of NS's antimicrobial actions and are not meant to be comprehensive.

2. Methodology

Literature was searched using electronic databases, including PubMed (http://www.ncbi.nlm.nih.gov/pubmed), ScienceDirect (http://www.sciencedirect.com/), Scopus (http://www.scopus.com/), Web of Science (https://apps.webofknowledge.com/), and advanced search in Google Scholar (http://scholar.google.com.my/), as well as recognized books, abstract, and thesis/dissertation. The following keywords are "*Nigella sativa*", "Black seed", Black cumin", "antimicrobial", "anti-bacterial", "antiviral", "antifungal", and "anti-parasitic" were used to search the literature. A combination of keywords was used in search engines during the literature survey from years 1951–2021. The relevant papers from the list of references of all retrieved available articles were also searched in the aforementioned databases mentioned above. Other relevant papers from the list of references of all available articles were also searched. All structures of chemical constituents have been drawn using ChemDraw Professional 16.0 software (PerkinElmer). The updated international union of pure and applied chemistry (IUPAC) names of isolated compounds of this plant were check-in the PubChem database.

3. *Nigella sativa*

3.1. Origin and global distribution

The origin of NS is not well documented. Most probably, NS is indigenous to the Mediterranean region [34]. As the seeds of this plant were first discovered in Tutankhamun's tomb in Egypt in the 18th dynasty (1549/1550–1292 BCE) [35], it implies that the origin of this plant is Egypt and its adjoining regions. However, NS is distributed globally and cultivated in many countries and regions of the world, including, but not limited to, the Middle Eastern Mediterranean region, Central and Southern Europe, the former Soviet Union (Baltic states, Central Asia, Eastern Europe, Russia, and Transcaucasia), Northern Africa, Sudan, Ethiopia, Kenya, Somalia, Djibouti, India, Pakistan, Bangladesh, Sri Lanka, Nepal, Iran, Syria, Turkey, and Saudi Arabia [36,37]. The plant is commercially grown in many north Indian states, and India is the world's largest producer and exporter of NS [36,38].

3.2. Taxonomic hierarchy

The taxonomic serial number of *N. sativa* is 506592 [39].

Taxonomic	Nomenclature	Characteristics
Kingdom	Plantae	Plantes, Planta, Vegetal, plants
Subkingdom	Viridiplantae	Green plants
Infrakingdom	Streptophyta	Land plants
Superdivision	Embryophyta	
Division	Tracheophyta	Vascular plants, tracheophytes
Subdivision	Spermatophytina	Spermatophytes, seed plants, phanérogames
Class	Magnoliopsida	
Superorder	Ranunculanae	
Order	Ranunculales	
Family	Ranunculaceae	Buttercups, boutons d'or, crowfoot
Genus	*Nigella* L.	
Species	*Nigella sativa* L.	

3.3. Plant description

NS is an erect, herbaceous flowering plant with a stiff and multi-branched stem and a well-developed taproot. The agro-morphological traits of NS are distinguishable from other herbaceous plants as well as different accessions of NS. The detailed agro-morphological traits of NS [38,40,41] are presented in Table 1. Fig. 1 shows the anatomy of NS [34]. The different body parts are also indicated clearly, including the cross and transverse sections of the fruits and seeds (Fig. 1).

Table 1
Agro-morphological traits of *Nigella sativa*.

Trait	Value/characteristics
Plant height	30–70 cm
Stem	
Diameter	0.65–0.98 cm
Shape	Angular
Leaves	
Arrangement	Alternate
Length	2.5–5.0 cm
Width	2.0–2.5 cm
Shape	Linear to lanceolate
Phyllotaxis	1–2
Pinna/rachis	5–6
Root	
Length	12.74 – 15.08 cm
Flower	
Color	Pink, white, yellow, pale blue, or pale purple color
Size	2.74 cm × 2.78 cm
Number of petals	5–10
Number of sepals	4–6
Seed	
Capsule size	0.4–1.7 cm
Seed/capsule	59.29 ± 3.2
Color	Black
Size	2.33 mm ± 0.1 × 1.14 mm ± 0.02
Shape	Acute, angles sharp, ovate, tetrangular, more tapering at the end.
Flowering and Fruiting time	January to April

3.4. Vernacular names

The vernacular name— in other words, local name, common name or non-Latin name—of a plant or animal is derived from the common native speech of everyday life that is distinguishable from the binomial nomenclature. This name originated based on plant morphology, habit and habitat, organoleptic properties, and therapeutic uses. Generally, medicinal plants are traded with their vernacular names [42]. Therefore, knowing the vernacular names is crucial for folk-taxonomic identification. At the same time, it provides information on plant morphology and gives information on habit and habitat, organoleptic properties, and therapeutic actions of the medicinal plant. However, this nomenclature does not always correspond one-to-one with scientific plant names. For example, both *Drypetes afzelii* and *Adansonia digitate* are known as *Kiri* to the Susu or Soso people of Guinea and Sierra Leone, West Africa [42]. Such complications are prevalent for vernacular names.

NS is commonly known as 'black seed' in English because the seeds usually turn black upon exposure to air. It is also referred to as 'Alhabahat Alsawda', 'Habbatus Sauda', and 'Alkamoun Alaswad', among the Muslim community, in reference to the colour of its seeds [43]. Other vernacular names of NS are presented in Table 2. The data were extracted from references [12,44,45].

3.5. Agronomic techniques

Conventionally, NS is propagated via seeds [46]. The growing season of this crop varies extensively depending on the geographical location of the cultivation area. It is grown as a winter crop in the tropics, whereas in temperate regions it is grown as a spring-summer or autumn crop [47]. Several studies suggested that seeds sown in early spring, with a 30 cm or 40 cm spacing and a depth of 2 cm, give a better yield [48] while the soil pH is 8.2 and rainfall is 790 mm [49].

The cultivation of NS is commonly seen in cold temperatures. However, seed dormancy is one of the significant setbacks in cultivating this plant [50]. Germination of NS seed is very low (12–15%), and stratification for three weeks has proven to be effective for breaking its dormancy and germination [46,48]. Furthermore, a temperature of 12–18 °C and 60% soil moisture are suitable for massive seed germination [46]. In order to increase its grain size and oil content, one or two irrigations are recommended at the flowering and seed formation stage [45].

There is a relationship of yield with genetic varieties of the same plant species [51]. Selection of the high yield of secondary metabolite varieties of NS and production through vegetative propagation is essential. Since seedlings have genetic variations, vegetative propagation, for example, adventitious rooting and plant tissue cultures [52,53], is recommended for mass-scale production. Environmental factors such as abiotic and biotic stress impact the yield of secondary metabolites [54–56]; these factors could also be considered for NS production. In some cases, abiotic stress, such as salinity stress, increases the yield of secondary lead compounds in plants [57], because stress conditions alter the metabolic pathway to accumulate the related metabolites to defend against adverse conditions [58,59]. NS plants manifest extreme variability in phytochemical profiles due to the adverse effects of changing climatic and environmental conditions [50,60]. For this reason, in vitro seed germination as well as vegetative propagation techniques are good choices for large-scale production of this plant with regular phytochemical profiles under aseptic and controlled conditions.

4. Ethnopharmacological uses

NS has been commonly used for medicinal and culinary purposes for many centuries [61]. Therefore, it is essential to study the ethnobotany of NS due to its wide use in the different parts of the world, from generation to generation. Due to traditional knowledge and customs, different communities have developed unique practices concerning NS and its medicinal uses.

4.1. Medicinal uses

Traditional medicine practices like Unani, Ayurveda [37], and the Arabian, Indian, and Chinese civilizations have used NS for a long time due to its numerous benefits [62]. For example, in Ayurveda, NS is known to balance Vata (movement energy) and Kapha (structure and lubrication energy), and increase Pitta (metabolism or digestion energy). The therapeutic uses of NS seeds mentioned in the Unani system include stomachic, laxative, carminative and galactagogue, and to counter inflammation, ascites, jaundice, piles, tertian fever, paralysis, and eye diseases. It is also used to combat headaches, cough and asthma, and to expel kidney stones.

4.2. Culinary and other traditional uses

NS seeds have a pungent, bitter taste and aroma. They are used as a condiment in curries in many cultures. NS is used as a flavouring additive in bread and pickles, and is also added to tea, coffee, casseroles etc. The grounded NS seeds are usually sprinkled on salads or mixed with honey. NS is an ingredient of the spice mixture *panch phoron*, and the dry-roasted seeds are sometimes used to flavour curries, pulses, and vegetables [37,63].

NS seeds have also been used as a liver tonic, diaphoretic, diuretic, stomachic, emmenagogue, and control for parasitic infections [37,64]. NS can be combined with other ingredients to cure diarrhoea, dyspepsia, reflux, obesity and dyspnea, and to freshen breath. The seeds are taken with buttermilk to treat persistent hiccups and treat anorexia, vomiting, oedema, and puerperal diseases [63,64]. They are useful against mercury poisoning, leprosy, flatulence, and polio [61,63], and can treat amenorrhea, dysmenorrhea, and skin eruptions. A tincture prepared from NS seeds has been proven to be useful [62]. It is also used as a vermifuge, and its oil extract is taken orally as a daily tonic due to its immunostimulant property [31,61].

Many people use the oil to treat skin conditions, including eczema,

psoriasis, and boils. Beeswax mixed with black seed oil is also used for skin infections, burns, joint pain relief, moisturizers, or anti-wrinkle agents [62,63]. The oil also has antiseptic properties and can be applied externally. To restore a lost sense of smell, dried pods are sniffed. They are used as insect repellents such as mothballs, and to preserve woollen fabrics from insect damage by scattering between the folds of fabrics [62].

4.3. Documentation of uses of Nigella sativa in religious and medical scripts

The names of medicinal plants, their formulation, and their importance has been mentioned in religious books [65,66]. NS is mentioned in books including The Prophetic Medicine and the Bible – two significant books across the old and new testaments (Isaiah 28:25,27 and Matthew 23:23). In the Bible, NS is cited more than any other plant. It is used in cancer treatment as well as in birth control [67]. NS is the curative "black cumin" in the Bible and is described as Melanthion by Hippocrates and Dioscorides, and as Gith by Pliny [68].

Prophet Mohammed (Peace Be Upon Him) narrated that NS seed can cure every ailment or illness except death (Al-Bhukhaari, 5688). Ibn Sina, also known as Avicenna in the west, who is famous for his book "The Canon of medicine," advised about the use of NS. It helps the body recover from dispiritedness and fatigue and boosts the body's energy [69]. Pedanius Dioscorides reported using NS seeds for food and therapeutic purposes to treat diseases of the eyes, toothache, and leprosy, to increase urine, and to repel snakes. In Babylonia, the NS plant was used externally to treat swelling and bruises, and internally to treat stomach problems. The physician Assaf started its medical uses to treat colds in the chest, head and body, nose infections and bright skin spots, as well as to increase semen and virility, and to improve hair growth. It also served as a pharmaceutical constituent against venomous creatures' stings and poisons [61]. In the history of Islamic medicine, NS is a "*miraculous* or *blessed"* plant. Muslim scholars went to tremendous lengths to explore the remedial features of this plant. Its importance is stated in "Prophetic Medicine" tradition. Aishah (may Allah be pleased with her) narrated that she heard the messenger of Allah (Peace Be Upon Him) saying:

"There is healing in black cumin for every disease, except as-saam". Ibn

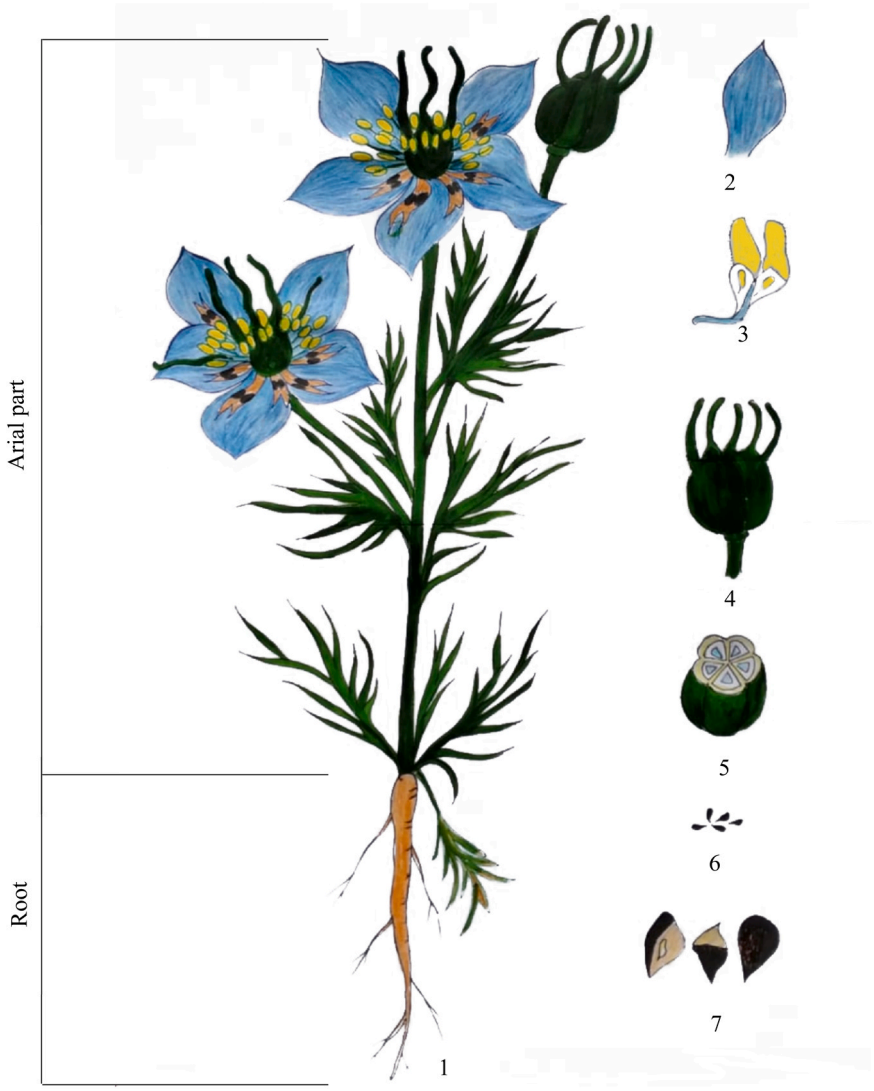

Fig. 1. *Nigella sativa* and its different body parts. 1. Habit, 2. Petal, 3. Stamen (front view), 4. Fruit, 5. Fruit (transverse section), 6. Seeds, and 7. Seeds (longitudinal and transverse section). (Artwork credit: Rabyea Jahan Mukti[1]).

