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# Phytochemical Composition, Antioxidant, Antimicrobial Potential and GC-MS Analysis of Crude and Partitioned Fractions of *Nigella sativa* Seed Extract

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**Abstract**

The study was aimed at investigating the phytochemical, antioxidant, antimicrobial potential and GC-MS analysis of crude and different fractions of *Nigella sativa* seed extract. Crude methanol extract of *N. sativa* seed was obtained, the phytochemical components, antioxidant and antimicrobial potential of the seed were evaluated and the crude extract was partitioned using different solvents. Antimicrobial activity was determined and GC-MS analysis of the fractions was conducted. Saponins, flavonoids, alkanoids (19.8, 41.6 and 82%), tannins, anthraquinones, terpenoids, steroids were present while cardiac glycosides were not detected. The hexane and chloroform fraction yielded 60.17 and 0.42% from the partitioned crude extract. DPPH (2, 2-dipheniyl-1-picrylhydrazyl), total phenolic content and ferric reducing antioxidant power (40.37, 20.1 and 48.4 %) increased in a dose-dependent manner. *N. sativa* fractions had varied antibacterial activity against the test bacteria and fungi. *Staphylococcus aureus*ATCC29213 and methicillin-resistant *S. epidermidis*3 were highly susceptible (60.0mm and 56.0mm) to chloroform fractions while *Trichophyton* sp. and *Candida tropicalis* had the highest susceptibility (50 mm) to the hexane fraction. A total of 24 distinct phyto-components were identified, and 9, 8, 7, 3 and 4 compounds were found in the hexane, methanol, ethyl acetate, chloroform fractions and the crude extract. The compounds were 2-Chloroethyl vinyl sulfide, 11-Octadecenoic acid, methyl ester, Cyclononene, 3,8-Dioxatricyclo[5.1.0.0(2,4)] octane, 4-ethenyl-, 7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin, Trimethylsilyl-di(timethylsiloxy)-silane among others. The crude, hexane and chloroform (%) fractions contained 99.3, 96.42 and 97.90% of Trimethylsilyl-di(timethylsiloxy)-silane. In conclusion, the *N. sativa* seed extract contains good phytochemicals, antioxidant and varied antimicrobial activities against the test pathogens.

**Keywords:** *Nigella sativa* seed extract, hexane fraction, phytochemical, antioxidant, Trimethylsilyl-di(-timethylsiloxy)-silane; *S. aureus* ATCC29213

**Резюме**

Целта на настоящата работа е проучване на фитохимичния, антиоксидантния, антимикробния потенциал, както и GC - MS анализ на суров/нативен екстракт от семена на *Nigella sativa* и различни негови фракции. Получен е изходен метанолов екстракт от семена на *N. sativa*, на който са определени фитохимичните компоненти и е оценен антиоксидантният и антимикробният потенциал. Суровият екстракт е фракциониран при използване на различни разтворители. Направен е антибактериален и GC-MS анализ на получените фракции.

Установено е наличие на сапонини, флавоноиди, алканоиди (съответно 19,8%, 41,6% и 82%), танини, антрахинони, терпеноиди, стероиди. Сърдечни гликозиди не са открити. Добивът на хексановата и хлороформната фракции е съответно 60.17% и 0.42% от изходния екстракт. Наблюдаваното увеличение на DPPH (2, 2-дифенил-1-пикрилхидразил), общото фенолно съдържание и FRAP (съответно 40.37%, 20.1% и 48.4%) е дозо-зависимо. Фракциите от екстракта на *N. sativa* показват различна антибактериална активност срещу тестваните бактерии и фунги. *Staphylococcus aureus ATCC29213* и резистентният към метицилин *S. epidermidis 3* са силно чувствителни (60.0

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mm и 56.0 mm) към хлороформните фракции, докато *Trichophyton sp*. и *Candida tropicalis* имат най-висока чувствителност (50 mm) към хексановата фракция. Идентифицирани са общо 24 различни фитокомпоненти, като 9, 8, 7, 3 и 4 съединения са открити в хексановата, метаноловата, етилацетатната, хлороформната фракции и суровия екстракт. Съединенията са 2-хлоретил винил сулфид, 11-октадеценова киселина, метилов естер, циклононен, 3,8-диоксатрицикло [5.1.0.0 (2, 4)] октан,4–етенил-7-хидрокси-3-(1, 1-диметилпроп-2-енил) кумарин, триметилсилил- ди (тиметилсилокси)-силан и др. Суровата, хексановата и хлороформната фракции съдържат съответно 99.3%, 96.42% и 97.90% триметилсилил-ди-(тиметилсилокси)-силан.

В заключение, може да се каже, че екстрактът от семена на *N. sativa* съдържа добри фитохимикали с антиоксидантен потенциал и разнообразна антибактериална активност срещу тестираните патогени.

## Introduction

The advent of antibiotic resistance amongst microorganisms coupled with their rapid spread has led to the birth of innumerable research papers on potential alternatives, which are not only sustainable and cost-effective but also eco-friendly and with the potential of large-scale production. In view of this current trend of development and spread of antibiotic resistance, traditional medicine (TM) may offer ample interesting prospects to combat drug resistance. In recent times, vast numbers of medicinal plants of ancient origin have been researched and found to possess immense potential to treat illnesses caused by bacterial species (Abdurohaman, 2015). The traditional systems of medicine, which include Ayurveda, traditional Chinese medicine (TCM), Kampo, Unani, and Siddha, have so far been unable to enter the mainstream medicine due to various reasons (Gupta *et al*., 2014). As a result of their efficacy, attempts to develop strong evidence-based standardization of these traditional systems of medicine have been made so that they can satisfy the criteria to fit into the modern medicinal framework.

