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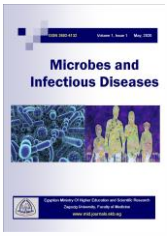

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Original article

Phytochemical constituents and antibacterial activity of *Nigella sativa* seeds against some pathogenic bacteria

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ABSTRACT

Background: This research investigates the antibacterial activity of *Nigella sativa* seeds against some bacteria isolated from clinical samples of patients attending Ahmadu Bello University Medical Centre, Zaria- Nigeria. **Aim:** This work aimed at determining the antibacterial activities of aqueous and methanolic extracts of *Nigella sativa* seeds against *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus*. **Methods:** Ten (10) clinical isolates of *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus* each were collected from Microbiology Laboratory of Ahmadu Bello University Medical Centre and were reconfirmed using culture, microscopy and some biochemical tests. The seed samples of *Nigella sativa* were purchased from herbal point Zaria, Kaduna State, Nigeria. **Results:** The phytochemical analysis of the extracts revealed the presence of carbohydrate, steroids, cardiac glycosides, saponins, flavonoids and alkaloids in both extracts. The highest antibacterial activity for aqueous extract of *Nigella sativa* seeds against *Salmonella* species was found to be 7.70±1.82 mm and for methanolic extracts was found to be 9.00±3.61 mm respectively. The highest antibacterial activity for aqueous extract of *Nigella sativa* seeds against *Escherichia coli* was found to be 8.10±2.17 mm and for the methanolic extract was found to be 6.00±0.00 mm indicating that it was not effective. Both methanolic and aqueous extracts of *Nigella sativa* seeds were not effective against *Staphylococcus aureus* (6.00±0.00mm). The minimum inhibitory concentration (MIC) of the aqueous extract of *Nigella sativa* seeds against *Escherichia coli* was 25mg/mL and the Minimum Bactericidal concentration (MBC) was 100mg/mL. While the minimum inhibitory concentration (MIC) of the methanolic extract *Nigella sativa* seeds against *Salmonella* species was 12.5mg/mL and the minimum bactericidal concentration (MBC) was 50mg/mL. **Conclusion:** Aqueous *Nigella sativa* seeds extract has antibacterial activity against *Salmonella* species and *Escherichia coli* but no activity against *Staphylococcus aureus*.

Introduction

Nigella sativa is a dicotyledonous of the family Ranunculaceae. It is an amazing herb with rich historical and religious background. The seeds of *Nigella sativa* are the source of the active ingredient of this plant. This species (sativa) has different names which are black seed, panacea (in old Latin which means; cure all) and in Arabic is called "Habbatus saudah" and "Habbatul Baraka" (Seed of blessing) [1]. In traditional medicine, *Nigella sativa* has been used for centuries to treat various illnesses including asthma, common cold, headache, nasal congestion, rheumatic disease, warts and many others [2]. More recently *Nigella sativa* has been used to treat conditions like infections, cancer, diabetes, hypotension, obesity, cardiovascular diseases and gastrointestinal problems [3]. *Nigella sativa* possesses antiviral, antioxidant, anti-inflammatory, anticoagulant, antihistaminic, immunomodulatory, broncho-

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dilatory, anti-tussive, antipyretic and analgesic activities [4]. It will be a potential herbal candidate to treat the patient with Covid-19 [5].

Salmonella species has been the major cause of human diseases in almost all parts of the world. The disease caused by this pathogenic microorganism is termed, Salmonellosis [6]. Salmonellosis is easily spread via faeco-oral route through eating contaminated food with faecal materials. It is the second leading cause of bacterial food borne illness in the world. There are two specific *Salmonella* species which are responsible for typhoid in humans. These are; *Salmonella typhi* and *Salmonella paratyphi* A and B. Similarly, sources of *Salmonella* species include fish, crocodile and aquatic habitat which are associated with human activities [7].