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Table 2
The vernacular name of *Nigella sativa*.

Language	Name	Language	Name
Arabic	Habatut Barakah Shooneez	**Hebrew**	Ketzakh
	Habba Sauda	**Indonesian**	Jintan hitam
	Habb al-barka,	**Kannada**	Krishna Jeerige
	Kamun eswid	**Malayalam**	Karim jerrakam
	Habbat al-barakah	**Marathi**	Kalonji Jire
Assamese	Kaljeera	**Persian**	Siah Dana
	Kolajeera		Siyâh dâne
Amharic	Tikur azmud		Siyah daneh
Bengali	Kalo jeeray	**Portuguese**	Cominho-negro
Bosnian	þurekot6	**Punjabi**	Kalvanji
Chinese	Hei Zhong Cao 黑種草	**Russian**	Chernushka
English	Fennel flower, Black cumins	**Sanskrit**	Krishana – Jiraka
	Love-in-amist		Upakunchika
	Nutmeg flower	**Spanish**	Ajenuz,
	Roman coriander		Arañuel
	Black caraway or blackseed	**Swedish**	Svartkummin
	Black sesame	**Tamil**	Karum jeerakam
French	Cheveux de Vénus		Karum cheerakam
	Nigelle	**Turkish**	Cörek out
	Nigelle de Crète	**Urdu**	Kalonji
	Toute épice		Mangrail
German	Schwarzkümmel		Kalaunji
Hindi	Kalonji		
	Kalaunji		
	Mangrail		

Shihab said: As-saam is death [Sahih Bukhari, Volume 7, Book 71, Hadith 591; Sunan Ibn Majah, Vol. 4, Book of Medicine, Hadith 3449].

In the Hadith above, the formulation of NS is also clearly stated: "Take five or seven (seeds) and grind them to a powder, then drop them into his nose with drops of olive oil, on this site and on this site" for the treatment of sickness. It is stated in the books of Seerah that Nabi-e-Akram (Peace Be Upon Him) himself used to take black seed for therapeutic purposes together with honey syrup [70,71]. NS was ascertained by Suhar Bakht and called habb-i-Sajzi, Sigzi grains.

The immense benefits of NS have been described by many ancient scholars, including ancient Greek physicians like Hippocrates (460–370 BCE) and Dioscorides (40–90 CE), Galen (129–200/216 CE), Muslim scholars like Al-Biruni (973–1048) and Ibn Sina (980–1037) (Table 3).

4.4. Use of Nigella sativa in modern medicine

Many of the traditional or historical uses of NS have been scientifically examined. The medicinal importance and scientific quest of this plant can be understood from the findings of an advanced search in the PubMed database (as of 5 January 2021), with the cumulative publication record presented in Fig. 2. A systematic search using the following keywords (*Nigella sativa*[Title/Abstract]) OR (black seed [Title/Abstract]) retrieved 1534 journal articles, of which 1065 were from Medline only. Further filtration revealed 1365 journal articles, of which 67 are clinical trials in the PubMed database. Among the (systemic) reviews, Medline covered only 97 out of 169 in PubMed. The plant has attracted more focus in recent years, as evidenced by the gradual upward trend of publications (Fig. 2). In the twentieth century, only 54 and 46 journal articles were published in the PubMed and Medline databases, respectively. This number has increased from the beginning of the twentieth century. In 2020 alone, 176 publications were included in PubMed, of which 17 articles are relevant to coronavirus disease (COVID-19). As NS has prominent antiviral compounds, such as TQ, extensive investigation is in progress, including clinical trials. It has been reported that the antiviral action of TQ is equivalent or likely superior to chloroquinone and hydroxychloroquinone. TQ demonstrated a good safety profile, whereas chloroquinone and hydroxychloroquinone showed toxicity effects [72,73].

Table 3
Health benefits of *Nigella sativa* reported in historical medicine.

Scholars	Period	Health benefits
Pharaoh Tutankhamen	1341–1323 BC	Epilepsy and cerebral malaria
Hippocrates	460–370 BC	Treat diseases of women and children, such as vaginal and oral thrush and skin conditions.
Dioscorides	40–90 AD	Cure headache, toothaches, nasal congestion, intestinal worms, and promote menstruation and milk production
Galen	129–210 AD	Hepatic and digestive disorders, common cold, and stimulants in a variety of conditions
Al-Biruni	973–1048	Excite body's energy and recover exhaustion or dissipatedness
Ibn Sina	980–1037	Stimulates body's energy, recover from fatigue or dispiritedness and cancer

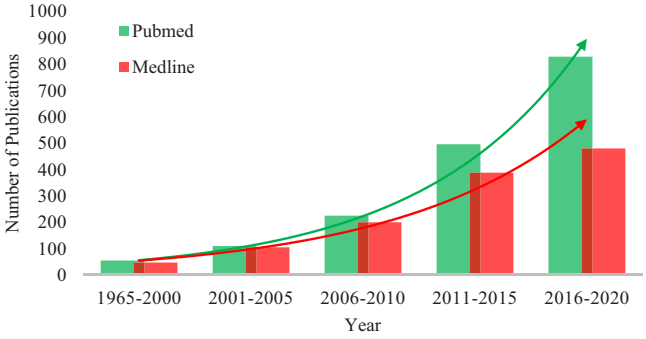


Fig. 2. *Nigella sativa* cumulative publication trends in PubMed database (as of 5 January 2021). Medline data were extracted to differentiate the trend, which is specific for biomedical and life science research.

Extant literature also provides much-needed evidence to support NS usage. For example, Bamosa, Kaatabi, Lebdaa, Elq and Al-Sultanb [74] reported that the grounded NS (supplement of 2 gm/day for three months in 94 uncontrolled types-2 diabetes mellitus patients) produced significant improvement in glycemia and diabetes control laboratory parameters. NS also showed mnemonic or nootropic properties. Among the elderly, its commercially available capsules (500 mg, taken for nine weeks) were shown to increase executive functions, as shown by various memory-related tests (letter cancellation, logical memory, trail making, digit span, and Stroop tests) [75]. Taking 500 mg NS capsules daily for four weeks as a nutritional supplement reduces anxiety, improves memory, and stabilizes mood [43]. Other clinical studies revealed that NS has activity against allergic diseases. Commercially available black seed oil capsules with the brand name Immerfit® were applied to patients with allergic diseases. Each capsule contained 500 mg NS oil with 1.5 mg β- carotene, 6 mg of vitamin E, and 70 μg biotin for stabilization. All study groups were given capsules at 40–80 mg/kg/day. The four studies showed that NS oil effectively relieved allergic disease symptoms (atopic, allergic rhinitis, bronchial asthma, eczema) [76].

5. Chemical compositions and phytocompounds

The following section outlines and discusses the chemical composition of NS, the activity of phytocompounds derived from NS, and structure–activity relationship of these active molecules.

5.1. Chemical compositions

NS seed contains a tiny amount of essential oils (0.4–1.49%), about 30–45% fixed oils and other compounds (Table 4). The concentration of other compounds varies. NS contains amino acids, proteins, carbohydrates as well as bioactive molecules.

The therapeutic efficacy of a plant depends on the phytocompounds

Table 4
Chemical compositions of *Nigella sativa*.

Compound	Yield (%)	Reference
Essential oil	0.4–1.49	[77]
Fixed oil	30–44.21	[77]
Thymoquinone	26.8–54.8	[78]
p-cymene	14.7–38.0	[78]
Longifolene	1.2–10.2	[78]
Thujene	1.3–10.1	[78]
Carvacrol	0.5–4.2	[78]
Cubebene	0.4–3	[78]
Pinene	0.2–2.4	[78]
Limonene	0.7–2.3	[78]
Sabinene	0.2–1.6	[78]
Oleic acid	25	[79,80]
Palmitic acid	18	[79,80]
Thymoquinone	3.5–8.7 mg/g	[79,80]

(secondary metabolites). Phytoconstituents are primary metabolites (involved with the life cycle, thus almost the same in all living cells) and secondary metabolites (products of subsidiary pathways, thus dealing with medicinal efficacy in the case of medicinal plants). They are not usually produced under the same conditions. In most cases, the specific function of the phytocompounds and their benefits to the organism itself are not yet known; however, they possess pharmacological properties that are beneficial for humans and animals' health care systems. The distribution of secondary metabolites differs dramatically from one plant to another, and even different accessions of the same species possess varying quantities and qualities of pharmacologically active compounds [5,81–83].

5.2. Phytocompounds

From NS, many phytoconstituents have been extracted, validated, and reported [44]. This review looked at available information on 171 isolated phytoconstituents of NS with structure (Fig. 3), part used, extraction method and isolation techniques (Table 5). Among these metabolites, only seven compounds exhibited antimicrobial activities in experimental studies (Table 6). Only a few other compounds showed other pharmacological properties. For example, α-Hederin has potential as an anticancer agent [84]. It is effective against *HEp-2* laryngeal cancer cells [85] and has antitumor properties [86]. It can also attenuate lung inflammation and improve cytokines [87]. Oleic acid and linoleic acid modulates the interaction of cardiac glycosides with the sodium pump [88], nigellidine possess immunomodulatory, anti-inflammatory and antioxidant activities [89], and longifolene is effective as an insect repellent [90]. But the pharmacological roles of a majority of the isolated pure compounds of NS in diverse health management are yet to be discovered, including IDs. However, the list of phytocompounds we have shown in this study might facilitate the researchers in drug discovery, mainly through computational biology, which can select target candidates with the highest potential for successful disease recovery using bioinformatic tools.

The highest number of metabolites has been isolated from seeds, followed by roots and shoots (Fig. 4a). These compounds were in different categories: alkaloids (**1–12**), fatty acids (**13–25**), polyphenols (**26–54**), phytosterols (**55–60**), terpenes and terpenoids (**61–151**), and others (**152–171**). Terpenes and terpenoids were the most predominant secondary metabolites, accounting for 53% of the identified molecules. Fig. 4b shows the prevalence of different types of phytocompounds of NS. For isolation of metabolites, different types of extraction methods, such as methanolic, ethanolic, n-hexane, chloroform, ethyl acetate, diethyl ether, hot and cold water, supercritical carbon dioxide, hydrodistillation and microwave distillation, have been used for different parts of the plant (Table 5).

Identification and characterization were done using various chromatography and spectroscopy methods, including thin-layer chromatography (TLC), column chromatography (CC), gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), ultra HPLC/MS-MS (UHPLC/MS-MS), reversed-phase HPLC (RP-HPLC), infrared spectroscopy (IR), nuclear magnetic resonance (NMR), mass spectrometry (MS), and X-ray crystallography (Table 5).

5.2.1. Alkaloids

A total of 12 alkaloids (**1–12**) were isolated from the seeds. The naturally occurring alkaloids of NS mainly have an indazole ring as a nucleus, such as migellicine and nigellidine. Nigellidine-4-O-sulfite is the first sulfated alkaloid of its type. Nigellimine and nigellimine n-oxide are isoquinoline alkaloids. Several dolabellane-type diterpene alkaloids, nigellamines A1–A5, were also isolated from NS [124,125]. Natural indazole-type alkaloids have only been obtained from NS, which means they are a potential taxonomic marker [126].

5.2.2. Fatty acids

NS possesses different types of fatty acids that can be determined using GC-MS. Linoleic acid is the most predominant fatty acid (56%). Oleic acid is the second-highest fatty acid present (24.6%). Palmitic acid, stearic acid, eicosadienoic acid, linolenic acid and some other acids are also present in small amounts in NS seed oil (**13–25**) [127]. The determined fatty acids were 8% of the total identified molecules. However, some of these molecules have shown potential modulating properties where the cardiac glycosides interact with the sodium pump [88].

