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ANTIBACTERIAL, ANTIOXIDANT, CYTOTOXICITY AND QUALITATIVE PHYTO-CHEMICAL EVALUATION OF SEED EXTRACTS OF *NIGELLA SATIVA* AND ITS SILVER NANOPARTICLES

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ABSTRACT: *Nigella sativa* is considered a miracle herb plant belongs to the Ranunculaceae family, inhabitant to South Asia and South-west Asia. The most important part of the *Nigella sativa* plant used by human beings are seeds, mostly as a spice because it contains a specific aroma. The seeds derived from *Nigella sativa* being exploited by people and used for thousands of years for culinary purposes, to cure various digestive disorders, recover from joint pains, obesity, common cold, headache, asthma, rheumatic diseases, etc. in India, Pakistan, Bangladesh, China, and Middle East countries. In the current study, the seed extracts of *Nigella sativa* were targeted for evaluation of qualitative biochemical analysis of phytochemicals, antibacterial, cytotoxicity, and antioxidant properties. Nanoparticles formulations derived as of the aqueous extract of seeds of *Nigella sativa* were developed and tested against selected microorganisms. FTIR and HPLC also performed to evaluate the functional group analysis and to detect the presence of a bioactive compound in the phytochemical extract. Cytotoxicity properties of methanolic and ethanolic seed extracts of *Nigella sativa* have been evaluated against three cell lines; A549, SW480, and HeLa. In the current findings, bioactive constituents present in extracts were alkaloids, flavonoids, saponins, tannins, and phenolic compounds which are responsible for antioxidant activities, antibacterial, and cytotoxic effects. *N. sativa* seeds contain high valuable bioactive molecules which can perform a very significant role in curing various diseases and in development of the green drug and can be explored for immunomodulatory effects.

INTRODUCTION: *Nigella sativa* is mostly named as black seed or black cumin or kalonji seeds, and in Middle East countries, it is traditionally called as “Haba al-barakah” meaning seeds of blessings, belongs to the plant family Ranunculaceae.

Nigella sativa (Kalonji) is an important spice with specific taste and aroma; these seeds are used in different food products for flavor like in savoury dishes, bread recipes, and pickles. For many years, this plant has been using in most of the Middle Eastern, Asian and Far Eastern countries in making spicy food and food preservation, also used for protection and for remediation of health in folklore medicine for treating various disorders^{1, 2}. It has been told by the prophet Muhammad, Abu Hurairah that “black cumin seeds can be used to treat all the ailments except for death.” *Nigella sativa* seeds have been exploited for dietary and

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<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(11).4922-31</p>	

therapeutic determinations in several countries¹. The seed extract has the anti-cancerous properties, and its oil increases the bile flow, has important medicinal property in paralysis, diabetes, back pain, and rheumatoid. It is also used for treating many ailments like fever, headache, asthma, common cold, and migraine, etc., color externally black and white internally. It also has a pungent aromatic smell due to thymoquinone and bitter^{1,2}. *N. sativa* seeds constitute oils, proteins, alkaloids, saponins and essential oil³. The *N. sativa* can be used as an immunostimulatory, hepatoprotective and nephroprotective agent due to various toxic substances present in the environment⁴.

There are so many reports that reveal the presences of pharmacologically active components in the *N. sativa* are mainly nigellone, dithymoquinone, thymoquinone, thymol, and thymohydroquinone. *Nigella sativa* seed composition includes protein 20-23%, carbohydrate 23.5 to 32.3%, moisture content 5.52 to 7.43%, and ash 3.77 to 4.92.⁵ Other important compounds obtained when essential oil derived from *Nigella sativa* seeds were analyzed by GC/MS includes *p*-cymene, carvacrol 4-terpineol, tanethol, α -pinene, and longifolene have also been reported. The seeds of *N. sativa* are enriched with unsaturated fatty acids that include linolenic acid as major fatty acid, oleic acid, dihomolinoleic acid, and eicodadienoic acid, while the major constituents of saturated fatty acids are palmitic and stearic acid. The major sterol present in the seeds is α -sitosterol, which ranges between 44% and 54% of the total sterols^{3,6,7,8}.

Various studies have been reported about antibacterial and antifungal effects of seed extracts of *N. sativa*⁹; a study on diethyl extract of *N. sativa* seeds with different concentrations has been done to determine the inhibitory capability of different microbes that include one of the pathogenic fungi *Candida albicans*; and some pathogenic group of bacteria, such as, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* have been reported. The chloroform and methanol extracts have shown to have high inhibitory properties contrary to *S. aureus*, *P. aeruginosa*, and *Candida albicans*^{10,11}. Thymoquinone and thymohydroquinone are major components of seeds of *N. sativa*, which have been reported effective against various gram-positive and gram-negative

bacteria^{12,13}. Silver nitrate is generally used to stimulate antimicrobial effect, but silver nanoparticles improve the surface area accessible for the exposed microbes. The silver nanoparticles mainly have an effect on lysis of microbial cells or transduction inhibition of microbial cells. There are physical, chemical, and biological methods illustrated in various literature to produce silver nanoparticles^{14,15}.

The most common physical and chemical approaches are applied and several of these approaches are constrained or practices lethal substances which are key aspects that make them 'not so favored' process for the synthesis of silver nanoparticles. Gold nanoparticles of essential oil of *N. sativa* shown cytotoxic effects and antibacterial effects⁹. Alternate, viable method *i.e.* biological means by exploiting microorganisms and plants to synthesize silver nanoparticles became trendy which are less toxic as well as cost-effective^{14,15}.