Staphylococcus is a genus of the Gram-positive cocci, family Staphylococcaceae and includes both coagulase positive and negative Staphylococci. It measures 0.5-0.7nm in diameter. The organism can occur singly, in pairs or in short chains with a strong tendency to form clusters. It is catalase positive and coagulase positive [8]. It is an aerobic organism that forms fairly large yellow and white colonies on nutrient rich agar media. The yellow colour of the colony is due to carotenoids that is produced by the organism. It is haemolytic on blood agar due to its production of 4 types of haemolysin [9].

Staphylococcus aureus (*S. aureus*) is one of the main pathogens that causes various diseases that are associated with wound infection, skin infection, soft tissue and other serious infections such as urinary tract infection, septicemia, osteomyelitis and endocarditis among others [10].

Escherichia coli (*E.coli*) belongs to the family Enterobacteriaceae, it is a Gram negative, lactose fermenter and non-sporing rod which inhabit the gastrointestinal tract of warm blooded animals as a normal flora. It is widely used as an indicator for the assessment of microbiological quality of water [11, 12]. It is the leading pathogen causing urinary tract infections and is among the most common pathogens causing blood stream infections, wounds, otitis media and other complications in humans. *E. coli* is also the most common cause of food and water borne human diarrhea and causes many deaths in children under the age of five years [13].

The aim of this study was to determine the antibacterial activities aqueous and methanolic extracts of *Nigella sativa* seed against *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus.*

Materials and methods

Study area

The research was carried out at Ahmadu Bello University, Zaria- Nigeria. Zaria is located in the North Western part of Nigeria with an estimated population of 408,198 in 2006 population census and with a total land area of 563km^3 (217sq m). Zaria is located at latitude 11.11128 and longitude 7.7227. Zaria has tropical savannah climate with warm weather year round, a wet season lasting from April to September, and a drier season from October to March [14].

Ethical clearance and approval

Ethical clearance was obtained from the University Health Service Centre, Ahmadu Bello University Medical Centre, Zaria- Nigeria.

Sample size

Non probability sampling was done where isolates of *Escherichia coli*, *Salmonella* species, and *Staphylococcus aureus* each were collected from the University Medical Centre, Ahmadu Bello University, Zaria-Nigeria.

Collection of isolates

Ten clinical isolates of *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus* each were collected from Microbiology Laboratory at Ahmadu Bello University Medical Centre, Zaria-Nigeria.

Plant material

The seed samples of *Nigella sativa* were purchased from Herbal point Zaria, Kaduna State-Nigeria.

Identification of plant materials

The *Nigella sativa* seeds was taken to the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria - Nigeria for authentication and a voucher number ABU060365 was given to the seeds.

Extraction of seed of *Nigella sativa*

Nigella sativa extraction was carried out by washing the seeds with sterile distilled water and allowed to air dry. The seeds were ground into powder.

Aqueous extraction of *Nigella sativa*

Sixty grams (60g) of the *Nigella sativa* seeds were ground and soaked in 400mL of sterile distilled water for 24hours. The mixture obtained was left in a sterile glass container and shaken to allow proper extraction and was filtered using a sterile muslin cloth after which the extract was obtained. The extract was kept in an evaporating dish at 70° C and allowed to evaporate after which the extract was obtained within 24hours, scrapped and stored in a bottle below ambient temperature until required [15].

Methanolic extraction of *Nigella sativa*

Sixty grams (60g) of the *Nigella sativa* seeds were ground and soaked in 400mL of methanol for 24hours. The mixture obtained was left in a sterile glass container and shaken to allow proper extraction and it was filtered using a sterile muslin cloth after which the extract was obtained. The extract was kept in an evaporating dish at 70° C and allowed to evaporate after which the extract was obtained within 24hours, scrapped and stored in a bottle below ambient temperature until required [15].

Qualitative phytochemical analysis

Test for flavonoids

Shinoda test

One mL of the stock was taken in a test tube and few drops of dilute NaOH solution was added. An intense yellow colour appeared in the test tube. Addition of few drops of dilute acid that change to colourless indicated the presence of flavonoids [16].