5.2.3. Polyphenols

A total of 29 polyphenols have been identified from the seeds, roots and shoots of NS. From roots and shoots, they were identified using RP-HPLC, and from seeds using the UHPLC-MS/MS method. Among 29 polyphenols, 17 compounds were phenolic acid (**26–42**), and the other 12 were flavonoids (**43–54**). Ferulic acid and sinapinic acid were the major phenolic acid esters identified from NS seed extract. In contrast, vanillic acid was the primary phenolic compound in shoots (143.21 mg/100 gm DW) and roots (89.94 mg/100 gm DW). Quercetin and kaempferol are the major flavonoid glycosides identified in NS seed extract, and catechin was the most abundant compound in roots (3.4 ± 0.74 mg/100 gm DW) and shoots (7.26 ± 0.97 mg/100 gm DW) [128,129].

5.2.4. Phytosterols

The total sterol concentration ranges from 1993.07 mg/kg to 2887.28 mg/kg in oil extracted from NS. β-sitosterol (44–54%) is the major sterol in NS seed oil. Stigmasterol (16.57–20.92%) is the second major sterol followed by δ-7-stigmasterol, δ-7-avenasterol, campesterol, and cholesterol (**55–60**) [130].

5.2.5. Terpenes and terpenoids

More than 50% of compounds among the identified phytocompounds of NS were terpenes and terpenoids (Fig. 4). We found 91 compounds belonging to the family of terpene (**61–151**). They include mono-terpene, di-terpene, sesquiterpenes, monoterpenoid alcohols and ketone. The major terpenes—TQ, trans-anethole, *p*-cymene, α-pinene and carvone—have been identified. TQ, the primary compound of NS, has a concentration of 388.61 μg/ml [117].

5.2.6. Others

A study showed that NS comprises 21% protein, 35.5% fat, 5.5% moisture, and 3.7% ash and carbohydrate (rhamnose, xylose, and arabinose) [127]. It also contains triterpene saponin (**171**) (α-hederin), phenylpropanoids (eugenol, ε-cinnamaldehyde) and some alkane hydrocarbons (n-nonane, dodecanal etc.) (**152–171**).

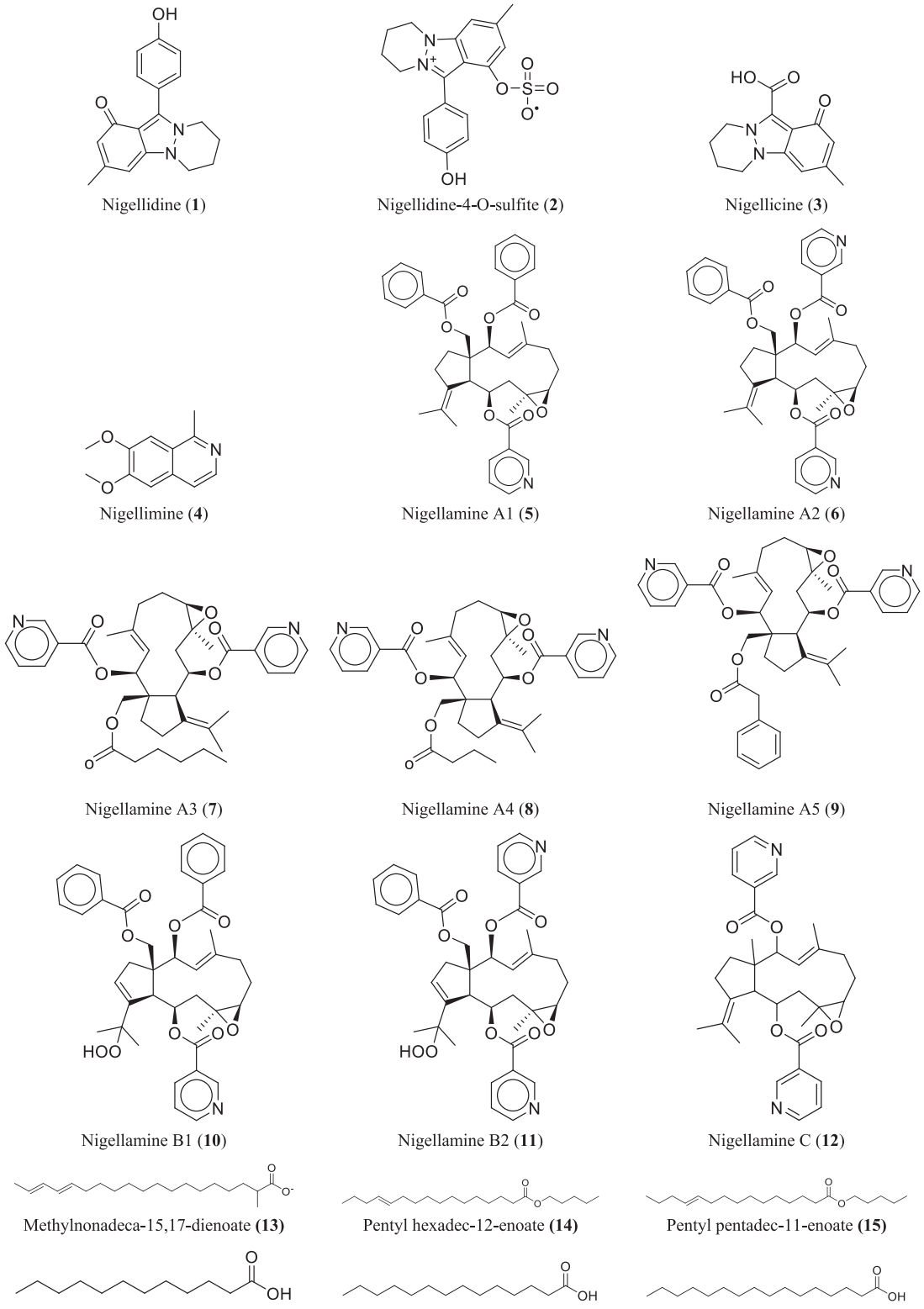


Fig. 3. Chemical structure of phytoconstituents of *Nigella sativa*. The compounds include alkaloids **(1–12)**, fatty acid (**13–25**), polyphenol (**26–54**), phytosterol (**55–60**), terpenes and terpenoid (**61–151**), and others (**152–171**).

6. Antimicrobial pharmacology of *Nigella sativa*

Different extracts of NS, NS oil and several bioactive molecules of NS have been effective against infections caused by microbes, as supported by numerous in vitro studies. They have distinguishable antibacterial, antiviral, antifungal and anti-parasitic activities, as discussed below.

6.1. Antibacterial activity

Various studies revealed the antibacterial potential of NS. In a recent

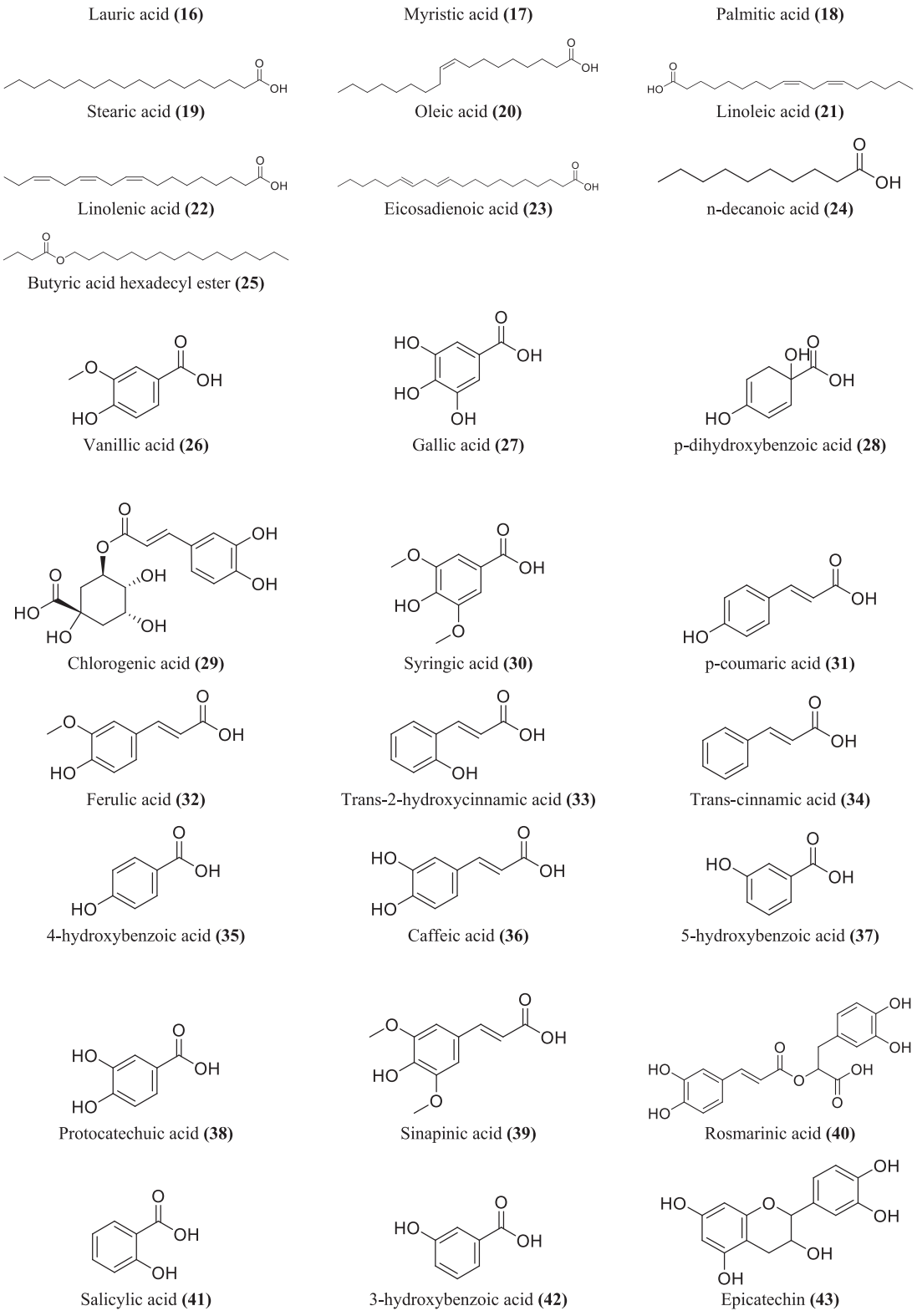


Fig. 3. (*continued*).

study, 0.5%, 1.0%, and 2.0% concentrations of NS oil were used in the agar diffusion technique against 24 spoilages, pathogenic, and lactic acid bacteria (LAB). The more effective concentration was 2.0% [131]. The lowest active concentration of NS oil was 0.5%. The concentration was not effective against *Escherichia coli, E. coli* O157:H7, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Lb. casei* ssp. *casei, Leu. pseudomesenteroides* or *Weissella paramesenteroides*. The most sensitive bacterium against all concentrations of NS oils was *Aeromonas hydrophila,* while the most resistant bacterium type was *Y. enterocolitica* (Table 7). In general, the antibacterial activity of fixed oils in NS samples against pathogenic bacteria and spoilage was higher than in LABs [131]. In another study of the antimicrobial activity of ethanol and hexane extracts of NS with the agar-well diffusion method, the extract's 1.5, 3.0, 4.5, and 6.0 mg/ml concentrations were tested and compared with

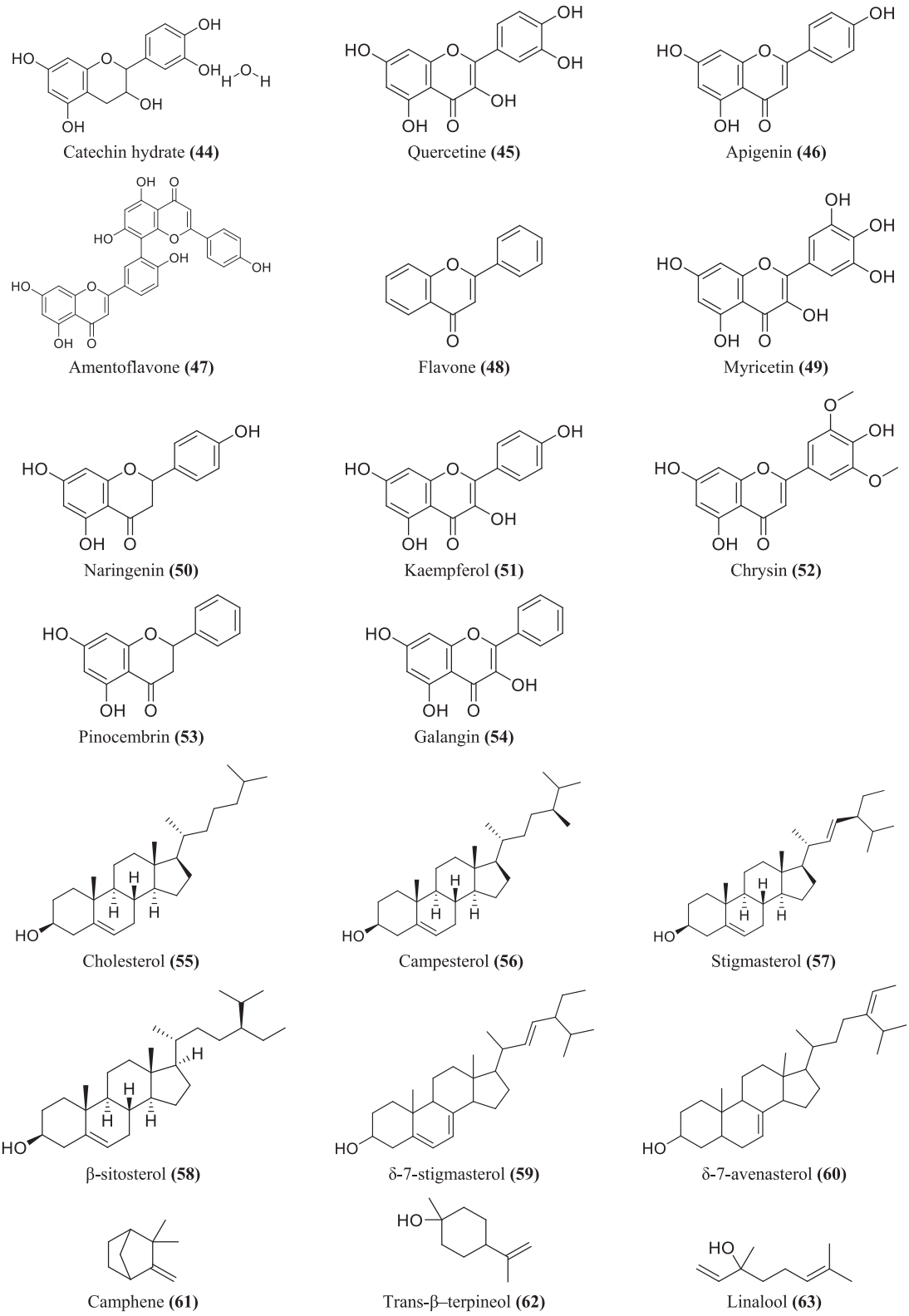
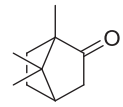