The bioactive ingredients of seeds of *Nigella sativa* have also been reported to valuable against various kinds of cancer around the world, like extracts of *Nigella sativa* shown inhibitory effects on chemical carcinogenesis skin cancer¹¹. The bioactive complexes existing in the seeds of *N. sativa* vary based on geographical conditions and different varieties grown around the world¹⁶. The principle phytoconstituent compound of *N. sativa* is thymoquinone, which has been reported as leishmanicidal and cytotoxic potential¹⁷. The seed composition of *N. sativa* has shown the prominent effect to treat cardiovascular dysfunction¹⁸.

One of the bioactive compound α -hederin present in seeds of *Nigella sativa* has been reported showing cytotoxic and immunopotential effects, antitumor activity in Lewis lung carcinoma in BDF1 mice^{3,19,20}, effects of volatile oil derived from *N. sativa* shown anticancerous effects on colon cancer in rat²¹, thymoquinone has potential to induce the apoptosis in myeloblastic leukemia a type of blood cancer, in human hepatoma HepG2 cells *N. sativa* shown cytotoxic effects²².

In one of the recent research studies, thymoquinone and other constituents of *N. sativa* have been evaluated for prevention of Alzheimer's disease and shown auspicious results²³.

The seed constituents of *N. sativa* can be evaluated against various metabolic disorders at clinical trial level²⁴. Various toxicity effects can be minimized by using seed constituents of *N. sativa*²⁵. In the current research study, three cell lines A549, SW480, and HeLa have been exposed to ethanolic and methanolic extracts to find out cytotoxic effects.

MATERIALS AND METHODS:

Collection of Seed Material: The seeds of *N. sativa* were obtained from the local marketplace of Phagwara, Punjab, India and acknowledged and approved for the experimental purpose by a taxonomist in the Department of Botany, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India.

Phytochemical Extracts from *Nigella sativa* Seeds: 300 gm of *N. sativa* seeds were properly washed with tap water slowly and followed by distilled water thrice to avoid any dust particle and microbes on seeds, and then kept over blotting paper for air dry at room temperature for 24 h. Next day, 150 gm of seeds were crumpled into powder with the aid of grinder and packed into thimble and extraction was done in 200 ml of water, acetone, and methanol respectively. The extraction was carried out for 18-20 cycles in Soxhlet apparatus followed by Whatman paper filtration to get a clear solution. The rotatory evaporator was used at 50 °C to concentrate the extracts and then stored at 4 °C and used later for phytochemical analysis and antibacterial activity analysis^{11,26}.

Phytochemical Analysis: The phytochemical investigations were executed with different reagents of phytochemical tests which determines the existence or nonexistence of alkaloids, flavonoids, saponins, sugars, steroids, glycosides and tannins in different extracts^{26,27}.

Test for Flavonoids: Sodium hydroxide (10% aqueous) was added in 2 ml of the extract. It was heated to get warm, which produces a yellow coloration. Add 3-4 drops of dilute HCl. The color change from yellow to a colorless denotes flavonoid is present.

Test for Steroids: Salkowaski test was conducted to analyze the presence of steroids in extracts. To perform steroid analysis, chloroform (1 ml) mixed

with concentrated sulphuric acid (1 ml), this mix of chloroform and sulphuric acid added in 5ml of extract. After shaking for a few seconds, red color is observed in the chloroform layer; this color formation indicates steroids are present in the extract.

Test for Alkaloids: 10 ml of the solvent extract is mixed with 5 ml, 1.5% (v/v) of hydrochloric acid, and then the concoction was sieved appropriately. This filtrate can be further used for testing the occurrence of alkaloids.

Dragendorff's Test: In 1 ml of filtrate, little drops of Dragendorff's reagent were added. It leads to form an orange-brown color precipitate that indicates alkaloids are present.

Mayer's Reagent: In 1 ml of filtered extract in a test tube addition of few drops of Mayer's reagent resulting into formation of a creamy color precipitate which determines the presence of alkaloids.

Evaluation for Tannin: To 5 ml of filtrate extract, 1 ml of 5% ferric chloride solution is added. It is allowed to react for a few seconds. Then a dark green color is obtained, which indicates tannin is present.

Evaluation for Saponins: 1 ml solution of the extract is diluted using 20 ml of double distilled water. The sample was shaken in the test tube for 15 min, the development of stable foam was observed; this reveals the presence of saponins in extracts.

Evaluation for Carbohydrates: Following tests were performed for qualitative evaluation of carbohydrates in different extracts.

Molisch's Test: To 2 ml of the extract test sample, 4-7 drops of Molisch's reagent was added. Then test tube was kept in slant position, and a small amount of concentrated H₂SO₄ acid was added slowly at downwards without shaking. A purple color ring development between sulphuric acid and the test layer reflects as a positive result.

Barfoed's Test: 3 ml of Barfoed's reagent added to 2 ml of extract in a test tube. After heating the solution to boil; the red brick precipitate was

observed, this indicates the presence of monosaccharide or disaccharide.

Reducing Sugar:

Benedict's Test: After addition of 5ml of Benedict's reagent to 10ml of extract, the mixture is boiled for 2 min, and development of a brick-red colored precipitate designates the incidence of carbohydrates.

Fehling's Test: Equal parts of Fehling's solution A and Fehling's solution B, i.e. 0.5 ml each was added to 10 ml of plant extract, and it was boiled for few minutes, the brick red color precipitate development indicating the existence of reducing sugar in the extract.

Test for Glycosides

Molisch's Test: 2-3 drops of Molisch's reagent was added in 2 ml of extract and mixed. Then 2 ml of concentrated sulphuric acid was added carefully through the side of the test tube. A reddish- violet ring formation specifies the presence of glycosides.