Test for saponins

Frothing test

A total of 0.5 g of the extract was dissolved in 10mL of sterile distilled water and was shaken vigorously for 30seconds and allowed to set for an hour. The occurrence of honey comb-like bubbles of at least 1cm in height and persisting for at least 30minutes indicated the presence of saponins [16]

Test for steroids

Lieberman Burchard's test

One mL of acetic anhydride was added to the extract followed by 1mL of concentrated sulphuric acid down the side of the tube. Colour change was observed immediately from red to purple which showed presence of triterpenes, while blue to green indicated the presence of steroids [16].

Test for tannins

Ferric Chloride test

A total of 0.5 mL of the extract was dissolved in 10mL of sterile distilled water and then filtered. Few drops of ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicated the presence of hydrolysable tannins and green precipitate indicated the presence of condensed tannins [15].

Test for carbohydrate

Molish's Test

A total of 0.5 g of the extract was added into a test tube. Two drops of Molish's reagents were added followed by few drops of concentrated $H2SO_4$. A purple to violet colour at the interface suggested the presence of carbohydrate [15].

Test for cardiac glycoside

Kella Kellani's test

A total of 0.5 g of the extract was dissolved in glacial acetic containing traces of ferric chloride. The test tube was held at 45 ℃. 1mL of concentrated $H2SO_4$ was added down the side of the test tube. A purple colour at the interface suggested the presence of cardiac glycoside [15].

Test for alkaloids

Meyer's test

Few drops of Meyer's reagent were added to 0.5g of the extract in a test tube. Appearance of creamy precipitate indicated the presence of alkaloids [16].

Test for anthraquinones

Bontrager's test

Ten milliliters of chloroform were added to 0.5g of the extract and then shaken. The mixture was then filtered and 5mL of 10% ammonia solution was added to the filtrate. The appearance of pink or cherry red colour at the lower layer indicated the presence of anthracenes [16].

Cultural characterization of isolates

The clinical isolates collected were reconfirmed by first culturing on Eosin Methylene Blue agar for *Escherichia coli*, Mannitol Salt agar for *Staphylococcus aureus* and Salmonella Shigella agar for *Salmonella* species. Colonies appearing bluish black with green metallic sheen on Eosin Methylene Blue agar were suspected to be *Escherichia coli*, those appearing golden yellow on Mannitol Salt agar were suspected to be

Staphylococcus aureus and those appearing colourless with or without black centers on Salmonella Shigella agar were suspected to be *Salmonella* species.

Gram staining (microscopy)

A smear of the isolates was made on grease free clean glass slide and allowed to air dry, heat fixed and the slide was flooded with crystal violet solution for 60 seconds after which it was washed with sterile distilled water and well drained to avoid diluting the mordant. It was flooded with iodine solution (Mordant) for 30 seconds. Then was washed with sterile distilled water immediately. It was then flooded with acetone which is the decolorizer and was washed immediately with sterile distilled water. The slide was flooded with safranin (counter stain) for 60 seconds, washed and was allowed to dry. It was then examined under the oil immersion objective lens [17].

Biochemical identification of the isolates

Catalase test

A loop was used to transfer colony onto the surface of a clean, dry glass slide. A drop of 3% H_2O_2 was dropped on the glass slide and observed for evolution of oxygen bubbles [18].

Coagulase test

A drop of physiological saline was placed on each end of the slide, with the loopful of a portion of the isolated colony emulsified in each drop to make two thick suspensions. A loopful of plasma was then added to one of the suspensions and mixed gently. Clumping of the organism was observed within 10 seconds indicating a positive coagulase test [18].

Indole test

A sterile test tube containing 4mL of tryptophan broth was inoculated aseptically by with 24 hours culture of the bacterial isolate. It was incubated at 37℃ for 24 hours. 0.5mL of Kovac's reagent was added to the broth culture. The presence of red ring indicated a positive result [19].

Methyl red test

Methyl Red Voges Proskauer (MRVP) broth was inoculated with a pure culture of the organism. Incubated at 37℃ for 24 hours. Five drops of methyl red reagent was added per 5mL of broth. Presence of red colour in the broth indicated positive [19].