*Nigella sativa* is an ancient plant commonly referred to as black seed, also known in English as black cumin, in Arabic and Hindi as Habbatul Barakah; Sonez, Habbatus Sauda, Kamun Aswad and Kalonji. Sanskrit: Krishna Jiraka, Persian: Siya Danah, (Chevallier, 1996; Ahmad *et al*., 2003). It has been used extensively in Iranian traditional medicine as a spice for cooking and preservative in many Asian countries including India (Anahita *et al*., 2016). *N. sativa* seeds are very rich and diverse in chemical composition. They contain amino acids, proteins, carbohydrates, fixed and volatile oils (Ozel *et al*., 2009). Most of the pharmacological activities are due to the quinone constituents present in the seed. Chopra *et al*. (1956) reported thymoquinone (TQ) as the main active constituent of the volatile oil of black seed. Aboutabl *et al*. (1986) reported the fixed oil and volatile oil of *N. sativa*.

The antimicrobial, antiviral, anti-parasitic and anthelmintic potential of *N. sativa* has been reported (El-Fatatry, 1975). The inhibitory potential of the seed extracts of *N. sativa* against some pathogenic bacterial and yeast has been reported (Topozada *et al*., 1965; Saxena and Vyas, 1986; Hanafy and Hatem, 1991).

In this study, the phytochemical composition, antioxidant and antimicrobial potential of crude *N. sativa* seed extract and different fractions (hexane, chloroform, ethyl acetate and methanol) against standard bacteria strains, methicillin-resistant *Staphylococci* and some test fungi was evaluated and the chemical composition of the crude and different fractions was determined using GC-MS analysis.

## Materials and Methods

### *Collection of samples and cultures*

Dried seeds of *N. sativa* (black seed) were obtained from Kano, northern Nigeria, and were authenticated at the Department of Botany Herbarium, University of Ibadan, Nigeria. Test bacteria: *Pseudomonas aerogenosa* ATCC 27853*, Staphylococcus aureus* ATCC 29213*, Bacillus* sp*, Escherichia coli* ATCC 11775*, E. coli* ATCC 35218*,* methicillin-resistant Staphylococci (*S. epidermidis* 1, 2 and 3, *S. saprophyticus* 1 and 2), and fungi (*Candida albicans, C. krusei, C. tropicalis, Trichophyton* sp., *Aspergillus niger*, *Penicillium* sp.) were obtained from the Department of Microbiology, University of Ibadan, Ibadan Oyo state.

### *Preparation of extracts*

The seeds were dried, milled, and a 2500 g milled sample was weighed into a macerating jar, then methanol was added and the mixture was stirred manually using a sterile glass rod and the stirring was repeated 8 hourly for 72 hrs of maceration. After maceration, the extract was decanted and filtered using Whatman's filter paper (No. 1). The filtrate was concentrated using a rotary evaporator under reduced pressure and low temperature.

After concentration in vacuum at 50°C, the percentage yield was calculated as follows:

$$\%\text{Yeild} = \frac{\text{weight of extract}}{\text{weight of plant material}} \times 100$$

*Qualitative and quantitative determination of phytochemicals in the crude extract of N. sativa seed*

Qualitative and quantitative phytochemical screening was carried out on the crude plant extract. Standard qualitative methods were used to test for alkanoids, flavonoids, saponins, tanins, anthraquinones, cardiac glycosides, terpenoids and steroids.

*Test for alkaloids, flavonoids, saponins, tannins, anthraquinones, cardiac glycosides, terpenoids and steroids*

The alkaloid, flavonoid and saponin content of the *N. sativa* crude extract was determined using standard methods (Raffauf, 1962; Harborne, 1989; Meda *et al*., 2005). Formation of precipitate indicated the presence of alkaloids while a yellowish white precipitate indicated the presence of alkaloids after two drops of Mayer's reagent had been added (Raffauf, 1962). A yellow coloration indicated the presence of flavonoids (Meda *et al*., 2005). Emulsion formation in frothing olive oil drop mixture after shaking indicated saponins formation (Harborne, 1989). A brownish green or blue black coloration indicated the presence of tannins (Trease and Evans, 1983). Formations of pink, red or violet coloration indicated the presence of anthraquinones (AOAC 2010). Formation of a violet ring below the brown ring in the acetic layer and a greenish ring formation throughout the thin layer indicated the presence of cardiac glycosides (Harborne, 1989). Formation of grey color indicated the presence of terpenoids. A brown-red ring at the interface between the two liquids was an indication of the presence of steroids (AOAC, 2010).

*Determination of total phenols, total alkaloids, flavonoids and saponins*

The total phenolic content of the samples was determined using the method of Singleton *et al*. (1999). The quantity of alkaloid was determined using the method of Harborne (1989). The total flavonoid content in *N. sativa* seed extract was determined according to the modified method of Uruquiaga and Leighton (2000). The total saponins content in *N. sativa* seed crude methanol extract was determined using the method of Obadoni and Ochuko (2001).

*Determination of antioxidant potential of the N. sativa seed extracts*

*DPPH radicals scavenging activity.* DPPH radicals scavenging of *N. sativa* seed crude methanol extract was determined according to the method of Irshad *et al*. (2012). Three milliliters DPPH solution in methanol (0.1mM) was mixed with 100 µL of different concentrations of the extracts (mg/mL). One hundred microliters methanol (without extracts) mixed with DPPH solution served as control. The absorbance of the mixture after incubation for 20 min at 37°C was taken at 515 nm. Ascorbic acid was used as positive reference. The experiment was carried out in triplicate. DPPH radical scavenging activity was calculated by using the following formula:

$$\%\ \text{inhibition} = [(AC - AE)\ AB/] \times 100$$

where AC = absorbance of the control and AE = absorbance of tested samples.

*Determination of total antioxidant capacity of N. sativa seed extract*

The total antioxidant activity of the methanol extract of *N. sativa* was determined (Mitsuda *et al.,* 1996) using sulphuric acid, sodium sulfate, ammonium molybdate and distilled water. The mixture was labeled as total antioxidant capacity (TAC). The extract was added to TAC and the absorbance was read at 695 nm after 15 minutes and ascorbic acid was used as standard, this was done according to the method of Mitsuda *et al.* (1996).