Free Radical Scavenging Assay: The stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) was used for the antioxidant activity of the plant extracts based on the scavenging effect on free radical activity. 0.394 mg of DPPH was used to prepare a 0.1 mM solution in 10 ml ethanol. 5 test samples with varying extract volume of 200 μ l, 400 μ l, 600 μ l, 800 μ l, and 1000 μ l were prepared. All the samples were made up to 2000 μ l by adding 1000 μ l 0.1 mM DPPH solution and distilled water. The reaction mixture was shaken vigorously and incubated for 30 min. The absorbances of samples were measured at 517 nm. A blank sample was prepared by adding ethanol and DPPH. The evaluation of radical scavenging activities of the tested samples, articulated as a percentage of inhibition was calculated as

$$\text{Percent inhibition of DPPH activity} = [(A_B - A_A) / A_B] \times 100$$

Where, A_A = absorbance values of the test sample & A_B = absorbance values of the blank sample^{26, 27, 29}.

A percentage inhibition versus volume curve was plotted to check the efficiency of plant extracts. In fig 6, antioxidant activity is shown for different concentration of extracts vs. DPPH.

Antibacterial Assay: The antibacterial screening was performed by disc diffusion method; discs were prepared using Whatman filter paper-41. About 20 ml of Mueller Hinton agar was poured into a petri dish, kept for few minutes for solidification; the bacterial strain was swabbed upon the agar plate. The sterilized discs were infused with silver nanoparticles solution and positive control standard antibiotic discs were placed inverted on the swabbed plate. The antimicrobial activity was measured after 18-24 h of incubation at 37 °C. The zone of inhibition produced by the plant extracts discs were measured and compared with the standard antibiotics (Chloramphenicol and Gentamycin) in millimeter (mm)^{12, 26, 28, 29}.

Test Organisms: *Bacillus subtilus* (121), *Salmonella typhimurium* (3231), *Bacillus subtilus* (441), *Salmonella enteric* (1164), *Staphylococcus aureus* (96), *Bacillus subtilis* (1305), *Staphylococcus aureus* (7443), *Escherichia coli* (40), *Bacillus cereus* (430), *Bacillus cereus* (2086). These microorganisms were procured from Institute of Microbial Technology (IMTECH) Chandigarh, India and stored as per standard protocol for further uses.

Biosynthesis of Silver Nanoparticles: Extraction of *Nigella sativa* seeds is done by homogenization using pestle and mortar. The extract is filtered using Whatman filter paper. To the filtrate 1mM, AgNO_3 is added and stirred or mixed for 15 min to achieve uniform proper mixing.



FIG. 1: (A) SEEDS OF *N. SATIVA*, (B) EXTRACTION BY THE HOMOGENIZATION, (C) AgNPs FROM *N. SATIVA* SEEDS

The reaction mixture was kept for 24 h at standard chamber temperature. Then the darker brown color was observed, which reveal the formation of silver nanoparticles. Then, it was collected by centrifugation at 4000 rpm for 10 min where the AgNPs were settled down as a pellet after which it was dissolved in toluene-water solution followed by an air dry. In **Fig. 1** seed of *N. sativa*, homogenization process and nanoparticles synthesis process is shown.

UV-Visible Spectrophotometer: The reduction of pure silver (Ag^+) ions leads to the production of silver nanoparticles which was observed by measuring the absorption of the reaction standard in the range of 200-600 nm wavelength using UV-Vis spectrophotometer.

FTIR: FTIR for aqueous extract of *Nigella sativa* seeds and silver nanoparticles produced from its aqueous extract was performed. Sample preparation was done by mixing a few drops of the sample with potassium bromide (KBr), and the pellet was prepared using hydraulic pressure press. Then FTIR measurement was performed, and respective functional groups to specific peaks were analyzed.

High-Performance Liquid Chromatography (HPLC): HPLC investigation was achieved at Herbal Health Research Consortium Pvt. Ltd., Amritsar, Punjab, India. In this current research study, HPLC was performed with two standards which was reported earlier were Gallic Acid and Thymoquinone. Technical grade standards were used as 99% purity. The buffer solution was organized by adding 0.36 g of KH_2PO_4 in 1000 ml of water + 0.5 ml of orthophosphoric acid.

Gallic Acid as Standard: For a methanolic extract of *Nigella sativa*, the test solution was prepared by adding 510.73 mg in 100 ml of water. The diluent used was 100 ml of water, and the standard solution of gallic acid was prepared by adding 11.35 mg in 50 ml of water.

Injection volume was fixed 5 μ l, the flow rate was fixed at 1.20 ml/minute, and the spectrum was measured at 270 nm as per the standard protocol of HPLC. Similarly, the test solution for ethanolic extract 180.77 mg dissolved in 100 ml of water and run with standard gallic acid, here retention time was 4.507 and 4.5 min.

Thymoquinone as Standard: Water and ethanolic seed extracts of *N. sativa* were analyzed with Thymoquinone as standard.

The assay was calculated by using the following formula:

$$\frac{\text{Area of test solution}}{\text{Area of standard}} \times \frac{\text{Weight of standard (mg)}}{\text{Dilution factor (ml)}} \times \frac{\text{Vol of water (100 ml)}}{\text{Weight of test extract (mg)}} \times \text{STD Purity \%}$$

Cytotoxicity Assay: Cytotoxic assay was performed at CSIR - Institute of Himalayan Bioresource Technology (IHBT), Palampur, Himachal Pradesh, India (SOP:-ACC/IHBT-1A). Cell lines were maintained in Dulbecco's modified Eagle medium (DMEM), cytotoxic effects of *N. sativa* methanolic and ethanolic extracts were evaluated in a defined dose of 200 mg which was dissolved in dimethyl sulfoxide (DMSO). Cell lines were exposed to these extracts for 48 hours at 37 °C in 5% CO_2 incubator and compared with Vinblastin as positive control and DMSO as a negative control. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-biphenyl tetrazolium bromide (MTT) assay was performed to estimate the percent cell viability, after the formation of formazan due to cleavage of MTT by metabolically active cells was measured to quantify at 540 and 630 nm by using a spectrophotometer. The growth inhibition rate was calculated as the percentage of parallel negative controls by the following formula:

$$\% \text{ cell inhibition} = 100 - \{(At - Ab)/(Ac - Ab)\} \times 100$$

Where At is the absorbance value of test compound, Ab is the absorbance value of the blank, and Ac is the absorbance value of negative control. Data was prepared as per triplet of experiment observation using Microsoft Excel and all data are presented as a mean value with its standard deviation indicated (mean \pm SD). The morphological changes in treated cells with the test compounds at 48 h were observed, and images were captured using fluorescent microscope (Nikon Eclipse Ti-S) at 10X.