Voges Proskauer test

Few drops of alpha-naphthol and potassium hydroxide was added to the Voges Proskauer broth with which was inoculated with a 24hour culture of the bacteria. A cherry red colour indicated a positive result, while a yellow brown colour indicated a negative result [20].

Citrate utilization test

Simmon citrate agar was inoculated with a pure culture of the organism. Incubated aerobically at 37℃ for 24hours. Change from green to blue along the slant indicated positive test [19].

Motility test

With a sterile straight needle, a colony of 24hours culture was picked and inoculated into motility medium by stabbing straight down into the center of the medium in a test tube to about half the depth. It was incubated at 37ºC for 24hours, motility was observed afterwards [19].

Urease test

The surface of the urea agar slant was streaked with a portion of the isolated colony. The cap was left loosely covered and incubated at 37ºC for 24hours. The presence of a pink colour was observed which indicated a positive urease test. [19].

Standardization of inoculum

McFarland standard (0.5) was prepared by mixing 9.95mL of 1% sulphuric acid and 0.05mL of Barium Chloride /H2O. The 1% Sulphuric acid was prepared by mixing 1mL of concentrated Sulphuric acid with 99mL of sterile distilled water while the 1% Barium chloride was prepared by dissolving 1g of solid Barium chloride in 100mL of sterile distilled water. The turbidity of the McFarland standard is as a result of the reaction between 1% Sulphuric acid and 1% Barium chloride [21].

Standardization of test organisms were done by emulsifying distinct colonies of isolates in a sterile bottle containing saline water. Their turbidity was compared with that of 0.5McFarland standard which is equivalent to 1.5×10^8 CFU/mL [21].

Preparation of test concentration

Five different concentrations of the extract (100mg/mL, 50mg/mL, 25mg/mL,12.5mg/mL and 6.25mg/mL) were prepared by dissolving 1g of the extract in 2mL of dimethyl sulfoxide (DMSO) and 8mL of sterile distilled water to give an initial

concentration of 100mg/mL. Half fold dilution was carried out serially into another 4 test tubes to give a concentration of 100mg/mL, 50mg/mL, 25mg/mL, 12mg/mL and 6.25mg/mL [22].

Antibacterial susceptibility testing

Agar well diffusion method was used to evaluate the antimicrobial activity of plant extract. The agar plate surface was inoculated by spreading a 0.1mL of the inoculum over the entire agar surface. Five holes with a diameter of 6mm were bored aseptically with a sterile cork borer, and 0.1mL of each extract concentration was introduced into the wells, 0.1mL of Dimethyl sulfoxide (DMSO) was introduced into a different agar plate which served as the negative control and 0.1mL Ciprofloxacin (10µg) was introduced into a different plate which served as the positive control. The agar plate was incubated at 37℃ for 24hours. Zones of inhibition were measured in millimeters using a meter rule. This was done in triplicates [23].

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of each extract was determined according to the method described by [24]. MIC was evaluated on the plant extract that showed antibacterial activity on agar well diffusion assay. It was performed for the 5 concentrations of each extract in 100mg/mL, 50mg/mL, 25mg/mL 12.5mg/mL and 6.25mg/mL by employing double dilution up to the last dilution. Two milliliters of nutrient broth were added into a sterile test tube and 1mL of the extract was added to it and serial dilution was done with the last 1mL been discarded. Each organism (0.1mL) was separately suspended into the nutrient broth and incubated over night at 37℃. The minimum inhibitory concentration was recorded as the lowest concentration of the extract that didn't show any visible growth of the organism after overnight incubation.

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of the extract was determined using the method used by [25]. Samples were taken from the tubes with no visible growth on the minimum inhibitory concentration and was sub-cultured on to freshly prepared nutrient agar and incubated at 37℃ for 24hours. The MBC was taken as the lowest concentration of the extract that did not have bacterial growth on the surface of the agar.