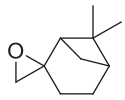
Fig. 3. (*continued*).

standard antibiotics (tetracycline, erythromycin, ciprofloxacin, and ampicillin). The ethanol extract showed an antibacterial effect against both Gram-negative and Gram-positive bacteria, and the extract showed remarkable and robust activity against *S. epidermidis, K. pneumonia* followed by *Bacillus cereus, Bacillus subtilis, E. coli,* and *Salmonella typhimurium* (Table 7). The extract showed the best effect against *B. subtilis*, followed by *B. cereus and Staphylococcus epidermidis*. The hexane extract was the most effective against *B. subtilis*, followed by *S. epidermidis* and *B. cereus* (Table 7) [132].

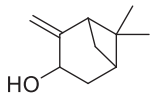
NS is also effective against Methicillin-resistant *Staphylococcus aureus* (MRSA), which is the most common pathogen encountered in laboratory practice as well as in clinics. MRSA has become a major health problem



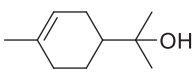
Camphor **(64)**



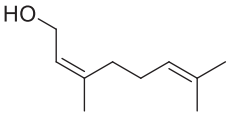
β-pinene oxide **(65)**



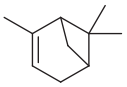
Cis-pinocarveol **(66)**



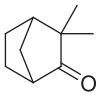
α-terpineol **(67)**



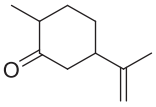
Nerol **(68)**



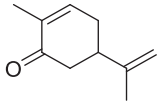
α-pinene **(69)**



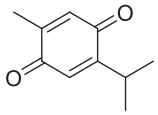
Fenchone **(70)**



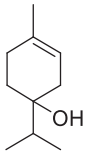
Dihydrocarvone **(71)**



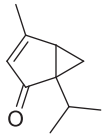
Carvone **(72)**



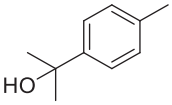
Thymoquinone **(73)**



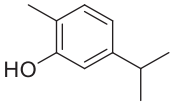
Terpinen-4-ol **(74)**



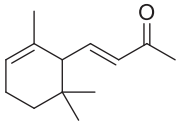
Umbellulone **(75)**



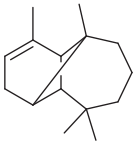
p-cymene-8-ol **(76)**



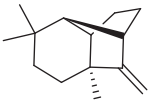
Carvacrol **(77)**



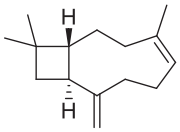
α-ionone **(78)**



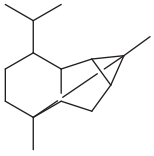
α-longipinene **(79)**



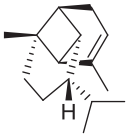
Longifolene **(80)**



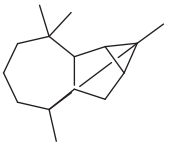
(Z)-caryophyllene **(81)**



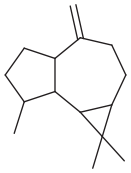
Cyclosativene **(82)**



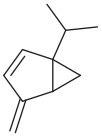
α-copaene **(83)**



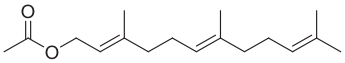
α-longicyclene **(84)**



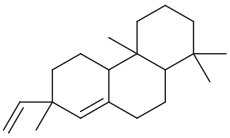
Aromadendrene **(85)**



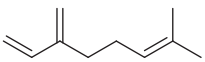
2,4,(10)-thujadiene **(86)**



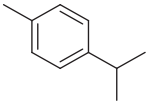
Dihydrofarnesyl acetate **(87)**



Pimaradiene **(88)**



Myrcene **(89)**



p-cymene **(90)**

Fig. 3. (*continued*).

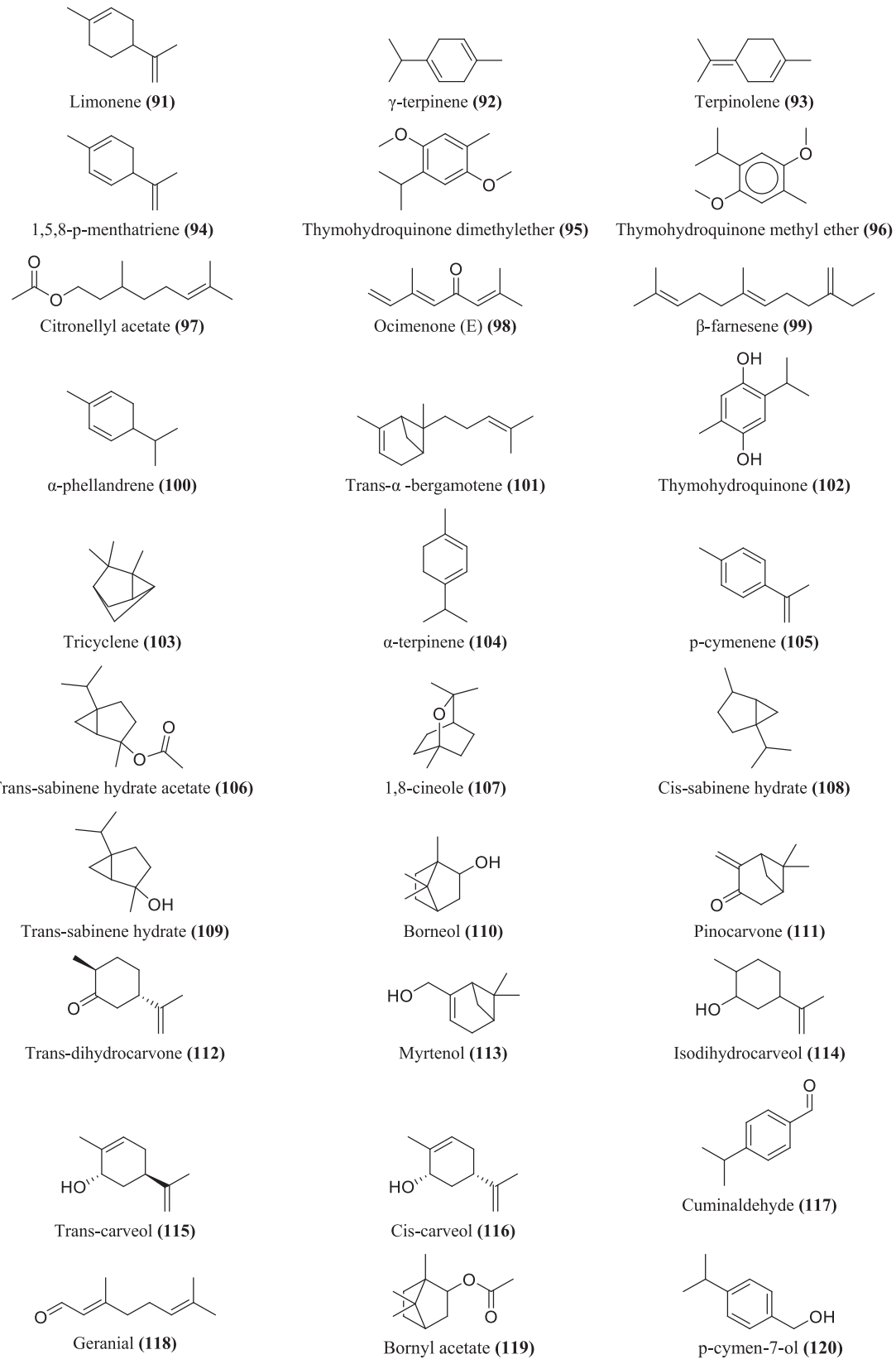


Fig. 3. (*continued*).

worldwide. There were 99 clinical isolates of MRSA and ATCC strain 25923 tested and compared with vancomycin (positive control). The study demonstrated that methanolic and chloroform NS extracts at 4 mg/disk completely inhibited MRSA strains with a significant zone size of > 12 mm. However, these strains were not inhibited at 0.5 mg/disk [133]. An ethanol extract of NS has been evaluated in vitro against *Streptococcus mutans* and *Streptococcus mitis*. In humans, these strains are known as causative agents of dental caries. The methanolic extract of NS

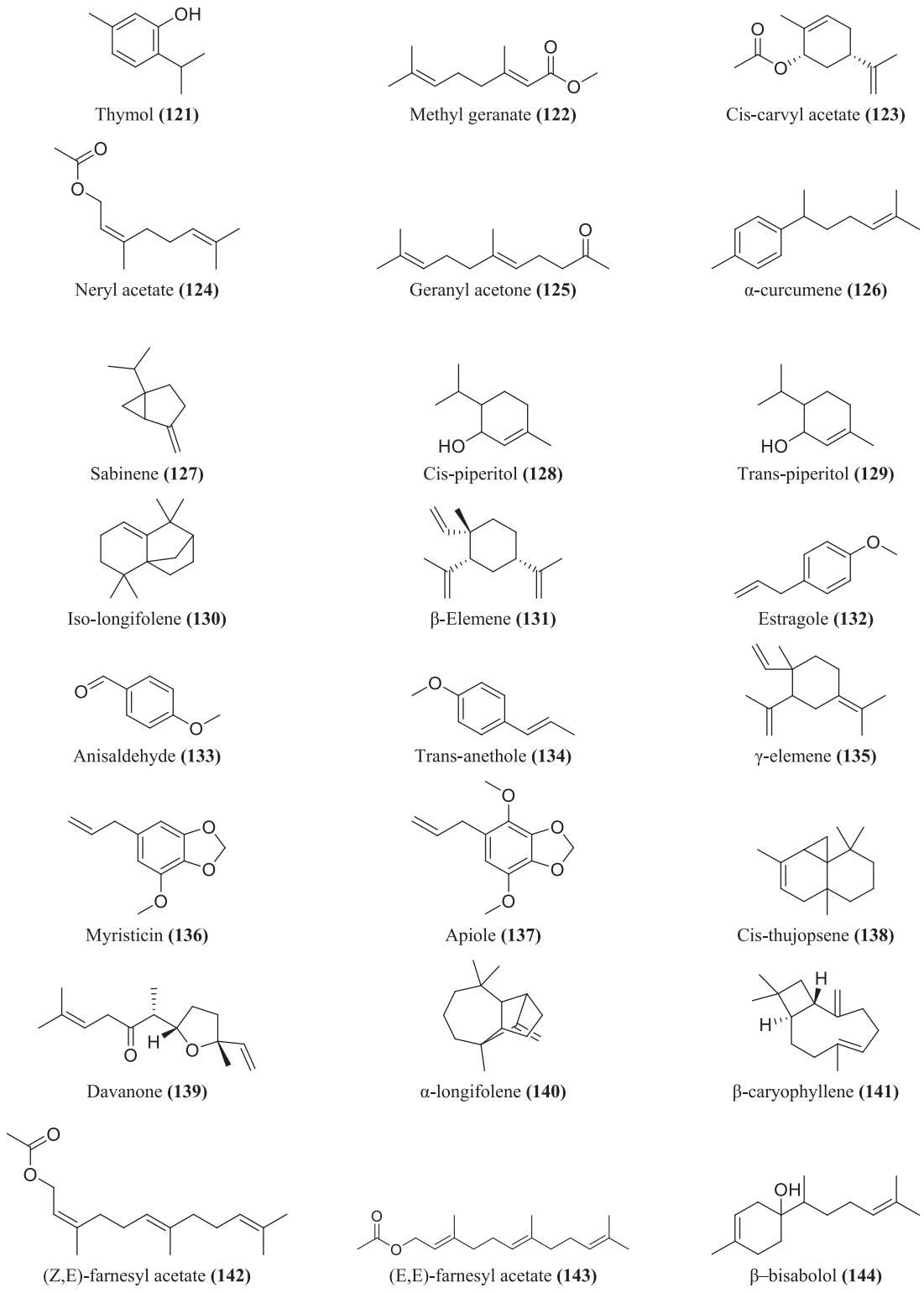


Fig. 3. (*continued*).

also showed pronounced antibacterial activity (Table 7) [134]. Another study revealed that NS was effective against antibiotic-resistant *Salmonella enterica*. Amoxicillin, chloramphenicol, tetracycline, gentamicin, and ampicillin-resistant *S. enterica* were also inhibited by methanol extract and NS seed oil in in vitro experiments [135].