In **Table 3**, % cytotoxicity has been shown calculating mean and standard deviation and in **Fig. 7**, % cytotoxicity of methanolic and ethanolic extracts of *Nigella sativa* have been shown with DMSO as negative control and vinblastine as a positive control.

RESULTS AND DISCUSSION:

Phytochemical Analysis: The qualitative phytochemical screening of the water, methanol, and acetone derived extracts of the *Nigella sativa* seeds, tasters exposed the occurrence of alkaloids, flavonoids, steroids and tannins. The occurrence of tannins is most expected to be accountable for the antioxidant and anti-inflammatory belongings

recorded for this plant and the alkaloids are accountable for additional therapeutic assets. Similar findings were also reported earlier by Naz, 2011, Mraihi et al., 2013; Ahmad et al., 2013 and Piras et al., 2013. In **Table 1**, phytochemical tests result is shown related to the presence and absence of phytoconstituents in seed extracts.

TABLE 1: QUALITATIVE PHYTOCHEMICAL EVALUATION OF THE WATER, METHANOL AND ACETONE EXTRACTS OF NIGELLA SATIVA SEEDS

Phytoconstituents	Test	Result	Water extract	Methanol extract	Acetone extract
Alkaloids	Mayer's test	Creamy color precipitate	+	+	+
	Dragendorff's test	Dark orange brown precipitate	+ +	+	+
Steroids	Salkowski's test	Red color	+	+	+
	Flavonoids test	Yellow (NaOH) to colorless (HCl)	+	+	+
Tannins	Ferric chloride	Dark green precipitate	+	+	+
Saponin	Saponin test	Foam formation	+	+	+
Glycosides	Molish's test	No purple ring formation	-	-	-
Proteins and amino acids	Biuret test	Violet color	+	+	+
	Reducing sugar	Benedict's test	-	-	-
	Fehling's test	No brick red color	-	-	-

+ = present, - = absent

Synthesis of Silver Nanoparticles:

Visual Observation: The color change from light to brownish black color was noted by virtual observation after addition of the AgNO₃ aqueous solution to the *Nigella sativa* aqueous extract after incubation for overnight at room temperature (25°C). This brownish-black color means the formation of AgNPs of the *Nigella sativa* seeds aqueous extract.

UV-Vis Spectroscopy: The absorption spectrum of AgNPs synthesized from *Nigella sativa* aqueous extract was observed in the range of 200-600 nm with the highest peak at 290 nm, which means the production of silver nanoparticles. In **Fig. 2** spectra for AgNPs derived from seed extracts of *N. sativa* is shown. The size of small things ranges between 1 nm to 1 mm as per their applications in living systems or the non-living world. In living systems, these nanostructures play a vital role in physiological activities.

Antibacterial Activity Screening: Zone of inhibition was observed clearly on a Petri plate with a lawn of bacterial strains. The zone observed was compared with standard antibiotics Gentamycin (Gen) and chloramphenicol (Chl). This antibacterial activity is due to the presence of phytochemicals and polyphenols like alkaloid,

phenol, tannin, a flavonoid that enhances integration to the cell wall or membrane helping to kill bacteria.

Here acetone extract was found to be more potent than water and methanol extracts. In **Table 2**, the zone of inhibition for different extracts and standard antibiotics against various microorganisms is mentioned in millimeter (mm). Graphical representations of antimicrobial effects of extracts, antibiotics, and silver nanoparticles are shown in **fig 3**. Our current research study also agrees with antimicrobial activity of extracts of *N. sativa* as per findings of Bourgou et al., 2012 and Forouzanfar et al., 2014.

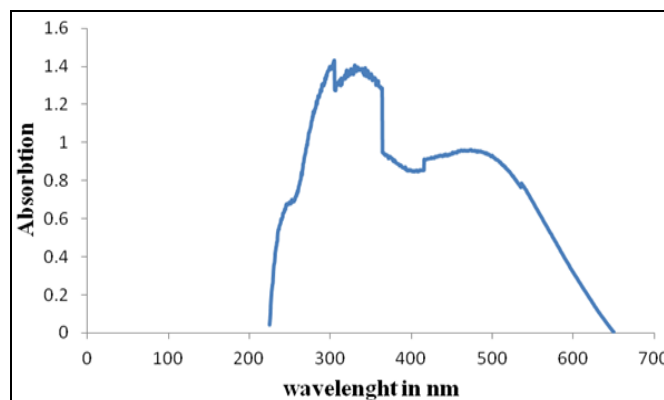
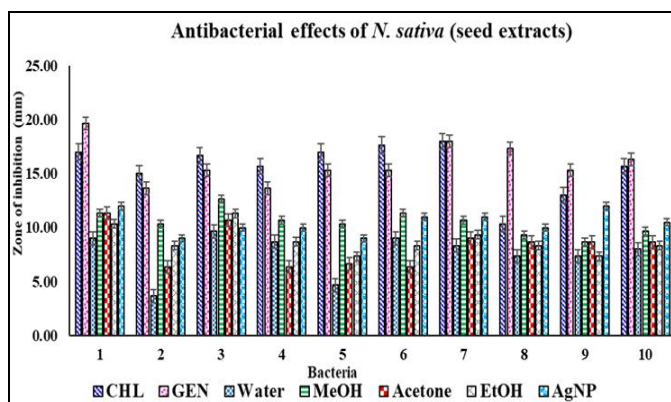