Results

Qualitative phytochemical constituents of *Nigella sativa* seeds

The aqueous and methanolic extract of *Nigella sativa* seeds contained carbohydrate, steroids, cardiac glycoside, saponins, flavonoids and alkaloids. Although, methanolic extract contained glycoside which was not contained in the aqueous extract. Likewise, tannin was found in aqueous extract and was absent in the methanolic extract of the plant. This was indicated in **Table (1)**.

The cultural, microscopic and biochemical properties of *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus*

Salmonella species appeared to be smooth and colourless with black center on Salmonella Shigella agar, Gram negative rods which were motile, citrate negative, methyl red positive, Voges Proskauer, urease and indole negative. *Escherichia coli* appeared as bluish black colonies with green metallic sheen on Eosin methylene blue agar, Gram negative rods which were motile, citrate, Voges Proskauer and urease negative, methyl red and indole positive. *Staphylococcus aureus* appeared as golden yellow colonies, Gram positive cocci, catalase and coagulase positive respectively as indicated in **Table (2)**.

Antibacterial activity (Mean ± Standard Deviation) of aqueous extract of *Nigella sativa* seeds on *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus*

Salmonella species had the highest antibacterial activity of 7.70 ±1.82 mm and lowest at 6.00 ±0.00 mm. For *Escherichia coli*, it had the highest antibacterial activity of 8.10 ±2.17mm and lowest of 6.00 ±0.00 mm with *Staphylococcus aureus* showing no activity at all concentrations (6.00 ±0.00 mm) as indicated in **Table (3)**.

Antibacterial activity (Mean ± Standard Deviation) of methanolic extract of *Nigella sativa* seeds on *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus*

Salmonella species had the highest antibacterial activity of 9.00±3.61mm and lowest at 6.00±0.00mm. *Escherichia coli* and *Staphylococcus aureus* showed no activity at all concentrations (6.00±0.00mm) as indicated in **Table (4)**.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous and methanolic extract of

Nigella sativa* on *Staphylococcus aureus*, *Salmonella* species and *Escherichia coli

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous and methanolic extract of *Nigella sativa* seeds against *Staphylococcus aureus*, *Salmonella* species and *Escherichia coli* were indicated in **Table (5)** with *Salmonella* species having an MIC at 25mg/mL and 50mg/mL with MBC at 100mg/mL. *Escherichia coli* also having an MIC at 25mg/mL and 50mg/mL with MBC at 100mg/mL.

Table 1. Phytochemical constituents of aqueous and methanolic extracts of *Nigella sativa* seeds

S/N	Phytochemical Compounds	Test	Aqueous Extract	Methanolic Extract
1	Carbohydrate	Molish's test	+	+
2	Glycosides	Fehling's test	–	+
3	Anthraquinones	Bontrager's test	–	–
4	Steroids	Lieberman Buchard's test	+	+
5	Cardaic glycoside	Killer killani's test	+	+
6	Saponins	Frothing test	+	+
7	Tannis	Ferric chloride test	+	–
8	Flavoniods	Shinoda test	+	+
9	Alkaloids	Dragendoff's test	+	+

Key: + = present, – = Absent

Table 2. Cultural, microscopic and biochemical characteristic of the organisms

Suspected Organism	Cultural characteristic	Gram reaction	Biochemical tests							
Salmonella species	Smooth and colourless colony with black center	Gram negative rod in clusters	CT	MR	VP	I	MOT	UR	CAT	COA
			–	+	–	–	+	–	NA	NA
Escherichia coli	Bluish black colonies with green metallic sheen	Gram negative rod	–	+	–	+	+	–	NA	NA
Staphylococcus aureus	Golden yellow colonies	Gram positive cocci	NA	NA	NA	NA	NA	NA	+	+

Key: + positive - Negative NA- Not applicable CT- Citrate MR- Methyl red VP- Voges Proskauer I- Indole MOT- Motility UR- Urease

Table 3. Antibacterial activity (Mean ± Standard Deviation) of aqueous extract of *Nigella sativa* seeds *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus*