The United States' Centers for Disease Control and Prevention (CDC) listed 18 bacteria as urgent threats, severe threats and concerning threats, and listed three bacteria on the watch list [148]. Among these infectious agents, *Clostridioides difficile, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, Salmonella Typhi, Enterobacteriaceae* and *Acinetobacter* as antibiotic-resistant bacteria were tested against different extracts of NS. These bacteria are responsible for various serious diseases. Fluoroquinolones-resistant *Clostridioides difficile* causes life-threatening diarrhoea. Carbapenems-resistant *Pseudomonas aeruginosa* is responsible for bloodstream, surgical site, urinary tract infections, pneumonia, and chronic lung disease [149].

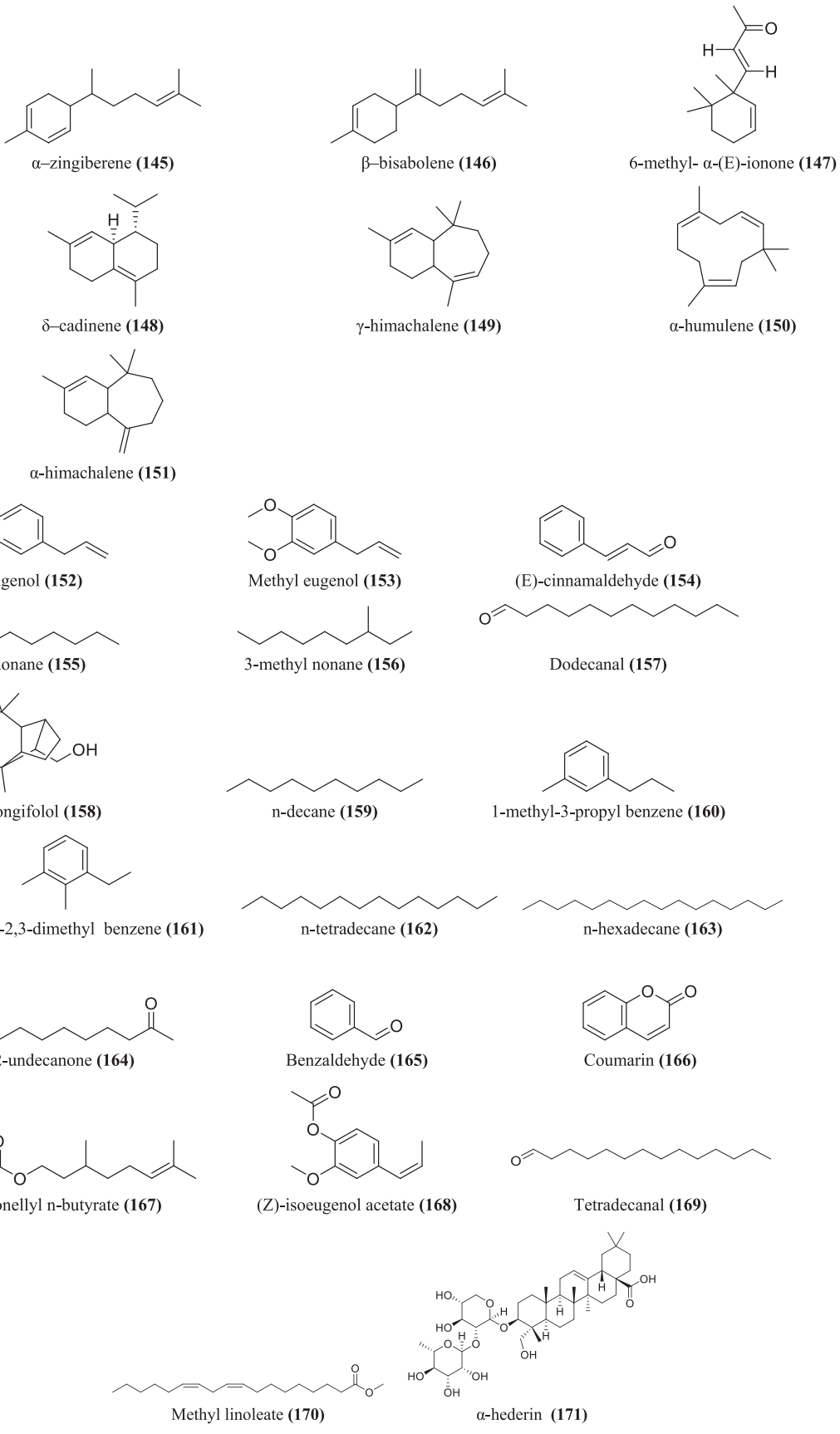


Fig. 3. (*continued*).

Table 5
Phytocompounds of *Nigella sativa*.

No.	Compound	Plant part	Solvent used/Method	Isolation and Identification	Reference
Alkaloid					
1	Nigellidine	Seed	Ethanol	TLC, CC	[91]
2	Nigellidine-4-O-sulfite	Seed	Methanol	NMR, MS, X-ray	[92]
3	Nigellicine	Seed	Chloroform	CC	[93]
4	Nigellimine	Seed	Chloroform	CC	[94]
5	Nigellamine A1	Seed	Methanol	HPLC	[95]
6	Nigellamine A2	Seed	Methanol	HPLC	[95]
7	Nigellamine A3	Seed	Methanol	HPLC	[96]
8	Nigellamine A4	Seed	Methanol	HPLC	[96]
9	Nigellamine A5	Seed	Methanol	HPLC	[96]
10	Nigellamine B1	Seed	Methanol	HPLC	[95]
11	Nigellamine B2	Seed	Methanol	HPLC	[95]
12	Nigellamine C	Seed	Methanol	HPLC	[96]
Fatty acid					
13	Methylnonadeca-15,17-dienoate	Seed	n-hexane	IR NMR, MS	[97]
14	Pentyl hexadec-12-enoate	Seed	n-hexane	IR NMR, MS	[97]
15	Pentyl pentadec-11-enoate	Seed	n-hexane	IR NMR, MS	[97]
16	Lauric acid	Seed	n-hexane	GC-MS	[98]
17	Myristic acid	Seed	n-hexane	GC-MS	[98]
18	Palmitic acid	Seed	n-hexane	GC-MS	[98]
19	Stearic acid	Seed	n-hexane	GC-MS	[98]
20	Oleic acid	Seed	n-hexane	GC-MS	[98]
21	Linoleic acid	Seed	n-hexane	GC-MS	[98]
22	Linolenic acid	Seed	n-hexane	GC-MS	[98]
23	Eicosadienoic acid	Seed	n-hexane	GC-MS	[98]
24	n-decanoic acid	Seed	MD, HD	GC-MS	[99]
25	Butyric acid hexadecyl ester	Seed	MD, HD	GC-MS	[99]
Polyphenol					
26	Vanillic acid	Root, shoot	Methanol	RP-HPLC	[100]
27	Gallic acid	Root, shoot	Methanol	RP-HPLC	[100]
28	*p*-dihydroxybenzoic acid	Roots	Methanol	RP-HPLC	[100]
29	Chlorogenic acid	Root, shoot	Methanol	RP-HPLC	[100]
30	Syringic acid	Root, shoot	Methanol	RP-HPLC	[100]
31	*p*-coumaric acid	Root	Methanol	RP-HPLC	[100]
32	Ferulic acid	Root	Methanol	RP-HPLC	[100]
33	Trans-2-hydroxycinnamic acid	Root, shoot	Methanol	RP-HPLC	[100]
34	Trans-cinnamic acid	Root, shoot	Methanol	RP-HPLC	[100]
35	4-hydroxybenzoic acid	Seed	Methanol	UHPLC/MS-MS	[101]
36	Caffeic acid	Seed	Methanol	UHPLC/MS-MS	[101]
37	5-hydroxybenzoic acid	Seed	Methanol	UHPLC/MS-MS	[101]
38	Protocatechuic acid	Seed	Methanol	UHPLC/MS-MS	[101]
39	Sinapinic acid	Seed	Methanol	UHPLC/MS-MS	[101]
40	Rosmarinic acid	Seed	Methanol	UHPLC/MS-MS	[101]
41	Salicylic acid	Seed	Methanol	UHPLC/MS-MS	[101]
42	3-hydroxybenzoic acid	Seed	Methanol	UHPLC/MS-MS	[101]
43	Epicatechin	Root, shoot	Methanol	RP-HPLC	[100]
44	Catechin hydrated	Root, shoot	Methanol	RP-HPLC	[100]
45	Quercetine	Root, shoot	Methanol	RP-HPLC	[100]
46	Apigenin	Root, shoot	Methanol	RP-HPLC	[100]
47	Amentoflavone	Root, shoot	Methanol	RP-HPLC	[100]
48	Flavone	Root, shoot	Methanol	RP-HPLC	[100]
49	Myricetin	Seed	Methanol	UHPLC/MS-MS	[101]
50	Naringenin	Seed	Methanol	UHPLC/MS-MS	[101]
51	Kaempferol	Seed	Methanol	UHPLC/MS-MS	[101]
52	Chrysin	Seed	Methanol	UHPLC/MS-MS	[101]
53	Pinocembrin	Seed	Methanol	UHPLC/MS-MS	[101]
54	Galangin	Seed	Methanol	UHPLC/MS-MS	[101]
Phytosterol					
55	Cholesterol	Seed	Hexane	GC-MS	[102]
56	Campesterol	Seed	Hexane	GC-MS	[102]
57	Stigmasterol	Seed	Hexane	GC-MS	[102]
58	β-sitosterol	Seed	Hexane	GC-MS	[102]
59	δ-7-stigmasterol	Seed	Hexane	GC-MS	[102]
60	δ-7-avenasterol	Seed	Hexane	GC-MS	[102]
Terpenes and Terpenoid					
61	Camphene	Seed	MD, HD	GC-MS	[99]
62	Trans- β –terpineol	Seed	MD, HD	GC-MS	[99]
63	Linalool	Seed	MD, HD	GC-MS	[99]
64	Camphor	Seed	MD, HD	GC-MS	[99]
65	β-pinene oxide	Seed	MD, HD	GC-MS	[99]
66	Cis-pinocarveol	Seed	MD, HD	GC-MS	[99]
67	α-terpineol	Seed	MD, HD	GC-MS	[99]
68	Nerol	Seed	HD	GC-MS	[103]
69	α-pinene	Seed	n-hexane	GC-MS	[98]

(*continued on next page*)

Table 5 (*continued*)