FIG. 2: UV SPECTRUM OF AgNPs DERIVED FROM SEED EXTRACTS OF N. SATIVA

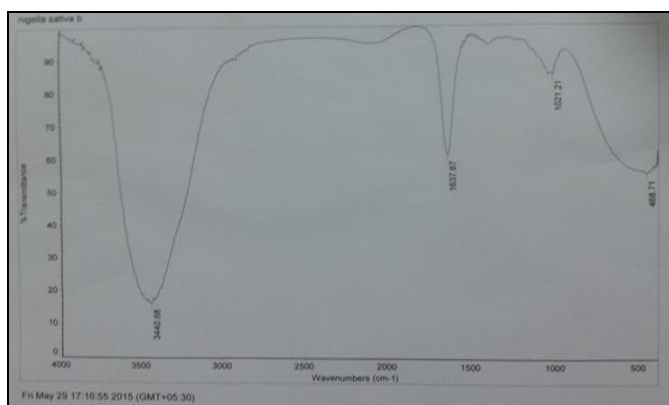
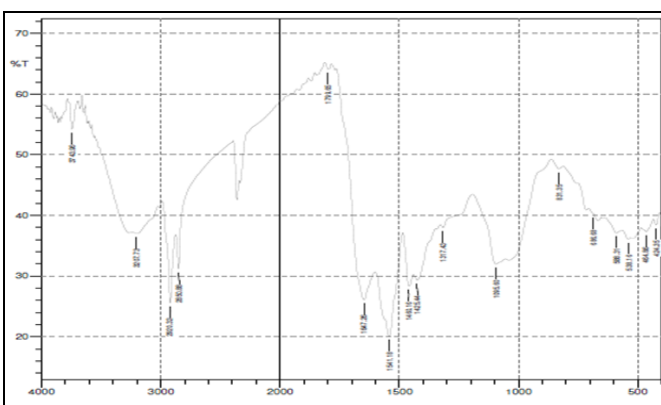
TABLE 2: COMPARISON OF ANTIBACTERIAL ACTIVITY TEST RESULT OF *NIGELLA SATIVA* SEEDS EXTRACT WITH CHLORAMPHENICOL AND GENTAMYCIN

Microorganisms	Chl	Gen	Water	Methanol	Acetone	AgNPs
<i>Bacillus subtilis</i> (121)	17.000	19.667	9.00	11.333	11.333	12
<i>Salmonella typhimurium</i> (3231)	15.000	13.667	3.667	10.333	6.333	9
<i>Bacillus subtilis</i> (441)	16.667	15.333	9.667	11.667	10.667	10
<i>Salmonella enteric</i> (1164)	15.667	13.667	8.667	10.667	6.333	10
<i>Staphylococcus aureus</i> (96)	17.000	15.333	4.667	10.333	6.667	9
<i>Bacillus Subtilis</i> (1305)	17.667	15.333	9.000	10.333	6.333	11
<i>Staphylococcus aureus</i> (7443)	18.000	18.000	8.333	10.667	9.000	11
<i>Escherichia coli</i> (40)	10.333	17.333	7.333	9.667	8.667	10
<i>Bacillus cereus</i> (430)	13.000	15.333	9.333	9.33	8.000	12
<i>Bacillus cereus</i> (2086)	15.667	16.333	6.33	9.667	8.333	10.5

Standard Error Mean \pm Standard Deviation.**FIG. 3: ANTIBACTERIAL ACTIVITY OF *N. SATIVA* EXTRACTS ASSESSMENT WITH STANDARD ANTIBIOTICS.** (In graph 1-10 is name of bacteria, 1- *Bacillus subtilis* (121), 2- *S. typhimurium* (3231), 3- *B. subtilis* (441), 4- *Salmonella enteric* (1164), 5- *Staphylococcus aureus* (96), 6- *Bacillus Subtilis* (1305), 7- *Staphylococcus aureus* (7443), 8- *Escherichia coli* (40), 9- *Bacillus cereus* (430), 10- *Bacillus cereus* (2086). Standard Error Mean \pm Standard Deviation)**FTIR Measurements:** The FTIR spectrum analysis was achieved to classify biomolecules

existing in aqueous extract of *Nigella sativa* seeds accountable for the development and stabilization of nanoparticles. The absorption spectrum of *Nigella sativa* was found at 3440.68 cm^{-1} , 1637.67 cm^{-1} , 1021.21 cm^{-1} and 468.71 cm^{-1} . The intense peak at 3440.68 cm^{-1} indicates the stretching O-H group, which confirms the presence of phenols, primary and secondary alcohols.

The peak at 1637.67 cm^{-1} indicates the presence of N-H bend due to primary amines. The peak at 1021.21 cm^{-1} is because of the C-N stretch of aliphatic amines. The amide (NH) and hydroxyl (OH) groups are responsible for stabilization of AgNPs of *Nigella sativa* seeds aqueous extract. In **Fig. 4A**, FTIR spectrum of an aqueous extract derived from seeds of *Nigella sativa* is shown and in **Fig. 4B**, AgNPs derived from seed extract of *Nigella sativa* is shown.

**A) FTIR spectrum of water extract of *Nigella sativa*****B) FTIR spectrum of AgNPs derived from *N. sativa*****FIG. 4: FTIR SPECTRUM: A) AQUEOUS EXTRACT B) AgNPs DERIVED FROM *N. SATIVA***

Antioxidant Activity: DPPH scavenging activity is shown in **Fig. 5** concerning different concentration of water extracts and silver

nanoparticles derived from water extracts. Scavenging activity is shown in the form of a percentage (%) of inhibition.