*Ferric reducing antioxidant potential (FRAP) assay of N. sativa seed extract*

The ferric reducing power of *N. sativa* crude methanol extract was determined according to the method of Irshad *et al*. (2012). For FRAP reagent preparation, 10 mLs of 300 mM acetate buffer (pH 3.6), 1 mL of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-striazine) in 40 mM HCl and 1 mL of 20 mM ferric chloride were mixed. One hundred microliters of different sample concentrations (100 - 1000 mg/mL) were added to 3 mL of prepared FRAP reagent. The absorbance of the reaction mixture after incubation for 30 min at 37°C was taken at 593 nm wavelength. The difference between the absorbance of the reaction mixture and the absorbance of the blank was used to calculate the FRAP value. Ascorbic acid was used as standard. FRAP value was expressed in terms of ascorbic acid equivalents (AAE) (Gupta and Kaur, 2015). The assay was done in triplicate.

*Partitioning of crude extract of N. sativa*

About 220 g of the crude extract of the seeds was weighed and transferred into a sterile beaker and subjected to bio-guided fractionation by solubilization in 100 mL of methanol. The sample was stirred to allow the extract to dissolve and 100 mL of water was added to dilute the concentration. This solution was transferred into a separating funnel fixed to a retort stand and defatted sequentially using hexane (5 x 200 mL) to obtain the hexane fraction. Subsequently, the solution was partitioned sequentially with ethyl acetate (5 x 200 mL) to obtain the equivalent fraction and with chloroform (5 x 200 mL) and then finally with methanol (5 x 200 mL) to obtain the chloroform and methanol fractions, respectively. Each fraction with the relic from the final hydro-methanol fraction was separated into separate jars and concentrated using a rotary evaporator and desiccated to obtain semi-liquid to dry fractions from each solvent. The fractions were stored in airtight containers in a refrigerator for later use (Jamil *et al.*, 2012).

*Antimicrobial potential of the crude and partitioned fractions of N. sativa seed extracts*

The antimicrobial potential of the crude extract as well as its fractions was tested using agar well diffusion method against the test bacteria, methicillin-resistant *Staphylococcus* strains and the test fungi. The suspension of the tested microorganism and spore suspension was seeded on Mueller - Hinton agar (Lab M Ltd., UK) plates. A sterile cork borer was used to cut uniform wells on the agar plate and the wells were filled with 10 µL of the extracts and fractions. The inoculated plates were incubated at 37°C for 24 hrs for bacteria and at 25°C for the fungi. After incubation, clear zones of inhibition around the wells were measured and recorded in diameters (mm).

*GC-MS analysis of the chemical composition of N. sativa seed extract and the fractions*

The crude and partitioned fractions of methanol seed extract of *N. sativa* were dissolved in petroleum ether. The soluble compound obtained from the organic fraction was re-dissolved in diethyl ether and subjected to GC/MS analysis. Gas chromatography-mass spectrometer (model: GCMS-Qp2010 Plus Shimadzu, Japan) made up of an auto sampler (AOC-20i) and a gas chromatograph interfaced to a mass spectrometer was used. Mass spectrum GC-MS interpretation for compound identification was done by comparing the spectrum of the unknown component in the extracted samples with the spectrum of the known components in the National Institute Standard and Technology (NIST) library. The name, molecular weight and structure of the components of the crude and partitioned fractions of *N. sativa* were ascertained.

**Results**

The crude extract of *N. sativa* seeds was obtained through the solvent extraction method using methanol as solvent. Two thousand five hundred grams of the dried seeds yielded 247.553 g of the crude methanol extract as shown in Table 1 after double extraction.

**Table 1**: The total yield of *N. sativa* seeds crude methanolic extracts

| Plant sample | Yield (g) | Yield (%) |
|---|---|---|
| *N. sativa* seeds | 2500.00 | - |
| Plant extract | 247.533 | 9.90 |

Table 2 shows the qualitative phytochemical composition of the *N. sativa* seeds. *N. sativa* seed crude methanol extract contains some bioactive components, such as saponins, flavonoids, tannins, alkanoids, anthraquinones, terpenoids and steroids, however, cardiac glycosides were not detected. The quantitative analysis of the *N. sativa* seeds crude methanol extract is shown in Table 3. Flavonoids were found to be the most predominant phytochemical, accounting for 41.6%, saponins and alkaloids were the other bioactive compounds with a yield of 19.8% and 8.2%, respectively. All the other unidentified phytochemicals accounted for the outstanding 30.1%.

**Table 2**: Qualitative and Quantitative phytochemical composition of *Nigella sativa seeds*

| Phytochemical Parameters | Qualitative Test | Quantitative Test |
|---|---|---|
| Alkanoids | + | 8.2 |
| Flavonoids | ++ | 41.6 |
| Saponins | +++ | 19.8 |
| Tanins | ++ | - |
| Anthraquinones | ++ | - |
| Cardiac glycosides | - | - |
| Terpenoids | + | - |
| Steroids | + | - |

Key: (-) = Not detected; (+) = Trace;
(++) = Present; (+++) = Prominent

*Antioxidant potential of the crude methanol extract of N. sativa*

The DPPH scavenging potential of the crude methanol extract of *N. sativa* is shown in Fig. 1a. The free radical scavenging activity of the extract increased significantly in a dose-dependent man-

ner with an increase in the concentration of the extract (100 – 1000 µg/mL). The DPPH scavenging activity ranged from 21.32 to 40.37%, the highest activity was observed at the 1000 µg/mL. Ascorbic acid exhibited higher activity compared to the extract within the concentration range used in this research.