No.	Compound	Plant part	Solvent used/Method	Isolation and Identification	Reference
70	Fenchone	Seed	n-hexane	GC-MS	[98]
71	Dihydrocarvone	Seed	n-hexane	GC-MS	[98]
72	Carvone	Seed	n-hexane	GC-MS	[98,99]
73	Thymoquinone	Seed	n-hexane	GC-MS	[98,99,104]
74	Terpinen-4-ol	Seed	n-hexane	GC-MS	[98]
75	Umbellulone	Seed	MD, HD	GC-MS	[99]
76	*p*-cymene-8-ol	Seed	n-hexane	GC-MS	[98]
77	Carvacrol	Seed	n-hexane	GC-MS	[98]
78	α-ionone	Seed	MD, HD	GC-MS	[99]
79	α -longipinene	Seed	n-hexane	GC-MS	[98,104]
80	Longifolene	Seed	n-hexane	GC-MS	[98,99]
81	(*Z*)-caryophyllene	Seed	Sup-CO2	GC-MS	[104]
82	Cyclosativene	Seed	Sup-CO2	GC-MS	[104]
83	α -copaene	Seed	Sup-CO2	GC-MS	[104]
84	α -longicyclene	Seed	Sup-CO2	GC-MS	[104]
85	Aromadendrene	Seed	Sup-CO2	GC-MS	[104]
86	2,4,(10)-thujadiene	Seed	Sup-CO2	GC-MS	
87	Dihydrofarnesyl acetate	Seed	Sup-CO2	GC-MS	[104]
88	Pimaradiene	Seed	Sup-CO2	GC-MS	[104]
89	Myrcene	Seed	n-hexane	GC-MS	[98]
90	*p*-cymene	Seed	n-hexane	GC-MS	[98]
91	Limonene	Seed	n-hexane	GC-MS	[98,104]
92	Γ-terpinene	Seed	n-hexane	GC-MS	[98]
93	Terpinolene	Seed	Sup-CO2	GC-MS	[104]
94	1,5,8-*p*-menthatriene	Seed	Sup-CO2	GC-MS	[104]
95	Thymohydroquinone dimethylether	Seed	Sup-CO2	GC-MS	[104]
96	Thymohydroquinone methyl ether	Seed	Sup-CO2	GC-MS	[104]
97	Citronellyl acetate	Seed	Sup-CO2	GC-MS	[104]
98	Ocimenone (E)	Seed	Sup-CO2	GC-MS	[104]
99	β-farnesene	Seed	MD, HD	GC-MS	[99]
100	α -phellandrene	Seed	n-hexane	GC-MS	[98]
101	Trans-α-bergamotene	Seed	MD, HD	GC-MS	[99]
102	Thymohydroquinone	Seed	MD, HD	GC-MS	[99]
103	Tricyclene	Seed	Sup-CO2	GC-MS	[104]
104	α-terpinene	Seed	Sup-CO2	GC-MS	[104]
105	*p*-cymenene	Seed	MD, HD	GC-MS	[99]
106	Trans-sabinene hydrate acetate	Seed	MD, HD	GC-MS	[99]
107	1,8-cineole	Seed	Sup-CO2	GC-MS	[104]
108	Cis-sabinene hydrate	Seed	Sup-CO2	GC-MS	[104]
109	Trans-sabinene hydrate	Seed	Sup-CO2	GC-MS	[104]
110	Borneol	Seed	Sup-CO2	GC-MS	[104]
111	Pinocarvone	Seed	Sup-CO2	GC-MS	[104]
112	Trans-dihydrocarvone	Seed	Sup-CO2	GC-MS	[104]
113	Myrtenol	Seed	MD, HD	GC-MS	[99]
114	Iso-dihydrocarveol	Seed	MD, HD	GC-MS	[99]
115	Trans-carveol	Seed	MD, HD	GC-MS	[99]
116	Cis-carveol	Seed	MD, HD	GC-MS	[99]
117	Cuminaldehyde	Seed	MD, HD	GC-MS	[99]
118	Geranial	Seed	MD, HD	GC-MS	[99]
119	Bornyl acetate	Seed	MD, HD	GC-MS	[99]
120	*p*-cymen-7-ol	Seed	MD, HD	GC-MS	[99]
121	Thymol	Seed	MD, HD	GC-MS	[99]
122	Methyl geranate	Seed	MD, HD	GC-MS	[99]
123	Cis-carvyl acetate	Seed	MD, HD	GC-MS	[99]
124	Neryl acetate	Seed	MD, HD	GC-MS	[99]
125	Geranyl acetone	Seed	MD, HD	GC-MS	[99]
126	α -curcumene	Seed	MD, HD	GC-MS	[99]
127	Sabinene	Seed	n-hexane	GC-MS	[98,104]
128	Cis-piperitol	Seed	MD, HD	GC-MS	[99]
129	Trans-piperitol	Seed	MD, HD	GC-MS	[99]
130	Iso-longifolene	Seed	MD, HD	GC-MS	[99]
131	β-Elemene	Seed	MD, HD	GC-MS	[99]
132	Estragole	Seed	n-hexane	GC-MS	[98]
133	Anisaldehyde	Seed	n-hexane	GC-MS	[98]
134	Trans-anethole	Seed	n-hexane	GC-MS	[98]
135	γ-Elemene	Seed	MD, HD	GC-MS	[99]
136	Myristicin	Seed	n-hexane	GC-MS	[98]
137	Apiole	Seed	n-hexane	GC-MS	[98]
138	Cis-thujopsene	Seed	MD, HD	GC-MS	[99]
139	Davanone	Seed	Sup-CO2	GC-MS	[104]
140	α-longifolene	Seed	Sup-CO2	GC-MS	[104]
141	β-caryophyllene	Seed	Sup-CO2	GC-MS	[104]
142	(*Z*,*E*)-farnesyl acetate	Seed	MD, HD	GC-MS	[99]
143	(E,E)-farnesyl acetate	Seed	MD, HD	GC-MS	[99]
144	β -bisabolol	Seed	MD, HD	GC-MS	[99]
145	α-zingiberene	Seed	MD, HD	GC-MS	[99]

(*continued on next page*)

Table 5 (*continued*)

No.	Compound	Plant part	Solvent used/Method	Isolation and Identification	Reference
146	β -bisabolene	Seed	MD, HD	GC-MS	[99]
147	6-methyl- α-(*E*)-ionone	Seed	MD, HD	GC-MS	[99]
148	δ-cadinene	Seed	MD, HD	GC-MS	[99]
149	γ-himachalene	Seed	MD, HD	GC-MS	[99]
150	α-humulene	Seed	MD, HD	GC-MS	[99]
151	α-himachalene	Seed	MD, HD	GC-MS	[99]
Others					
152	Eugenol	Seed	MD, HD	GC-MS	[99]
153	Methyl eugenol	Seed	MD, HD	GC-MS	[99]
154	(*E*)-cinnamaldehyde	Seed	MD, HD	GC-MS	[99]
155	n-nonane	Seed	n-hexane	GC-MS	[99]
156	3-methyl nonane	Seed	n-hexane	GC-MS	[98]
157	Dodecanal	Seed	MD, HD	GC-MS	[99]
158	Iso-longifolol	Seed	MD, HD	GC-MS	[99]
159	n-decane	Seed	n-hexane	GC-MS	[98]
160	1-methyl-3-propyl benzene	Seed	n-hexane	GC-MS	[98]
161	1-ethyl-2,3-dimethyl benzene	Seed	n-hexane	GC-MS	[98]
162	n-tetradecane	Seed	n-hexane	GC-MS	[98]
163	n-hexadecane	Seed	n-hexane	GC-MS	[98]
164	2-undecanone	Seed	Sup-CO2	GC-MS	[104]
165	Benzaldehyde	Seed	MD, HD	GC-MS	[99]
166	Coumarin	Seed	MD, HD	GC-MS	[99]
167	Citronellyl n-butyrate	Seed	MD, HD	GC-MS	[99]
168	(*Z*)-isoeugenol acetate	Seed	MD, HD	GC-MS	[99]
169	Tetradecanal	Seed	MD, HD	GC-MS	[99]
170	Methyl linoleate	Seed	MD, HD	GC-MS	[99]
171	α-hederin	Seed	Ethyl acetate	CC5	[105]

CC: Column chromatography, HPLC: High performance liquid chromatography; GC-MS: Gas Chromatography-Mass Spectrometry, MD: Microwave distillation, HD: Hydro-distillation, TLC: Thin layer chromatography, Sup-CO2: Super critical carbon dioxide, IR: Infrared spectroscopy, NMR: Nuclear magnetic resonance, MS: Mass Spectrometry, UHPLC/MS-MS: Ultra HPLC/MS-MS, RP-HPLC: Reversed-phase HPLC.

Table 6
Antimicrobial phytoconstituents of *Nigella sativa*.

No.	Compound	Antimicrobial activity	Other pharmacological activities
1.	Thymoquinone	Antibacterial [106,107], Antidermatophytic activity [108,109], Antifungal [110], Act as putative efflux pump inhibitors in Listeria monocytogenes [111]	Antiinflammatory [112, 113], anticancer, antitumor [114], Anticonvulsant effect [115], antileishmanial effects [116], anti-proliferative effect [117], Effective against HEp-2 laryngeal cancer cells [85], anti-inflammatory activities of TQ in PDA (pancreatic ductal adenocarcinoma) cells, [118], TQ as an inducer of cell cycle arrest and apoptosis in human colon cancer HCT-116 cells [119], Antioxidation activity [120], Antinociceptive effects [121]
2.	α-pinene	Bactericides [122]	Insect repellents [90]
3.	*p*-cymene	Antimicrobial [123], Bactericides [122]	Insect repellents [90]
4.	Longifolene		Insect repellents [90]
5.	Thymol	Antifungal [110]	Anti-inflammatory [113]
6.	Carvacrol	Act as putative efflux pump inhibitors in *Listeria monocytogenes* [111]	-
7.	Thymohydroquinone	Antibacterial activity [107], Antifungal activity [110]	-

Streptococcus pneumonia shows resistance to various antibiotics and is responsible for bacterial pneumonia, meningitis, bloodstream infections, and ear and sinus infections. NS essential oil and other phytochemicals (TQ, *p*-cymene, carvacrol) can modify the resistance potentiality of AMR bacteria and become sensitive. NS could be an excellent source of new antibacterial agents to achieve AMR stewardship.

6.1.1. *Mode of actions against bacteria*

Gram-positive and Gram-negative bacteria have multidrug efflux pumps (EPs), which assist bacterial populations to enhance their immunity and survive when exposed to antimicrobial substances. The leading cause of MDR bacteria is EPs, a transporter of proteins involved in the removal of drugs and toxic substances to the external environment from the interior of the cell (Fig. 5a) [150,151]. In *Listeria monocytogenes*, two efflux pumps have been identified [152]. One efflux pump called MdrL extrudes macrolides, cefotaxime, heavy metals, and EtBr. The other, Lde, extrudes fluoroquinolone, acridine orange, and EtBr [153,154]. NS oil, TQ, carvacrol, and its active compounds are efficient modulators of antibiotic resistance in L. *monocytogenes*. TQ and essential oil inhibit efflux pumps, increase the accumulation of antibiotics in the bacterial cells, and at lower doses, enhance their effectiveness [23]. TQ also intervenes in its antibacterial effect by producing ROS, prompting oxidative stress and cell apoptosis (Fig. 5b) [22]. On the other hand, *p*-cymene has no antimicrobial activity. However, it increases the membrane permeability, thereby promoting the influx of antimicrobial substances [23].

6.2. *Antiviral and potential anti-SARS-CoV-2 effects with the mode of actions*

NS increases the helper T cell (T4 and T8) suppressor ratio and natural killer cells (NK) cell activity. It results in improved immunity, so shows a suppressing effect against human immune deficiency virus protease [44]. NS exhibits anti-influenza virus activity. It plays a pivotal role in diminishing the pathogenic effects by inducing efficient immune

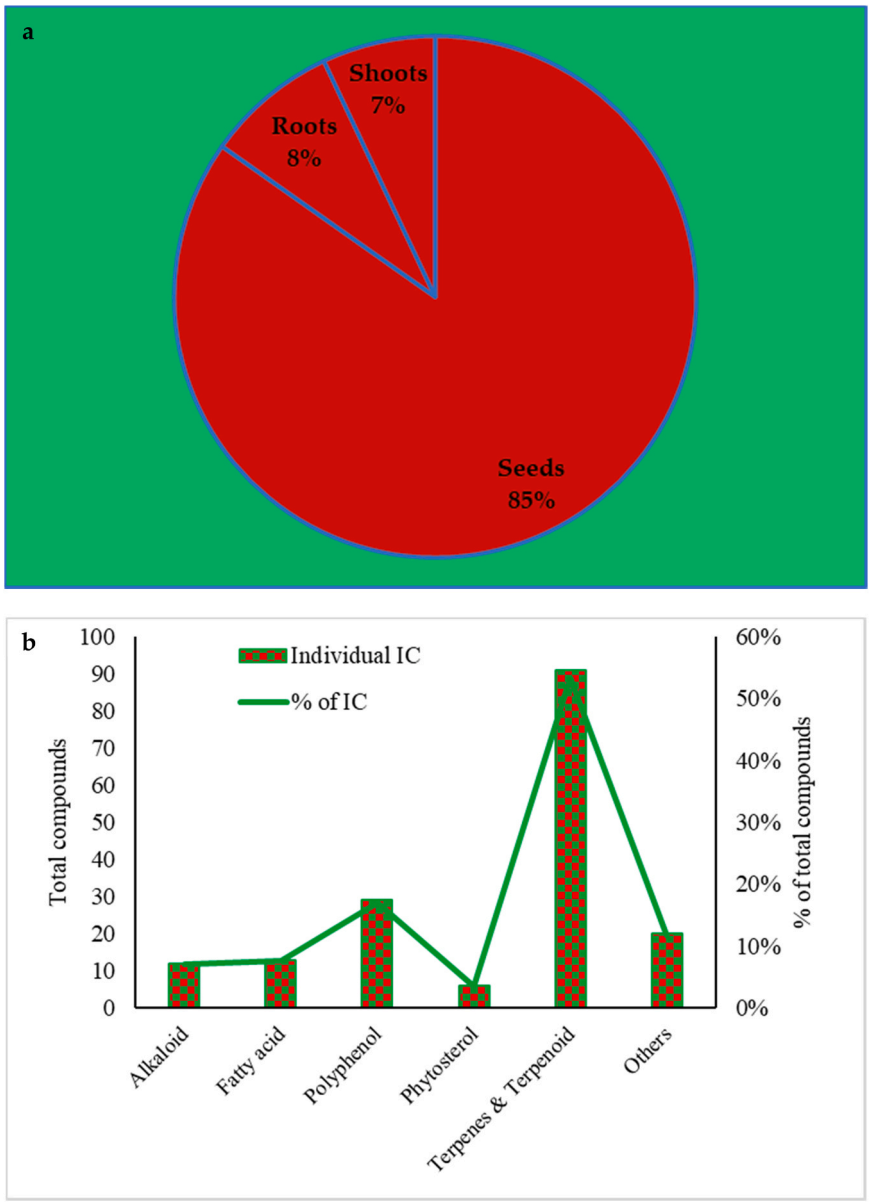


Fig. 4. Phytoconstituents of *Nigella sativa*. (a) shows different parts of *N. sativa* that are used for the isolation of compounds, (b) shows types of identified compounds (IC) and the percent value of each type of identified compound.

responses [155]. Patients suffering from hepatitis C virus (HCV) infection exhibited notable improvement in HCV viral load using oil extracted from NS seed [69]. Regaining and seroreversion of HIV-positive patients can be achieved by using NS seeds [156]. NS oil potentially binds with murine cytomegalovirus that showed inhibition of viral growth in a rat model [157]. The ethanolic extracts of NS successfully reduced the viral load and death in embryonic chicken eggs against the Newcastle disease virus [158]. Recent studies revealed that NS has a high potential as a herbal antiviral agent against novel coronavirus [159]. DL-Arabinose is an important phytochemical found in NS with effective antiviral activity [160].