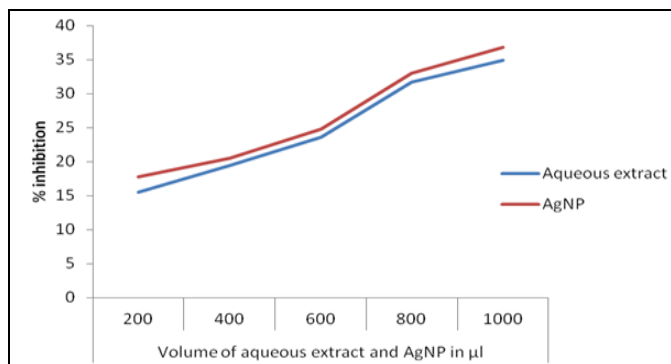


FIG. 5: % INHIBITION OF NIGELLA SATIVA AQUEOUS EXTRACT AND IT'S AgNPs

HPLC Analysis:

i) The calculation for a methanolic extract of *N. sativa* with Gallic acid as standard, as shown in Fig. 6A).

$$2738007/5816657 \times 11.35/50 \times 100/510.73 \times 99 = 2.07\%$$

ii) The calculation for ethanolic extract of *Nigella sativa* with Gallic acid as standard, as shown in Fig. 6B).

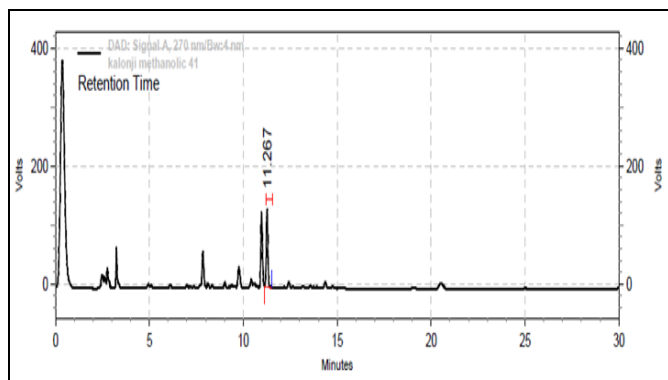
$$3155738/5816657 \times 11.35/50 \times 100/180.77 \times 99 = 6.74\%$$

iii) The calculation for water extract of *Nigella sativa* with Thymoquinone as standard as shown in Fig. 6C).

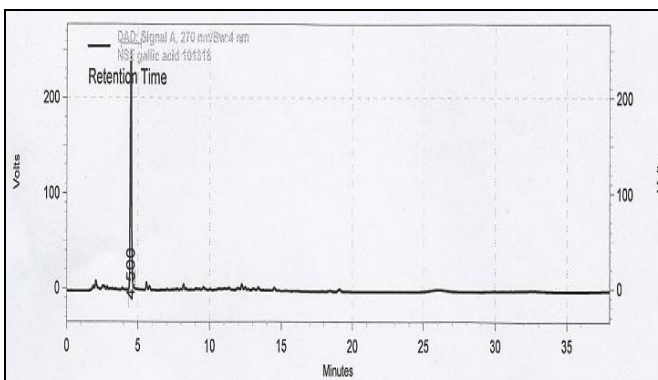
$$1534232/2102843 \times 5.2/10 \times 1/10 \times 10/10.20 \times 99 = 3.68\%$$

iv) The calculation for ethanolic extract of *Nigella sativa* with Thymoquinone as standard, as shown in Fig. 6D).

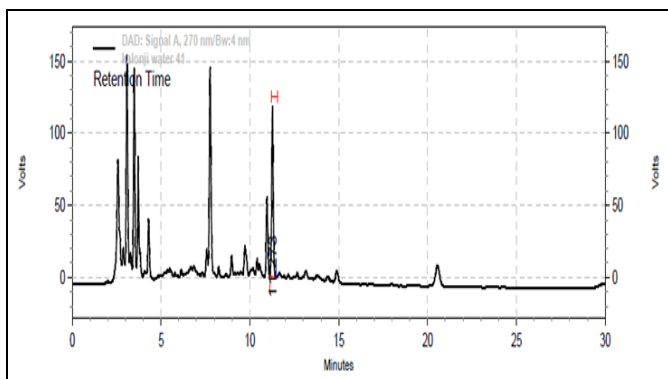
$$181336/2102843 \times 5.2/10 \times 1/10 \times 10/11.3 \times 99 = 3.93\%$$



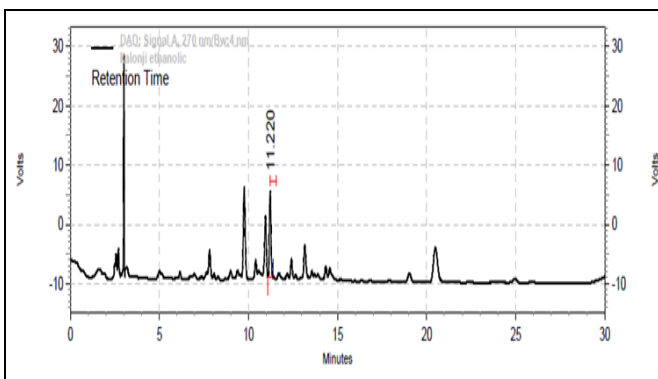
A) Methanolic extract of *N. sativa* with Gallic acid as standard



B) Ethanolic extract of *Nigella sativa* with standard gallic acid



C) Water extract of *N. sativa* with Thymoquinone as standard



D) Ethanolic extract of *N. sativa* with thymoquinone as standard

FIG. 6: A, B, C & D ARE HPLC SPECTRUM OF EXTRACTS OF *N. SATIVA* WITH STANDARD GALLIC ACID AND THYMOQUINONE

TABLE 3: % CYTOTOXICITY RESULTS OF METHANOLIC AND ETHANOLIC EXTRACTS OF *N. SATIVA*

Sample	Concentration	SW480	A549	HeLa
Vinblastine (IHB-NPP-FF-1374/1375/1376)	8.1098 µg/ml	95.4 ± 0.3	85.5 ± 3.4	79.7 ± 0.5
NSM	200 µg/ml	99.3 ± 0.8	99.3 ± 0.1	35.6 ± 1.2
NSE	200 µg/ml	0.0 ± 2.9	0.0 ± 1.3	0.0 ± 5.6