The TPC of *N. sativa* seed crude methanol extract is shown in Fig. 1b. The TPC of the extract increased in a dose-dependent manner (100 – 1000 µg/mL). TPC ranged from 1.1 to 20.1% with the highest activity recorded at 1000 µg/mL. TPC of the extract was less than that of the standard. Gallic acid for all the concentrations was used in this research.

Figure 1c shows the FRAP of *N. sativa* seed crude methanol extract using ascorbic acid as the standard. The extract did not exhibit FRAP activity at 2.5 – 5µg/mL. FRAP activity increased in a dose-dependent manner from 10 – 100 µg/mL. The activity ranged from 6.6 to 48.4%. The reducing power of the extract was however lower than that of the standard for all concentrations used.

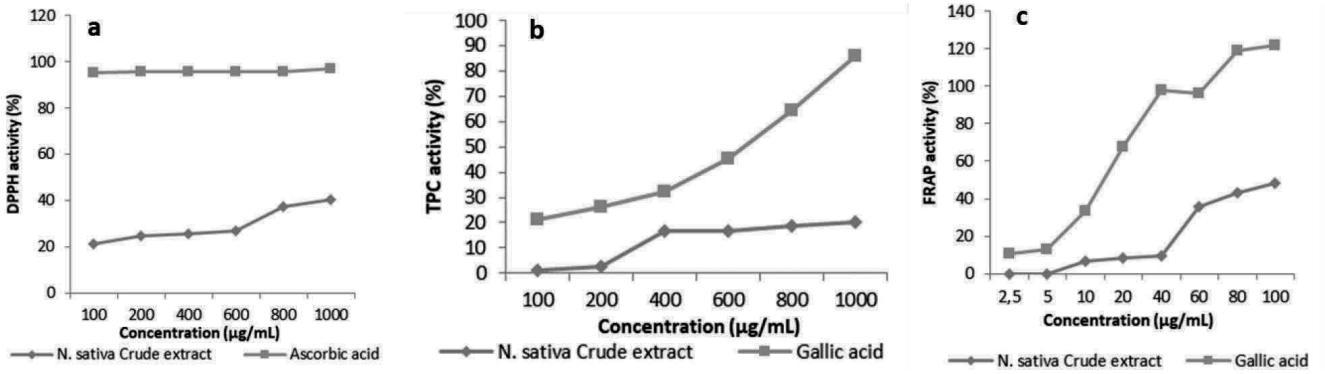



**Fig. 1**. Antioxidant potential of crude methanol extract of *N. sativa*: (a) DPPH activity; (b) Total phenolic content; (c) FRAP (%)

*Partitioning of the crude methanol extract of N. sativa seed*

The crude methanol extract of *N. sativa* seed was partitioned into fractions using different solvents: hexane, ethyl acetate, methanol and chloroform. The percentage yield of each *N. sativa* seed extract partitioned fraction (HEXFNs, CHLFNs, ETACFNs and METHFNs) is shown in Table 3. HEXFNs had the highest yield (60.17%) and CHLFNs had the least yield (0.42%).

Figure 2 shows the thin layer chromatograph of the fractions from the partitioned *N. sativa* seed extract using a polar solvent as the mobile phase. It was observed that the hexane fraction traveled the fastest and showed distinct bands indicating the presence of other compounds within that fraction. The hexane fraction was followed by the ethyl acetate fraction, which also showed distinct bands.

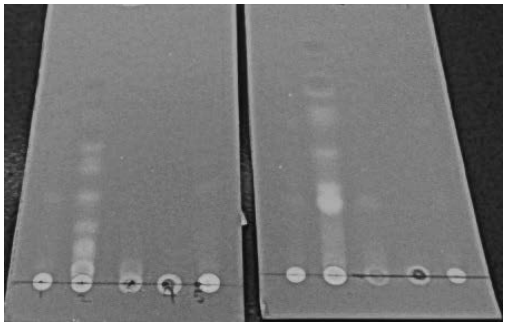


**Fig. 2.** Thin layer chromatograph of the fractions from the partitioned *N. sativa* seed extract. 1: chloroform fraction; 2: hexane fraction; 3: ethyl acetate fraction; 4: methanol fraction.

*Antimicrobial activity of crude and partitioned fractions of N. sativa seed extracts*

The antibacterial activity of the crude and fractions of *N. sativa* seed against the test bacteria is shown in Fig. 3a. The antimicrobial activity of the CMENs against the test bacteria ranged from 2.0 to 18.0 mm. *S. aureus* ATCC29213 had the highest susceptibility while *E. coli* ATTC22775 had the lowest. *P. aeruginosa* ATCC27853 was resistant to the extract. Antibacterial activity of HEXFNs and CHFLNs against the test bacteria ranged from 8.0 to 14.0 mm and 2.0 – 56.0 mm. *B*. sp. had the highest susceptibility to HEXFNs. *E. coli* ATCC35218 and *E. coli* ATCC11775 were not susceptible to HEXFNs. *S. aureus* ATCC29213 had the highest susceptibility to CHFLNs. All the test bacterial pathogens were susceptible to CHFLNs.

The antibacterial activity of ETACFNs and METFNs against the test bacteria ranged from 4.0 to 8.0 mm and from 0.0 to 2.0mm. *E. coli* ATCC11775 and *P. aeruginosa* ATCC27853 were not susceptible to ETACFNs. METHFNs did not exhibit antagonistic activity against most of the test bacteria. Only *S. aureus* ATCC 29213 was suscep-