Concentration (mg/mL)	Zone of inhibition (Mean ±S.D) in mm		
	Salmonella species	*Escherichia coli*	*Staphylococcus aureus*
100	7.70± 1.82	8.10 ± 2.17	6.00 ± 0.00
50	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
25	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
12.5	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
DMSO	6.00± 0.00	6.00 ± 0.00	6.00 ± 0.00
Ciprofloxacin (10µg)	36.00 ± 0.00	42.00 ± 0.00	36.00 ± 0.00

KEY: DMSO –dimethyl sulfoxide mm- millimetres S.D- Standard deviation

Table 4. Antibacterial activity (Mean± Standard Deviation) of methanolic extract of *Nigella sativa* seeds on *Salmonella* species, *Escherichia coli* and *Staphylococcus aureus*

Concentration (mg/mL)	**Zone of inhibition (Mean ±S.D) in mm**		
	Salmonella species	*Escherichia coli*	*Staphylococcus aureus*
100	9.00 ± 3.61	6.00 ± 0.00	6.00 ± 0.00
50	7.70 ± 2.83	6.00 ± 0.00	6.00 ± 0.00
25	7.10 ± 2.30	6.00 ± 0.00	6.00± 0.00
12.5	7.00 ± 2.05	6.00 ± 0.00	6.00 ± 0.00
DMSO	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Ciprofloxacin (10µg)	36.00 ± 0.00	42.00 ± 0.00	36.00 ± 0.00

KEY: DSMO –dimethyl sulfoxide mm- millimetres S.D- Standard deviation

Table 5. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous and methanolic extract of *Nigella sativa* on *Staphylococcus aureus*, *Salmonella* species and *Escherichia coli*

Concentration in mg/mL														
Organism	***Salmonella* species**					***Escherichia coli***					***Staphylococcus aureus***			
ID	Aqueous		Methanolic		ID	Aqueous		Methanolic		ID	Aqueous		Methanolic	
	MIC	MBC	MIC	MBC		MIC	MBC	MIC	MBC		MIC	MBC	MIC	MBC
SS1	-	-	-	-	EC1	-	-	-	-	SA1	-	-	-	-
SS2	25	-	-	-	EC2	-	-	-	-	SA2	-	-	-	-
SS3	25	100	-	-	EC3	-	-	-	-	SA3	-	-	-	-
SS4	-	-	50	-	EC4	50	-	-	-	SA4	-	-	-	-
SS5	-	-	-	-	EC5	25	100	-	-	SA5	-	-	-	-
SS6	25	50	25	100	EC6	-	-	-	-	SA6	-	-	-	-
SS7	50	-	50	100	EC7	50	-	-	-	SA7	-	-	-	-
SS8	-	-	50	-	EC8	-	-	-	-	SA8	-	-	-	-
SS9	-	-	50	-	EC9	50	-	-	-	SA9	-	-	-	-
SS10	-	-	-	-	EC10	50	100	-	-	SA10	-	-	-	-

KEY: - No activity MIC- Minimum inhibitory concentration MBC- Minimum bactericidal concentration ID- Isolate Code

Discussion

The phytochemical constituents of the aqueous and methanolic extracts of *Nigella sativa* seeds revealed the presence of carbohydrate, cardiac glycoside, saponins, steroids, flavonoids and alkaloid which were active against *Salmonella* species and *Escherichia coli* and did not show any activity against *Staphylococcus aureus* this result is in accordance with the work of **Balogun et al.** [26], and **Khan et al.** [27]. This antibacterial activity is as a result of these constituents specifically flavonoid which was found to have antimicrobial effect against wide range of microorganisms. Flavonoids and many other phenolic components have been reported to be effective antioxidants, anticancer, antibacterial, cardio protective agents, anti-inflammation, immune system promoting, and confer skin protection from ultraviolet radiation [28]. Alkaloids contained in the plant extracts were also found to have antimicrobial activity because of their unique chemical structures [29]. They inhibit bacterial growth through a variety of mechanisms, including inhibition of the bacterial nucleic acid and protein synthesis, modifications of the bacterial cell membrane permeability, damage of the cell membrane and cell wall, inhibition of bacterial metabolism and efflux pumps [30]. Saponin contain a steroidal group with one or more sugar(s) attached. These chemical structures determine their biological properties as natural, non-ionic detergents which have cytotoxic, hemolytic, molluscicidal, anti-inflammatory, anti-fungal, anti-yeast, anti-bacterial and anti-viral activities [31].