A recent study suggested that NS could be considered a natural substitute for chloroquine because it contains several bioactive components such as TQ, dithymoquinone, thymohydroquinone, and nigellimine activity, augmented by a Zn supplement [161]. Another *in-silico* study by molecular docking suggested that nigerlidine and α-hederin are the main compounds from NS that can prevent COVID-19 with the same or better score than drugs under clinical trials [162]. In another *in-silico* study, nigellidine showed the highest binding affinity to N terminus protease (−7.61) and nucleocapsid and the main protease necessary for viral maturation in protein structures, RNA packaging, and other functions. Moreover, binding to IL1 and TNF-α receptors may reduce cytokine storm after viral infection [24]. α-hederin (−6.265 kcal/mol) and thymohydroquinone (−5.466 kcal/mol) are identified as suitable binding ligands to ACE2 receptors in the human system responsible for virus entry [25]. Among other compounds, dithymoquinone (DTQ), also known as nigellone, shows a binding affinity of − 8.6 kcal/mol compared to a positive control (chloroquine, −7.2 kcal/mol), and has the promising ability of binding at the SARS-CoV-2:ACE2 interface to disrupt viral host interaction [159].

Oral administration of honey with NS reduced the severity of clinical symptoms, earlier viral clearance, and reduced mortality in COVID-19 patients [163]. Taibah University started a novel evidence-based approach, "TaibUVID", for COVID-19 treatment. A single TaibUVID dose includes one large spoonful of *Nigella sativa* oil (or 2 g of *Nigella sativa* seeds) mixed with one gram of ground anthemis hyaline and one large spoonful of natural honey. This mixture is to be chewed in the mouth and swallowed orally for COVID-19 close contacts and patients. It

Table 7
In vitro antibacterial activity of *Nigella sativa* extracts.

Plant part	Extract	No. test MOs	Inhibited MOs	Type	MEIC	MIC	ZOI (mm or %)	Remarks	Reference
Seed	n-H	24	*A. hydrophila* ATCC 7965*	-ve	2%	ND	37.5 mm		[131]
			B. cereus FMC 19	+ve			29.5 mm		
			B. subtilis IMG 22	+ve			27 mm		
			Corynebacterium xerosis UC 9165	+ve			32.5 mm		
			Enterobacter aerogenes CCM 2531	-ve			23.6 mm		
			Enterococcus faecalis ATCC 15753	+ve			19.5 mm		
			E. coli DM	-ve			19.3 mm		
			E. coli O157:H7 KUEN 1461	-ve			24 mm		
			K. pneumoniae FMC 5	-ve			18.8 mm		
			Listeria monocytogenes Scott A	+ve			21.5 mm		
			Mycobacterium smegmatis RUT	+ve			32.2 mm		
			Proteus vulgaris FMC 1	-ve			35 mm		
			P. aeruginosa ATCC 27853	-ve			21.2 mm		
			Pseudomonas fluorescens EU	-ve			21.4 mm		
			S. typhimurium	-ve			24.4 mm		
			S. aureus Cowan 1	-ve			29.5 mm		
			Y. enterocolitica EU	+ve			16.5 mm		
			Streptococcus salivarius	+ve			24 mm		
			Lactobacillus delbrueckii	+ve			24.5 mm		
			Lb. casei ssp. casei K64	+ve			20.8 mm		
			Lactobacillus. Paracasei	+ve			21 mm		
			Leu. pseudomesenteroides E83	+ve			19.3 mm		
			Leuconostoc gelidum E26	+ve			19.6 mm		
			Weissella paramesenteroides E95	+ve			23.3 mm		
Seed	E	8	*S. epidermidis**	+ve	6 mg/ml	ND	15 mm	NoA against *P. aerogenosa* and *E. aerogens*	[132]
			K. pneumonia	-ve			ND		
			B. cereus	+ve			8.0 mm		
			B. subtilis	+ve			7.0 mm		
			E. coli	-ve			ND		
			S. typhimurium	-ve			9.0 mm		
	n-H		*S. epidermidis*	+ve			19 mm	NoA against *P. aerogenosa, E. aerogens, E. coli* and *S. typhimurium*	
			K. pneumonia	-ve			ND		
			B. cereus	+ve			11 mm		
			B. subtilis	+ve			26 mm		
Seed	$CHCl_3$, M	99	*S. aureus*	+ve	4 mg/disc	0.2–0.5 mg/ml	> 12 mm		[133]
Seed	M	2	*S. mutans*	+ve	ND	ND	12.7 mm		[134]
			S. mitis	+ve			10.4 mm		
Seed	EO	1	*S. enetrica*	-ve	ND	> 1000±322.7 μg/ml	20–14 mm		[135]
	M		*S. enetrica*	-ve	ND	≥ 562.5 μg/ml	35–17 mm		
Seed	M	5	*S. aureus**	+ve	100 mg/ml	ND	25 mm	NoA against*E. coli, K. pneumonia* and *B. cereus*	[136]
			P. aeruginosa	-ve			21 mm		
Seed	$CHCl_3$	6	*S. aureus* ATCC 103207	+ve	18 mg/ml	ND	19 mm	NoA against*V. cholerae El Tor* and *K. pneumoniae ATCC 13883*	[137]
			B. cereus ATCC 6623	+ve			16 mm		
			B. subtilis ATCC 27853	+ve			26 mm		
			Vibrio cholerae El Tor	-ve			20 mm		
			S. aureus ATCC 103207	+ve			26 mm		
			B. cereus ATCC 6623	+ve			25 mm		
	E		*B. subtilis* ATCC 27853	+ve			28 mm	NoA against *K. pneumoniae ATCC 13883*	
			E. coli ATCC 12079	-ve			21 mm		
			V. cholerae El Tor	-ve			24 mm		
Seed	E	4	*B. subtilis*	+ve	ND	12.5 mg/ml	18 mm		[29]
			S. aureus	+ve			16 mm		
			E. coli	-ve			20 mm		
			P. aeruginosa	-ve			16 mm		
Seed	M	2	*S. mutans**	+ve	ND	0.2 mg /ml	22.3 mm		[138]

(*continued on next page*)

Table 7 (*continued*)

Plant part	Extract	No. test MOs	Inhibited MOs	Type	MEIC	MIC	ZOI (mm or %)	Remarks	Reference
			Lactobacillus acidophilus	+ve		05 mg/ml	18.6 mm		
	H_2O		*S. mutans**	+ve		0.3 mg /ml	15.3 mm		
			L. acidophilus	+ve		0.3 mg/ml	12.3 mm		
Seed	EA	2	*S. aureus**	+ve	ND	62.5 µg/ml	10.6 mm		[139]
			Propionibacterium acnes	+ve		ND	9.0 mm		
Seed	EO	1	*Chlamydia trachomatis D*	-ve	ND	6.25 and 3.12 µM	ND	MIC of carvacro, thymol was 6.25 and *p*-cymene, TQ was 3.12 µM	[140]
Seed	DEE	4	*S. aureus*	+ve	25–400 µg	ND	21 mm	*S. typhimurium* was resistant	[141]
			E. coli	-ve	/disc		25 mm		
			P. aeruginosa	-ve			25 mm		
Seed	M	2	*E. coli*	-ve	1.2 g/kg	ND	100 %		[142]
			S. aureus	+ve	2.14 g/kg		87.5 %		
	$CHCl_3$		*E. coli*	-ve	2.6 mg/kg		100 %		
			S. aureus	+ve	2.6 mg/kg		100 %		
	EO		*E. coli*	-ve	0.3 g/kg		100 %		
			S. aureus	+ve	0.3 g/kg		100 %		
Seed, fruit	cH_2O	5	*E. coli**	-ve	ND	ND	23 mm		[143]
			P. aeruginosa	-ve			23 mm		
			Salmonella typhi	-ve			23 mm		
			E. faecalis	+ve			23 mm		
	hH_2O		*E. coli**	-ve			22 mm		
			P. aeruginosa	-ve			22 mm		
			Salmonella typhae	-ve			22 mm		
			E. faecalis	+ve			22 mm		
	M		*E. coli**	-ve			23 mm	NoA against *S. aureus*	
			P. aeruginosa	-ve			23 mm		
			Salmonella typhae	-ve			23 mm		
			E. faecalis	+ve			23 mm		
Seed	M	6	*E. coli**	-ve	ND	1.125 mg/ml	ND	NoA against *B. cereus*	[144]
			P. aeruginosa	-ve		1.125 mg/ml			
			*S. aureus**	+ve		0.56 mg/ml			
			S. typhi	-ve		1.125 mg/ml			
			B. subtilis	+ve		1.125 mg/ml			
	E		*P. aurigenisa*	-ve		1.125 mg/m		NoA against *E. coli*	
			S. aureus	+ve		0.56 mg/ml			
			*S. typhi**	-ve		1.125 mg/ml			
			B. cereus	+ve		1.125 mg/ml			
			B. subtilis	+ve		1.125 mg/ml			
TQ	DMSO, H_2O	4	*Clostridium difficile**	+ve	160 µg/ml	10–40 µg/ml	ND		[145]
			Clostridium perfringens	+ve		80–160 µg/ml			
			Bacteroides fragilis	-ve		80–160 µg/ml			
			Bacteroides thetaiotaomicron	-ve		80–160 µg/ml			
Seed	M	2	*Porphyromonas gingivalis*	-ve	50 mg/ml	25 mg/ml	5.4 mm		[146]
			Prevotella intermedia	-ve		25 mg/ml	10.1 mm		
Seed	E	1	*Streptococcus pyogenes*	+ve	ND	12.5 mg/ml	6.33 mm		[30]
Plant powder	E	3	*K. pneumoniae*	-ve	50 mg/100 ml	ND	14 mm		[32]
			*Acinetobacter baumannii**	-ve			17 mm		
			P. aeruginosa	-ve			11 mm		
Seed	M	4	*S. pyogenes**	+ve	50 mg/ml	ND	19 mm		[147]
			P. aeruginosa	-ve			15 mm		
			K. pneumoniae	-ve			15 mm		
			P. vulgaris	-ve			15 mm		

*Antibiotic resistant species, ǂIndirect immunofluorescence assay. Other test assays were disc diffusion method; MEIC: Most effective inhibitory concentration; MIC: Minimum inhibitory concentration; MOs: Number of test microorganisms; ND: Not determined; ZOI: Zone of Inhibition, DMSO: Dimethylsulfoxide, EO: Essential Oil, EA: Ethyl acetate, TQ: Thymoquinone, $CHCl_3$: Chloroform, cH_2O: Cold water, hH_2O: Hot water, M: Methanol, E: Ethanol, DEE: Diethyl ether, NoA: No activity, +ve: Positive bacteria, -ve: Negative bacteria.

is a promising evidence-based approach to decrease fatalities and rapidly end the COVID-19 pandemic [164].