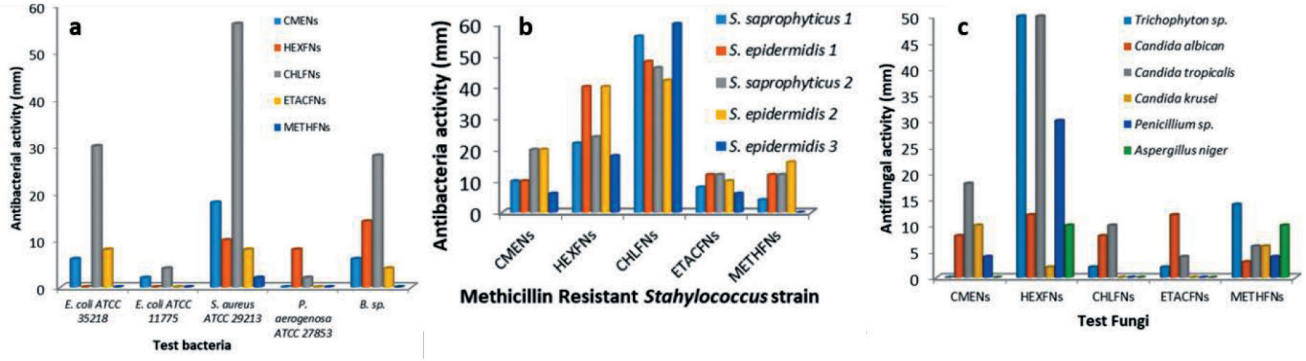



**Fig. 3.** Antimicrobial activities of crude and partitioned fractions of *N. sativa* seeds against (a) test bacteria; (b) methicillin-resistant *Staphylococcus* strains; (c) test fungi

tible to METFNs. The antibacterial activity of the CMENs against the test methicillin-resistant *Staph.* strains ranged from 6.0 to 20.0mm (Fig. 3b). *S. saprophyticus* 2 and *S. epidermidis* 2 had the highest susceptibility (20.0 mm) while *S. epidermidis* 3 had the least susceptibility. The antibacterial activity of HEXFNs and CHLFNs against the methicillin-resistant *Staph.* strains ranged from 18.0 to 40.0mm and 46.0 – 60.0mm, respectively. *S. epidermidis* 1and 2 had the highest susceptibility to HEXTNs. All test methicillin-resistant *Staphylococcus* strains were highly susceptible to CHLFNs, and *S. epidermidis* 3 had the highest susceptibility.

The antibacterial activity of ETACFNs and METHFNs against the methicillin-resistant strains ranged from 8 to 16.0 mm and from 4.0 to 16.0mm. *S. epidermidis* 1, *S. saprophyticus* 2 and *S. epidermidis* 2 had the highest susceptibility while *S. epidermidis* 3 was not susceptible to ETACFNs and METHFNS.

The crude extract and different fractions of *N. sativa* seed had varied antagonistic effect against most of the test bacteria and methicillin-resistant *Staphylococcus* strains. CHLFNs exhibited the highest antibacterial activity against nearly all of the test bacteria. *P. aeruginosa* ATCC27853 was not susceptible to CMENs, ETACFNs and METHFNs.

The antifungal activity of the crude and partitioned fractions of *N. sativa* seed extract against the test fungi is shown in Fig. 3c. All the test fungi showed varied susceptibility to the fractions of *N. sativa*. CMENs had antifungal activity against 66.67% of the test fungi while 33.3% were resistant. *C. tropicalis* had the highest susceptibility (18.0 mm) while *Penicillium* sp. had the lowest. HEXFNs exhibited antifungal activity against all the test fungi and the antifungal activity was in the range from 2.0 to 50.0 mm, with *Trichophyton* sp. having the highest susceptibility. CHLFNs had antifungal activity against 33.3% of the test fungi, of which *C. tropicalis* had the highest susceptibility (10.0 mm). *C. krusei, Penicillium* sp. and *A. niger* were not susceptible to CHLFNs. The antifungal activity of ETACFNs ranged from 2.0 to 12.0 mm, where *C. albican* had the highest susceptibility. METHFNs had antifugal activity against all the test fungi, of which *Trichophyton* sp. had the highest susceptibility (14.0 mm). *Trichophyton* sp. was susceptible to all the fractions except CMENs while *C. albican and C. tropicalis* were susceptible to all the fractions. *A. niger* was not susceptible to CMENs, CHLFNs, ETACFNs and METHFNs.

*GC-MS analysis of the crude and partitioned fractions of N. sativa seed extract*

The GC-MS analysis of crude and partitioned fractions of *N. sativa* seed extracts is shown in Table 3 a-e. Thirty-one peaks indicating the presence of 24 compounds were detected. The peak, retention time (mins), compound name, chemical formula, chemical structure, molar mass (g/mol) and peak area (%) of the chemical compounds identified from the crude and partitioned fractions of *N. sativa* seed extracts are shown in Table 4 a-e. 4, 8, 7, 9 and 3 different chemical compounds were identified from CMENs, METHFNs, ETACFNs, HEXFNs and CHLFNs, respectively. Among the 24 compounds identified, the HEXFNs had the highest number of compounds (9 compounds) identified. The CHLFNs had the least number of compounds with a total of 3 compounds identified.

The GC-MS analysis revealed the presence of 4 compounds: 9-Octadecenal (0.30%), 3-Octyne, 6-methyl- (0.21%), 9, 12-Octadecadienal (linolein aldehyde) (0.18%) and Trimethylsilyl-di(-timethylsiloxy)-silane (99.30%) in the crude methanol extracts of *N. sativa* seed extracts (CMENs). 7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin (0.11%), 2,6-Pyridinediamine (1.66%), 1-Nonylcycloheptane (0.25%), Cyclopentaneundecanoic acid (0.10%), 7,11-Hexadecadienal (0.12%), Oleic