SEM ± SD

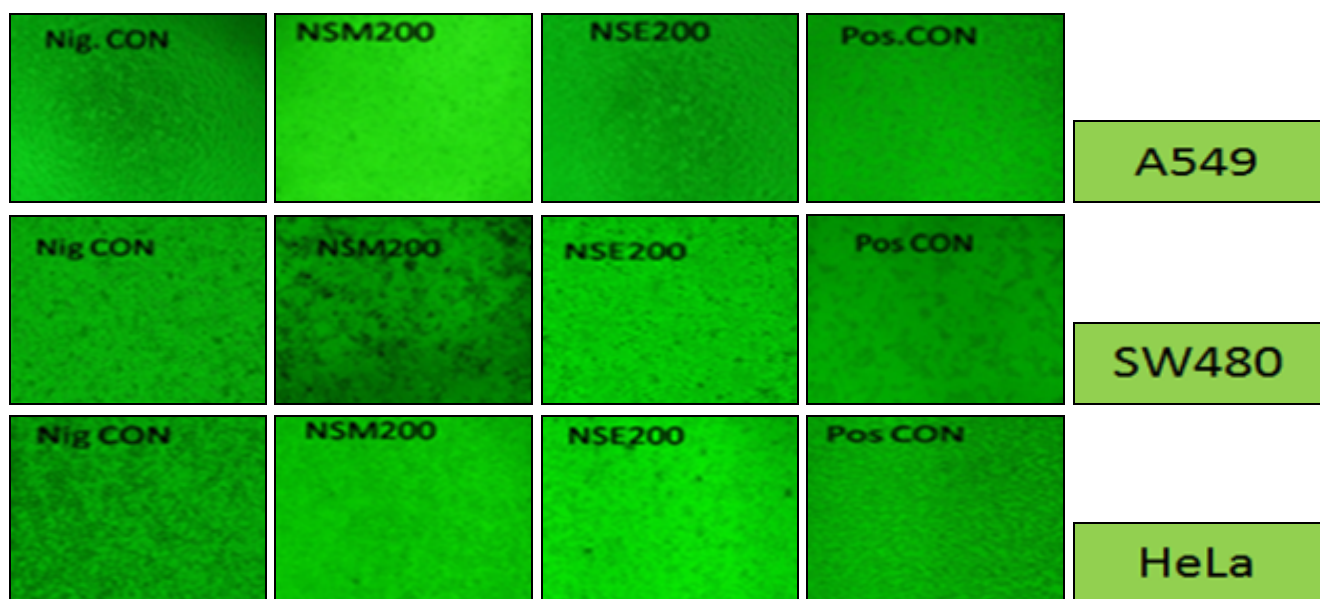


FIG. 7: IMAGES OF DIFFERENT CELL LINES EXPOSED WITH NSM AND NSE EXTRACTS FOR 48 h

CONCLUSION: Extracts of *Nigella sativa* are found to contain various bioactive compounds like alkaloid, flavonoid, steroid, and tannin. Concentrated extracts of *Nigella sativa* seeds can act as effective antimicrobial activity against various bacterial strains. Acetone extract is found to be more potent in antibacterial activity. Silver nanoparticles from aqueous extract were synthesized and confirmed by UV spectrophotometer and FTIR. Presence of phenols, -OH, -NH in aqueous extract of *Nigella sativa* seeds render to synthesize active AgNPs. DPPH absorbs free hydrogen ion (H^+) from aqueous extracts, and the color changes from deep violet to yellowish colour and shows the highest absorption at 517 nm.

More absorption of H^+ leads to more yellowish color hence less absorption spectrum indicating higher % inhibition. From this, we can conclude that both the extracts of *Nigella sativa* and aqueous-AgNPs has high antioxidant activity, and it increases as volume increases while AgNPs bear more free radical scavenging property. HPLC analysis has revealed that the presence of gallic acid and thymoquinone in seed extracts, which play a varied role in metabolic activities. In *in-vitro* cytotoxicity methanolic extracts have been found more effective while ethanolic extracts have not shown significant cytotoxic effects on cell lines used in the current study. So, *Nigella sativa* extracts can be explored further in immunomodulation activities, like antitoxic effects,

anticancerous effects in *in-vitro* studies as well as in *in-vivo* conditions.

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REFERENCES:

1. Hajra N: *Nigella sativa*: the miraculous herb. Pak J Biochem Mol Biol 2011; 44 (1): 44-48.
2. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Damanhoury ZA and Anwar F: A review on the therapeutic potential of *Nigella sativa*: a miracle herb. Asian Pac J Trop Biomed 2013; 3: 337-52.
3. Piras A, Rosa A, Marongiu B, Porcedda S, Falconieri D, Dessì MA, Ozcelik B and Koca U: Chemical composition and in vitro bioactivity of the volatile and fixed oils of *Nigella sativa* L. extracted by supercritical carbon dioxide. Industrial Crops and Products 2013; 46: 317-23.
4. Srinivasan K: Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: traditional uses, chemical constituents, and nutraceutical effects. Food Quality and Safety 2018; 2(1): 1-16.
5. Forouzanfar F, Bazzaz BSF and Hosseinzadeh H: Black cumin (*Nigella sativa*) and its constituent (thymoquinone):