6.3. Antifungal effects with the mode of actions

NS has an inhibitory effect against different pathogenic yeasts (Table 8). The presence of β-sitosterol and oleic acid in the oil composition of NS is considered to be responsible for its antifungal activity [165]. In addition, S. Gupta [166] stated that different components of NS oil, such as stigmasterol and β-sitosterol, show antifungal properties against some pathogenic yeasts such as *Candida tropicalis, Candida albicans,* and *Geotrichum candidum*. An in vivo study in mice showed an antifungal activity of NS seeds' aqueous extract against *Candida albicans* responsible for candidiasis [167]. Another study [168] showed that the candidacidal pathway in mice neutrophils is nitric oxide (NO)-dependent. There is a possibility that the active ingredient(s) of the plant extract may stimulate granulocytes and monocytes to form nitric oxide, leading to excellent antifungal activity, which in turn kills *C. albicans* [169]. The

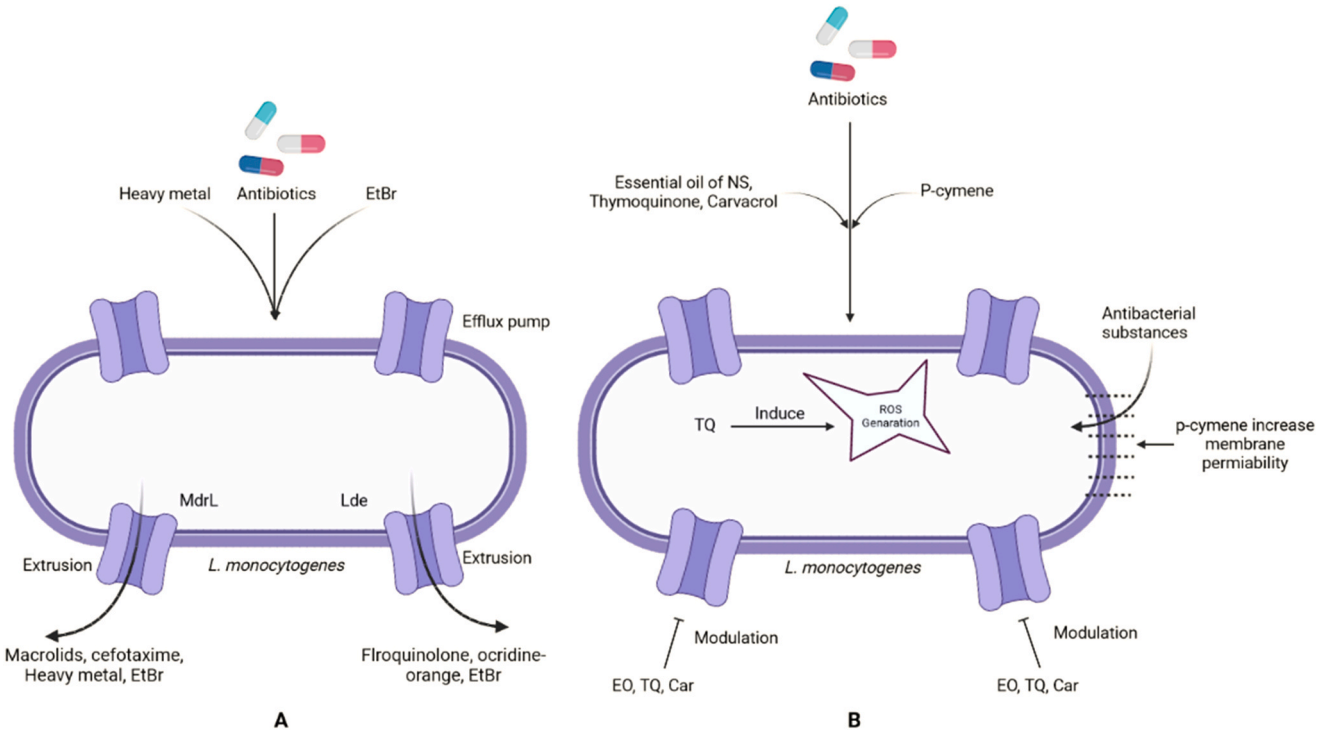


Fig. 5. Mechanism of antibiotic resistance modulation by *Nigella sativa*. A) L. *monocytogenes* extrude macrolides, cefotaxime, heavy metals, EtBr and MdrL and fluoroquinolone, acridine orange, EtBr by Lde efflux pump on the bacterial cell surface. B) EO, TQ, Car modulate MdrL and Lde efflux pump and inhibit to extrude antibiotics. TQ also increases ROS generation, which prompts oxidative stress and cell demise. *P*-cymene increases the membrane permeability and increasing the influx of antimicrobial substances. EtBr: Ethidium Bromide, EO: Essential oil, TQ: thymoquinone, Car: carvacrol.

Table 8
Antifungal activity of *Nigella sativa*.

	Fungus	Extract	Method	MIC	Reference
Pathogenic yeasts	*C. albicans*	Essential oil	BM	2300 μg/ml	[171]
		Essential oil	BM	IC_{90}: 4.916 mg/ml	[165]
	C. dubliniensis	Essential oil	BM	IC_{90}: 5.183 mg/ml	[165]
	C. glabrata	Essential oil	BM	IC_{90}: 5.992 mg/ml	[165]
	C. krusei	Essential oil	BM	IC_{90}: 4.939 mg/ml	[165]
Dermatophytes	*Trichophyton mentagrophytes*	Essential oil	BM	2 ± 0.6 mg/ml	[172]
		Ether	ADD	40 mg/ml	[173]
		Methanol	BM	8 mg/ml	[108,123]
		Aqueous	BM	IC_{50}: 16 mg/ml	[108]
	Trichophyton rubrum	Essential oil	BM	4 ± 1.1 mg/ml	[172]
		Ether	ADD	40 mg/ml	[173]
	Epidermophyton floccosum	Essential oil	BM	2 ± 0.6 mg/ml	[172]
		Ether	ADD	40 mg/ml	[173]
	Microsporum gypseum	Essential oil	BM	2 ± 0.6 mg/ml	[172]
		Methanol	BM	IC_{50}: 4 mg/ml	[108]
		Aqueous	BM	IC_{50}: 8 mg/ml	[108]
	Microsporum canis	Essential oil	BM	4 ± 1.1 mg/ml	[172]
		Methanol	BM	IC_{50}: 4 mg/ml	[108]
		Aqueous	BM	IC_{50}: 8 mg/ml	[108]
		Ether	ADD	10 mg/ml	[173]
	Trichophyton interdigitale	Ether	ADD	40 mg/ml	[173]
Non-dermatophyte filamentous fungi	*Aspergillus fumigatus*	Essential oil	BM	IC_{90}: 1.5 mg/ml	[174]
			BM	IC_{90}: 1.5 mg/ml	
	A. flavus	Essential oil	BM	IC_{90}: 1.5 mg/ml	[174]
			BM	IC_{90}: 2 mg/ml	

BM: Broth-Macrodilution, ADD: Agar Disk Diffusion, MIC: Minimum Inhibitory Concentration, IC_{50}: Half-maximal inhibitory concentration, IC_{90}: Ninety percent-maximal inhibitory concentration.

anti-yeast activity of NS seed extract is maximised at pH 7 and 30 °C [170].

The essential oil of NS possesses higher antidermatophytic activity [175]. Furthermore, an ether extract of NS seed and its active component TQ showed some antifungal activity against dermatophytes: four species of *Trichophyton rubrum* and one each of *T. interdigitale*, *T. mentagrophytes*, *Microsporum cani*, and *Epidermophyton floccosum.* The minimum inhibitory concentration (MIC) of the ether extract of NS was between 10 and 40 mg/ml, while that of TQ was 0.125 and 0.25 mg/ml, inhibiting 80–100% fungal growth. [176]. NS oil exhibited significant effectiveness against non-dermatophytic filamentous fungi such as *Drechslera hawiinesis, Alternaria alternate,* and *F. moniliforme,* which were

inhibited entirely at 0.1% and 0.15% concentration [177].

In a study, NS showed maximum inhibition (76.1%) at 10% concentration against *Macrophomina phaseolina*, one of the most damaging phytopathogenic fungi causing charcoal rot disease (Iqbal et al., 2014). Ns-D1 and Ns-D2 are two novel antifungal defensins, isolated from seeds of NS, that exhibit intense divergent antifungal activity towards several phytopathogenic fungi [178].

The essential oil of NS (1.5 mg/ml) inhibits the production of mycelia (67.4%) and aflatoxin in *Aspergillus parasiticus* [26]. A study by Khosravi indicated that when *Aspergillus flavus* and *Aspergillus fumigates* were exposed to NS oils (2 mg/ml), they exhibited detachment of the fibrilar layer of the cell wall. The researcher also observed that the plasma membrane was separated from the cell wall, the cytoplasm was disrupted, and subsequently the cytoplasm was vacuolated, before the vacuole fused. Overall, oil from NS showed moderate to weak activity against aflatoxin-producing fungi [179].

6.4. Anti-parasitic activity with the mode of actions

Studies have indicated that NS possesses significant anti-parasitic (anti-helminths, anticestodals, and anti-schistosomal) properties. NS seeds showed significant anticestodal activities. These easy-to-grow and affordable indigenous plant-based drugs are safe agents for treating children with roundworm and tapeworm infestations. The most effective oral doses were 40 and 50 mg per kg of body weight with no serious adverse effects [180]. The methanolic extract of NS seeds (1250 mg/kg) suppressed *Plasmodium yoelii* infection at a suppression rate of 94%, whereas the most effective synthetic drug, chloroquinone, had an 86% suppression rate. A 400 mg/kg of aqueous suspensions and oil emulsions of NS seeds were used to treat coccidiosis in rabbits. The emulsion has higher concentrations of alkaloid nigellicine that has a deadly influence on parasites [181]. NS oil effectively reduced the number of *Schistosoma mansoni* worms in the liver and reduced the total number of ova that accumulated in both the intestine and the liver. It has also been shown to be effective against other helminths such as *Hymenolepis nana* [182]. NS was tested against cercariae, miracidia, and adult worms of *Schistosoma mansoni*. It has demonstrated strong biocidal activity against all the stages of the parasite as well as an effect on the egg-laying activity of the adult female worms [28]. However, the molecular mechanisms of biocidal action of NS against parasites are yet be investigated.

7. Safety and toxicity

TQ is the main constituent of the volatile oil of NS seeds. Its seed extract and its constituents appear to have a low level of toxicity [31]. After oral administration of TQ, the demonstrated toxicity level was negligible. Oral TQ is bio-transformed into more minor toxic metabolites in the gastrointestinal tract or metabolized in the liver into dihydro-thymoquinone. After administration, the toxicity increases due to the complete absorption of TQ into the systemic circulation [183]. The treatment of male albino rats with an intraperitoneal administration of phosphate-buffered saline (PBS) or 200 mg/kg cyclophosphamide and then intragastric administration of NS oil or TQ on alternate days for 12 days, initiated 6 h before or after a cyclophosphamide injection, showed that the treatment with cyclophosphamide induced significant toxicity while treatment with NS oil or TQ induced a significant reduction in overall toxicity [184]. The high values of oral and intraperitoneal lethal doses of NS fixed oil at 28.8 ml/kg of body weight show its low acute toxicity [185]. No toxicity was observed when NS oil was administered in different doses up to 10 ml/kg of body weight [186].

The acute toxicity of TQ is relatively low, and it is well-tolerated, generally, when given sub-chronically in drinking water at doses that are about 12 times the cytoprotective dose [187]. Another study involved oral administration of the aqueous, chloroform, and methanol extracts of NS seeds, in 6, 9, 14, and 21 g/kg. The methanol extracts in all four doses and chloroform extract at 21 g/kg significantly decreased animals' weight. The study of the hepatic toxicity of the extracts at 6 g/kg/day for 14 consecutive days, as well as a hepatic histological study, revealed no abnormal activity of ALP, SGPT, and SGOT in blood [188]. The supplementation of NS up to 1 g/kg for 28 days produced no changes in liver enzyme level and no toxicity effect on liver function [189]. TQ is beneficial for the prevention and protection of cisplatin nephrotoxicity in rodents. The oral administration of TQ (50 mg/L in drinking water) for five days before and five days after a single injection of cisplatin (5 mg/L in rats and 7 or 14 mg/l in mice) also greatly improved cisplatin-induced nephrotoxicity [190]. The subacute toxicity evaluations of the aqueous, chloroform and methanol extracts of NS at 6 g/kg showed no toxicity. However, the chronic toxicity study found slightly toxic 2 ml/kg NS fixed oil [191]. The high antioxidant potential of TQ is responsible for the reduction in related nephropathy toxicity [184].

8. Challenges and future directions

NS has shown promising results against numerous chronic IDs, yet further investigation should be conducted using human clinical trials with standardized preparation and verification for its full therapeutic potency. Further *in silico* studies, preclinical, experimental evidence, and prospective studies are proposed to evaluate the phytotherapy potential of NS, particularly for emerging and novel diseases such as COVID-19. NS oil and essential oils for topical anti-ageing skincare formulations are also recommended due to their various pharmacological activities such as antioxidant, anti-ageing, antibacterial, anti-inflammatory, anti-carcinogenic, anti-allergy, and moisturizing properties. Other novel extraction techniques, such as natural deep eutectic solvents, could be explored to identify new compounds from the various parts of NS plants, especially the seed.

9. Conclusion

This review presents a succinct summary of the antimicrobial effects of NS, a medieval armamentarium worth further clinical exploration in modern medicine. Various crude extracts from different solvents have been reportedly established as antibacterial, antifungal, antiviral, and anti-parasitic agents. NS oil, TQ, carvacrol, and other active compounds are efficient modulators of AMR bacteria. NS oil and TQ inhibited efflux pumps, resulting in the high accumulation of antibiotics in bacterial cells. Ultimately bacteria become sensitive and die. This action can be valid even at lower doses. TQ facilitates the production of ROS, promotes oxidative stress, and thereby occurs cell apoptosis. Another potent compound of NS, *p*-cymene increases the bacterial membrane permeability, thereby promoting the influx of antimicrobial substances. NS oil, TQ, carvacrol and *p*-cymene could be potential antibacterial agents. Phytochemicals, nutritionally vital constituents, PUFA as well as highly active volatile compounds such as *p*-cymene, TQ, α-thujene, carvacrol, β-pinene, limonene, methyl linoleate, sabinene, D-limonene, 4,5-epoxy-1-isopropyl-4- methyl-1-cyclohexene, and 4-terpineol found in NS could exert a medicinal value when processed and used correctly.

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MSH, AS, AD, MAM, AA, IAA, and ZU prepared the manuscript. MSH, LCM, BHG and MMRS critically edited the draft. AS, AD, MAM, and AA prepared chemical structure. MSH and LCM supervised and critically evaluated the manuscript for scientific quality. MSH conceptualized, designed the diagrams, and project administration. All authors read the manuscript and approved it.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability

Data is contained within the article.

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