**Table 3a**: GC-MC of the Methanol fraction of *N. sativa* seed extracts (METHFNs)

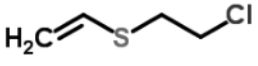
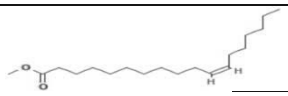
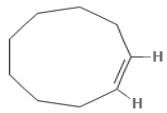
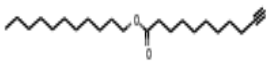
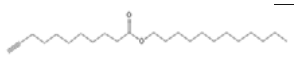
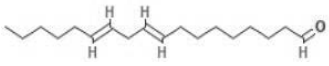
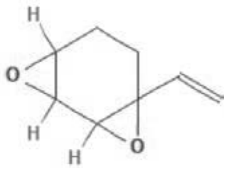
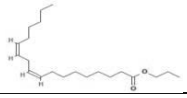
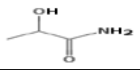
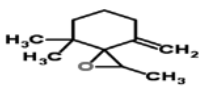
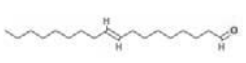
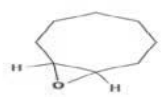
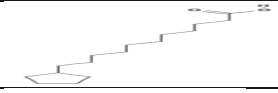
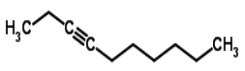
| № | Retention time [mins] | Compound name | Chemical formula | Chemical structure | Molar mass [g/mol] | Peak Area [%] |
|---|---|---|---|---|---|---|
| 1. | 34.103 | 2-Chloroethyl vinyl sulfide | $C_4H_7ClS$ |  | 122.616 | 0.58 |
| 2. | 55.974 | 11-Octadecenoic acid, methyl ester | $C_{19}H_{36}O_2$ |  | 296.4879 | 0.10 |
| 3. | 59.820 | Cyclononene | $C_9H_{16}$ |  | 124.227 | 0.19 |
| 4. | 61.835 | Undec-10-ynoic acid, undecyl ester | $C_{22}H_{40}O_2$ |  | 336.55 | 0.27 |
| 5. | 62.385 | Undec-10-ynoic acid, dodecyl ester | $C_{23}H_{42}O_2$ |  | 350.5784 | 0.32 |
| 6. | 67.037 | 9,12-Octadecadienal | $C_{18}H_{32}O$ |  | 264.453 | 0.17 |
|  | 67.404 | 3,8-Dioxatricyclo[5.1.0.0(2,4)] octane, 4-ethenyl- | $C_8H_{10}O_2$ |  | 138.166 | 0.47 |
|  | 96.602 | n-Propyl 9,12-octadecadienoate | $C_{21}H_{38}O_2$ |  | 322.533 | 96.90 |
|  | **Total** | - | - |  | - | **99.00** |

**Table 3b**: GC-MC of the Ethyl Acetate fraction of *N. sativa* seed extract (ETACFNs)

| Peaks | Retention time [mins] | Compound name | Chemical formula | Chemical Structure | Molar mass [g/mol] | Peak Area (%) |
|---|---|---|---|---|---|---|
| 1. | 12.305 | Propanamide | $C_3H_7NO$ |  | 73.095 | 0.20 |
| 2. | 38.169 | 1-Oxaspiro[2.5]octane, 2,4,4-trimethyl-8-methylene- | $C_{11}H_{18}O$ |  | 166.264 | 0.16 |
| 3. | 59.014 | Octan-2-one | $C_8H_{16}O$ |  | 128.215 | 0.19 |
| 4. | 61.872 | 9-Octadecenal | $C_{18}H_{34}O$ |  | 266.469 | 0.23 |
| 5. | 67.037 | 9-Oxabicyclo [6.1.0]nonane | $C_8H_{14}O$ |  | 126.199 | 0.18 |
| 6. | 67.404 | Cyclopentaneundecanoic acid | $C_{16}H_{30}O_2$ |  | 254.414 | 0.50 |
| 7. | 98.067 | 4-Decyne | $C_{10}H_{18}$ |  | 138.24992 | 98.51 |
|  | **Total** | - | - |  | - | **99.97** |

Acid(0.68%), cis-13-Octadecenoic acid (0.48%), 9-Octadecenal (0.18%) and Trimethylsilyl-di(-timethylsiloxy)-silane (96.425) were present in the hexane fraction of *N. sativa* seed extracts (HEXFNs).9,12-Octadecadienoyl chloride, (Z,Z)- (linoleic acid) (1.86%), 9,12-Octadecadienal (linolein aldehyde) (0.24%) and Trimethylsilyl-di(-timethylsiloxy)-silane (97.90%) were identified in the chloroform fraction of *N. sativa* seed extracts (CHLFNs). Propanamide (0.20%), 1-Oxaspiro[2.5] octane, 2,4,4-trimethyl-8-methylene- (0.16%), Octan-2-one (0.19%), 9-Octadecenal (0.23%), 9-Oxabicyclo[6.1.0]nonane(0.18%), Cyclopentaneundecanoic acid(0.50%) and 4-Decyne (98.51%) were

**Table 3c**: GC-MC of the Hexane fraction of *N. sativa* seed extract (HEXFNs)

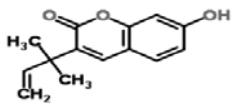
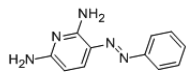
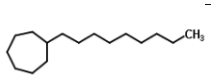
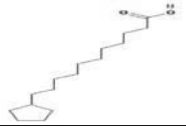
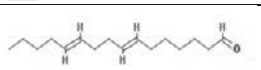
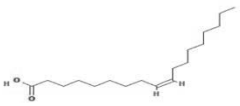
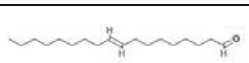
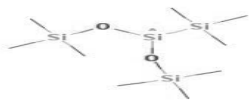
| Peaks | Retention time [mins] | Compound name | Chemical formula | Chemical structure | Molar mass [g/mol] | Peak Area [%] |
|---|---|---|---|---|---|---|
| 1 | 59.49 | 7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin | $C_{14}H_{14}O_3$ |  | 230.26 | 0.11 |
| 2 | 60.22 | 2,6-Pyridinediamine | $C_5H_7N_3$ |  | 109.13 | 1.66 |
| 3 | 62.97 | 1-Nonylcycloheptane | $C_{16}H_{32}$ |  | 224.43 | 0.25 |
| 4 | 63.70 | Cyclopentaneundecanoic acid | $C_{16}H_{30}O_2$ |  | 254.41 | 0.10 |
| 5 | 65.02 | 7,11-Hexadecadienal | $C_{16}H_{28}O$ |  | 236.39 | 0.12 |
| 6 | 67.00 | Oleic Acid | $C_{18}H_{34}O_2$ |  | 282.47 | 0.68 |
| 7 | 71.87 | cis-13-Octadecenoic acid | $C_{18}H_{34}O_2$ |  | 282.47 | 0.48 |
| 8 | 72.24 | 9-Octadecenal | $C_{18}H_{34}O$ |  | 266.47 | 0.18 |
| 9 | 94.07 | Trimethylsilyl-di(timethylsiloxy)-silane | $C_9H_{27}O_2Si_4$ |  | 279.65 | 96.42 |
| | **Total** | - | - | | - | **100** |