- a review on antimicrobial effects. Iranian Journal of Basic Medical Sciences 2014; 17 (12): 929-38.
6. ALHaj NA, Shamsudin MN, Alipiah NM, Zamri HF, Bustamam A, Ibrahim S and Abdullah R: Characterization of *Nigella sativa* L. essential oil loaded solid lipid nanoparticles. American Journal of Pharmacology and Toxicology 2010; 5(1): 52-57.
 7. Khader M and Eckl PM: Thymoquinone: An emerging natural drug with a wide range of medical applications. Iran J Basic Med Sci 2014; 17(12): 950-57.
 8. Khan MA and Afzal M: Chemical composition of *Nigella sativa* L.: Part 2 recent advances. Inflammopharmacology 2016; 24: 67-79.
 9. Manju S, Malaikozhundan B, Vijayakumar S, Shanthi S, Jaishabanu A, Ekambaram P and Vaseeharan B: Antibacterial, antibiofilm and cytotoxic effects of *Nigella sativa* essential oil coated gold nanoparticles. Microbial Pathogenesis 2016; 91: 129-35.
 10. Singh S, Das SS, Singh G, Schuff C, de Lampasona MP and Catalán CA: Composition, *in-vitro* antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black cumin seeds (*Nigella sativa* L.). Bio Med Research International 2014; 1-10.
 11. Emeka LB, Emeka PM and Khan TM: Antimicrobial activity of *Nigella sativa* L. seed oil against multi-drug resistant *Staphylococcus aureus* isolated from diabetic wounds. Pakistan Journal of Pharmaceutical Sciences, 2015; 28(6): 1985-90.
 12. Halawani E: Antibacterial activity of thymoquinone and thymohydroquinone of *Nigella sativa* L. and Their Interaction with Some Antibiotics, Advances in Biological Research 2009; 3: 148-52.
 13. Shaaban HA, Sadek Z, Edris AE and Saad-Hussein A: Analysis and antibacterial activity of *Nigella sativa* essential oil formulated in microemulsion system. Journal of Oleo Science 2015; 64(2): 223-32.
 14. Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paraliker KM and Balasubramanya RH: Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Mater Lett 2007; 61: 1413-18.
 15. Karmakar S, Kundu S and Kundu K: Bioconversion of silver salt into silver nanoparticles using different microorganisms. Artificial Cells, Nanomedicine and Biotechnology 2010; 38 (5): 259-66.
 16. Ahmad N, Ahmad R, Al-Layly A, Al-Shawi H, Al-Ali A, Amir M and Mostaf A: Ultra-High-Performance Liquid Chromatography-based identification and quantification of thymoquinone in *Nigella sativa* extract from different geographical regions. Pharmacognosy Magazine 2018; 14(57): 471-80.
 17. Mahmoudvand H, Tavakoli R, Sharififar F, Minaie K, Ezatpour B, Jahanbakhsh S and Sharifi I: Leishmanicidal and cytotoxic activities of *Nigella sativa* and its active principle, thymoquinone. Pharmaceutical Biology 2015; 53(7): 1052-57.
 18. Shakeri F, Khazaei M and Boskabady MH: Cardiovascular Effects of *Nigella sativa* L. and its constituents. Indian Journal of Pharmaceutical Sciences 2018; 80(6): 983-71.
 19. Swamy SM and Tan BK: Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seed. J Ethnopharmacol 2000; 70: 1-7.
 20. Randhawa MA and Alghamdi MS: Anticancer activity of *Nigella sativa* (black seed)-a review. The American Journal of Chinese Medicine 2011; 39(06): 1075-91.
 21. Salim EI and Fukushima S: Chemopreventive potential of volatile oil from black cumin (*Nigella sativa* L.) seeds against rat colon carcinogenesis. Nutr Cancer 2003; 45: 195-02.
 22. Thabrew MI, Mitry RR and Morsy MA: Cytotoxic effects of a decoction of *Nigella sativa*, *Hemidesmus indicus* and *Smilax glabra* on human hepatoma HepG2 cells. Life Sci 2005; 77: 1319-30.
 23. Cascella M, Bimonte S, Barbieri A, Del Vecchio V, Muzio MR, Vitale A, Benincasa G, Ferriello AB, Azzariti A, Arra C and Cuomo A: Dissecting the potential roles of *Nigella sativa* and its constituent thymoquinone on the prevention and the progression of Alzheimer's disease. Frontiers in Aging Neuroscience 2018; 10: 16.
 24. Tavakkoli A, Mahdian V, Razavi BM and Hosseinzadeh H: Review on clinical trials of black seed (*Nigella sativa*) and its active constituent, thymoquinone. Journal of Pharmacopuncture 2017; 20(3): 179.
 25. Canayakin D, Bayir Y, Kolic Baygutalp N, Sezen Karaoglan E, Atmaca HT, Kocak Ozgeris FB, Keles MS and Halici Z: Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of *Nigella sativa*. Pharmaceutical biology 2016; 54(10): 2082-91.
 26. Kumar A, Anu, Mohan A, Sharma NR and Rehman H: Antibacterial, antioxidant analysis of phytochemical extracts derived from seeds of *Syzygium cumini* L. against pathogenic bacteria. Research J Pharm and Tech 2017; 10(8): 2707-14.
 27. Kazemi M: Phytochemical composition, antioxidant, anti-inflammatory and antimicrobial activity of *Nigella sativa* L. essential oil. Journal of Essential Oil Bearing Plants 2014; 17(5): 1002-11.
 28. Bourgou S, Pichette A, Marzouk B and Legault J: Antioxidant, anti-inflammatory, anticancer and antibacterial activities of extracts from *Nigella sativa* (Black Cumin) plant parts. J Food Biochem 2012; 36: 539-46.
 29. Mraih F, Journi M, Chérif JK and Trabelsi-Ayadi M: Characterization of three *Nigella sativa* L. crude oil species, measures of their antioxidant activity by DPPH. Journal of Biologically Active Products from Nature 2013; 3(3): 208-15.

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