This is also similar to the findings of **Balogun et al.** [26], which was carried out in Maiduguri, Nigeria which revealed that aqueous extract had no antibacterial activity on *S. aureus*, and methanolic extract had antibacterial activity on *Salmonella* species.

On the contrary, the findings of **Ozlem et al.** [32], showed that methanolic and ethanolic extracts of *Nigella sativa* seeds showed antimicrobial effect on wide range of bacteria and fungus. He used 14 bacteria and 1 fungus with inhibition zones of between 7-11 mm for *Staphylococcus aureus* which was found to be more sensitive among the bacteria, while on the other hand, *Salmonella* species and *Escherichia coli* were found to be more resistant bacteria against *Nigella sativa* seed extracts. The result of this study is also contrary to that of **Kabir**, [33] who showed that aqueous extract had no antibacterial activity on *Salmonella* species and this might be due to absence of Tannins in his aqueous extract which was found to be present in this research study. Tannin-rich plant extract have shown high antimicrobial effects and their antibacterial activity depends on conditions such as pH, temperature, type of solvent, and action time [34].

Also, the findings of this study contradicts the findings of **Usman et al.** [35], which was carried out in Kano, Nigeria who found out that methanolic extract of *Nigella sativa* seeds have antibacterial activity on *S. aureus* and no antibacterial activity on *E.coli* . This is due to difference in the methodology of extraction, the different concentrations used, the method adopted for antimicrobial susceptibility test, solvent used in extraction, difference in the strain of the organism and geographic location from which the seeds were sourced.

Salmonella species appeared as smooth and colourless colonies with black center on Salmonella Shigella agar; Gram negative small rods; motile bacteria which is similar to the findings of **Mobley**, [36]. *E. coli* appeared as bluish black colonies with green metallic sheen on Eosin Methylene Blue agar, Gram negative rod, motile bacteria which is similar to the findings of **Tong et al**. [8]. *Staphylococcus aureus* appeared as golden yellow colonies on Mannitol Salt agar, Gram positive cocci bacteria, which were catalase and coagulase positive which is also similar to the findings of **Kim et al**. [11].

A number of factors could influence the agar well diffusion technique one of which is inoculum size. The smaller the inoculum size, the higher the possibility of falsely large inhibition zones while the bigger inoculum size, the higher the possibility of falsely smaller zones. Therefore, there is need for proper matching of the inoculum suspension with 0.5 McFarland standard [37].

The minimum inhibitory concentration (MIC) of both methanolic and aqueous extracts for *Salmonella* species was found to be 25mg/mL and 50mg/mL respectively and for *Escherichia coli* it was also found to be 25mg/mL and 50mg/mL respectively. The minimum bactericidal concentration (MBC) of both extracts was found to be 50mg/mL and 100mg\mL for *Salmonella* species and *Escherichia coli* which is in contrary to the findings of **Usman et al.** [35]; both extracts were found to be more effective on Gram negative than Gram positive bacteria.

Conclusion

The methanolic and aqueous extract of seeds of *Nigella sativa* were found to contain important phytochemicals such as carbohydrate, cardiac glycoside, saponins, steroids, alkaloids and flavonoids. The antibacterial activity of both aqueous and methanolic extracts of *Nigella sativa* were effective against *Escherichia coli* and *Salmonella* species. The minimum inhibitory concentration (MIC) of both methanolic and aqueous extracts for *Salmonella* species was found to be 25mg/mL and 50mg/mL respectively and for *Escherichia coli* it was also found to be 25mg/mL and 50mg/mL respectively. The minimum bactericidal concentration (MBC) of both extracts was found to be 50mg/mL and 100mg\mL for *Salmonella* species and *Escherichia coli*.

Competing interest

None declared.

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