**Table 3d**: GC-MC of the Chloroform fraction of *Nigella sativa* seed extract (CHLFNs)

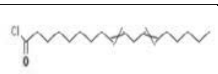
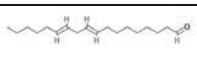
| Peaks | Retention time [mins] | Compound name | Chemical formula | Chemical Structure | Molar mass [g/mol[ | Peak Area [%] |
|---|---|---|---|---|---|---|
| 1. | 60.736 | 9,12-Octadecadienoyl chloride, (Z,Z)- (linoleic acid) | $C_{18}H_{31}ClO$ |  | 298.895 | 1.86 |
| 2. | 66.964 | 9,12-Octadecadienal (linolein aldehyde) | $C_{18}H_{32}O$ |  | 264.453 | 0.24 |
| 3. | 94.07 | Trimethylsilyl-di(timethylsiloxy)-silane | $C_9H_{27}O_2Si_4$ |  | 279.65 | 97.90 |
| | **Total** | | - | | - | **100** |

**Table 3e**: GC-MC of the Crude Methanolic extract of *N. sativa* seed extracts (CMENs)

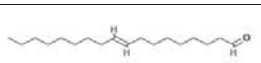
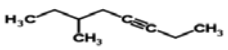
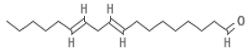
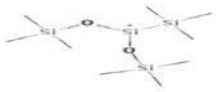
| Peaks | Retention time [mins] | Compound name | Chemical formula | Chemical structure | Molar mass [g/mol] | Peak Area [%] |
|---|---|---|---|---|---|---|
| 1. | 61.542 | 9-Octadecenal | $C_{18}H_{34}O$ |  | 266.47 | 0.30 |
| 2. | 67.038 | 3-Octyne, 6-methyl- | $C_8H_{14}$ |  | 110.2 | 0.21 |
| 3. | 67.367 | 9,12-Octadecadienal (linolein aldehyde) | $C_{18}H_{32}O$ |  | 264.453 | 0.18 |
| 4. | 91.290 | Trimethylsilyl-di(timethylsiloxy)-silane | $C_9H_{27}O_2Si_4$ |  | 279.65 | 99.30 |
| | Total | - | - | | - | 99.99 |

**Table 4**: Comparative evaluation of chemical compounds present in the crude and partitioned fractions of *N. sativa* seed extract

| No. | Compound name | Crude and partitioned fractions of N. sativa seed extract | | | | |
|---|---|---|---|---|---|---|
| | | CMENs | METHFNs | ETACFNs | HEXFNs | CHLFNs |
| 1. | 2-Chloroethyl vinyl sulfide | - | + | - | - | - |
| 2. | 11-Octadecenoic acid, methyl ester | - | + | - | - | - |
| 3. | Cyclononene | - | + | - | - | - |
| 4. | Undec-10-ynoic acid, undecyl ester | - | + | - | - | - |
| 5. | Undec-10-ynoic acid, dodecyl ester | - | + | - | - | - |
| 6. | 9,12-Octadecadienal | + | + | - | - | + |
| 7. | 3,8-Dioxatricyclo[5.1.0.0(2,4)] octane, 4-ethenyl- | - | + | - | - | - |
| 8. | n-Propyl 9,12-octadecadienoate | - | + | - | - | - |
| 9. | Propanamide | - | - | + | - | - |
| 10. | 1-Oxaspiro[2.5]octane, 2,4,4-trimetyl-8-methylene- | - | - | + | - | - |
| 11. | Octan-2-one | - | - | + | - | - |
| 12. | 9-Octadecenal | + | - | + | - | - |
| 13. | 9-Oxabicyclo[6.1.0]nonane | - | - | + | - | - |
| 14. | Cyclopentaneundecanoic acid | - | - | + | + | - |
| 15. | 4-Decyne | - | - | + | - | - |
| 16. | 7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin | - | - | - | + | - |
| 17. | 2,6-Pyridinediamine | - | - | - | + | - |
| 18. | 1-Nonylcycloheptane | - | - | - | + | - |
| 19. | 7,11-Hexadecadienal | - | - | - | + | - |
| 20. | Oleic Acid | - | - | - | + | - |
| 21. | cis-13-Octadecenoic acid | - | - | - | + | - |
| 22. | 9,12-Octadecadienoyl chloride, (Z,Z)-(linoleic acid) | - | - | - | - | + |
| 23. | Trimethylsilyl-di(timethylsiloxy)-silane | - | - | - | - | + |
| 24. | 3-Octyne, 6-methyl- | + | - | - | - | - |
| | Total | 3 | 8 | 7 | 7 | 3 |

identified in the ethyl acetate fraction of *N. sativa* seed extracts (ETACFNs). 2-Chloroethyl vinyl sulfide (0.58%), 11-Octadecenoic acid, methyl ester (0.10%), Cyclononene (0.19%), Undec-10-ynoic acid, undecyl ester (0.27%), Undec-10-ynoic acid, dodecyl ester (0.32%), 9,12-Octadecadienal (0.17%), 3,8-Dioxatricyclo[5.1.0.0(2,4)]octane, 4-ethenyl- (0.47%), n-Propyl 9,12-octadecadienoate (96.90%) were detected in the methanol fraction of *N. sativa* seed extracts (METHFNs).

Comparatively, Table 4 shows the compounds identified from the crude and partitioned fractions of *N. sativa* seed extract. Trimethylsilyl-di(-timethylsiloxy)-silane had the highest percentage of all the identified chemical compounds present in the CMENs (99.3%), HEXFNs (96.42%) and CHLFNs (97.90%) fractions, respectively. 4-Decyne (98.51%) has the highest percentage among the chemical compounds identified in ETACFNs. n-Propyl 9, 12-octadecadienoate (96.90%) has the highest percentage among the chemical compounds identified in METHFNs. Octadecadienal was identified from CMENs, METHFNs and CHLFNs fractions of *N. sativa* seed extract. 9-Octadecenal was identified from CMENs and ETACFNs fractions while cyclopentaneundecanoic acid was identified from ETACFNs and HEXFNs fractions of *N. sativa* seed extract.

**Discussion**

*N. sativa* is a useful medicinal plant with various applications and has been extensively researched (Khan *et al.*, 2011; Hussain and Hussain, 2016). In developing countries such as Nigeria people still depend heavily on traditional remedies as antidote against infectious diseases. The relatively low yield of the methanol extract of *N. sativa* obtained during this study may be attributed to the intrinsic characteristics of the particular seed purchased, the harvesting and processing conditions, and the extraction techniques (Abdurohaman, 2015). Nameer *et al*. (2016) and Anela *et al*. (2017) reported lower yield from solvent extraction when compared with other extraction techniques. The presence of different bioactive compound in the methanol seed extract of the *N. sativa* agreed with the report of Alaghemand *et al*. (2018) on leaf extracts of *N. sativa*. These bioactive compounds have been detected in many seeds with medicinal properties, such as sesame, dill, kalonji, flaxseed, chia, watermelon, pomegranate and pumpkin seeds as well as numerous other medicinal plants (Majorie, 1999; Gupta and Kaur, 2015).

The antioxidant, anticancer, analgesic, intercalating agents, flavoring and antimicrobial functions of the phytochemicals (saponins, tannins, alkaloids, terpenoids) have been reported (Majorie, 1999; Peng *et al*., 2015). Also, their biological properties, such as anti-apoptosis, anti-aging, anti-inflammatory, cardiovascular protection and improvement of the endothelial function have been reported (Majorie, 1999; Abdurohaman, 2015). The abundance of flavonoids reported in this study agreed with the report of Ishtiaq *et al*. (2013) on *N. sativa* methanol extracts. The methanol seed extract of *N. sativa* has antioxidant activity in varied concentrations. The high DPPH activity recorded in ascorbic acid compared to the methanol extract is similar to the report of Peschel *et al*. (2007) and Saleh *et al*. (2018). Several authors have reported similar observations using crude methanol extracts from different plants and plant products (Bourgou *et al*., 2008; Rajha *et al*., 2014).

The high antiradical activity of the crude methanol extract may be attributed to the presence of high phenolic content that perhaps functions as a free radical scavenger (Bourgou *et al*., 2008; Mousa-Ayoub *et al*., 2011).

The ferric ion scavenging activity (FRAP) of the extracts was dose-dependent. A similar direct dose-concentration relationship was reported by Chaudry *et al*. (2007) when pectin, and manganese were used as elicitors to determine the FRAP scavenging activity of *N. sativa* seed extracts. The susceptibility of the test pathogens to the crude methanol extract of *N. sativa* may be due to the presence of phytochemicals with antagonistic activity. This was in accordance with the findings of some researchers on the bioactivity of *N. sativa* seed extract against some pathogenic bacteria (Ishtiaq *et al*., 2013; Anela *et al*., 2017; Saleh *et al*., 2018). Enomoto *et al*. (2001) attributed the antimicrobial activity of the crude methanol extract of this seed to the presence of phenolic compounds responsible for phenolic toxicity against microbes, including enzyme inhibition by the oxidized compounds, probably via non-specific interactions with the proteins.

The high antimicrobial activity of the fractions against the test pathogen was similar to the findings of Gowhar *et al*. (2009), who reported the potency of the chloroform fraction of *N. sativa* seed in apoptosis in HeLa cells. The susceptibility of *S. aureus* ATCC29213 and resistance of *E. coli* ATCC 35218 to the fractions agrees with the findings of Alam *et al*. (2010), who reported susceptibility of *S. aureus* ATCC 103207 and resistance of *E. coli* ATCC12079

to chloroform fraction of *N. sativa* extract. Moreover, Hosseinzadeh *et al*. (2007) reported that there was no significant difference in the antibacterial activity of either chloroform or methanol fraction of *N. sativa* extract against some *S. aureus* and *E. coli.*

The susceptibility of the methicillin-resistant *Staphylococci* to the crude methanol extract of the seeds agrees with the findings of Erdogrul *et al*. (2008) and Saleh *et al*. (2018). High susceptibility of *C. albicans* and resistance of *Trichophyton* sp. to the extracts contrast with the report of Mohamed (2016). Detection of different chemical compounds in the samples by GCMS analysis is in line with the work of Nazeer and George (2017), who reported on compounds such as the chemical components of n-hexane extracts of *N. sativa* seeds. Trimethylsilyl-di(timethylsiloxy)-silane has been reported to have antimicrobial properties (Nagati *et al*., 2012).

## Conclusion

The crude methanol seed extract of *N. sativa* contains important bioactive compounds with antioxidant properties. The crude and the partitioned fractions exhibited varied and broad spectrum of antibacterial activity against some of the test standard bacteria strain, methicillin-resistant *Staphylococcus* spp. and some test fungi. The crude extract and the fractions of *N. sativa* seed extracts contain some important compounds.

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