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EVALUATION OF ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF THE CRUDE EXTRACT OF *NIGELLA SATIVA* SEEDS ON THE BACTERIAL ISOLATES OF WOUND

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ABSTRACT

Background: *Nigella sativa* (Black cumin, *Habbatussawda*) is a medicinal herb used in the treatment of many infectious and non infectious ailments. **Objective:** To investigate the effect of the antibacterial activities of the crude extracts of this plant was compared against that of Ciprofloxacin and Pefloxacin on seven wound associated bacteria. **Materials and Methods:** This study was conducted as cross sectional experimental study. The susceptibility pattern of the crude extract, fractions and the standard antibiotics were determined using Kirby-Bauer disc diffusion technique. MIC of the extract, fractions and the standard antibiotics were determined using Macro broth dilution techniques. Separation of the crude extract was carried out using column chromatography and the LD_{50} of the extract was determined using Lorke's method. Data were analyzed using SPSS version 20.0 (California, Inc., USA) and $p < 0.05$ was considered significant. **Results**- Highly significant antibacterial activity was observed with the Methanolic seed extract on Coagulase negative

Staph (at 10, 5 and 1mg/disc, F = 0.001, F = 12.4). No any significant activity was observed with the crude diethyl ether extract at all concentrations. The most highly insignificant activity was observed on *P. aeruginosa* (F = 0.074, P = 2.10). Similarly, no any significant difference was observed between the MICs and MBCs of the crude Methanolic seed extract when compared with that of Ciprofloxacin. An oral acute toxicity studies had shown an LD_{50} of the crude Methanolic seed extract to be >5000mg. **Conclusion:** Findings of the present study revealed that the overall result of the present study provided evidence that the crude extracts of *Nigella Sativa seeds* as well as some of the fractions (especially TF5; fifth fraction of *N.sativas*) could be considered as a potential source of novel antibacterial agents which may be employed to forestall the present antibiotic resistance menace pending further work on drug development processes.

KEYWORDS: Antibacterial activity. Nigella Sativa Seeds, Bacterial isolates of Wound.

INTRODUCTION

Nigella sativa is an annual growing plant in the dry temperature regions of the world and belong to the family Rananclaceae. The plant was found to be of economic and agricultural importance and has the ability to resist pathogenic micro-organism. According to research conducted which revealed that N.staiva has multi-actions in almost all known ailments of various body systems of humans, which include bronchitis, asthma, cough, hypertension, diabetes, fever and headache.[1,2]

Since ancient civilization, natural sources especially plants are used as medicinal therapy because they contain several components which are believed to cure various infectious diseases. the biodiversity of plants provides an important source of chemical compounds, which have many therapeutic application such as antiviral, antibacterial, antifungal and anticancer activities.[3]

Nigella sativa is a herbaceous plant which is better known as black seed, a habitat of Southeast Asia and Mediterranean 144 countries. Indian folks used this plant as a food preservative as well as a protective and curative treatment for numerous disorders.[4] The black seeds contain 36–38% fixed oil, with proteins, alkaloids, saponins and essential oils making up the rest of the composition.[5] Although black seed extract or oil has been reported to possess antimicrobial activity[1], antioxidant activity[5], antitumor activity[6] and a stimulatory effect on the immune system[7], its full potential as an antimicrobial agent has not

been exploited. this current study was conducted to investigate the antibacterial activity of the seed extract of Nigella sativa against pathogenic isolates of bacteria. the results of this study may further strengthen the recommendation for the use of ethno-medicine in the treatment and control of microbial infections.

On the other hand, plants are traditionally proved to be a rich source of novel drug compound. The herbal mixtures have made large contributions to human health and well-being.[8] A wide variety of secondary metabolites such as tannins, tarpenoids, flavonoids, alkaloids, quinines etc. are endowed with antimicrobial properties.[9,10] The black seed (*Nigella sativa*) which is known throughout the world with different names such as seed of blessing (*habbat-ul baraka*), *habbatussauda* (in hausa), black cumin, black caraway, Kalonji etc is claimed to be one of these agents proven to have multi range of actions covering both antibacterial[11] and anticestodal effects.[12] It grows widely in the Western Asia and the Mediterranean region.

In Nigeria, *N. sativa* seeds and its oil have been used traditionally in combination with other medicinal plants (honey, olive oil etc) in the remedy of various ailments ranging from digestive disorders, gynaecological disorders, Asthma, dyspnoea and wound.[13]

Among various medicinal spices Nigella sativa L. (family Ranunculaceae) has emerged as a miraculous herb with a wide spectrum of pharmacological activities. N. sativa seeds are most extensively studied, both phytochemically and pharmacologically. Both seeds and oils are known to possess various health properties like antitumor activity[15], antioxidant activity[16], anti-inflammatory activity[17], antibacterial activity[18], and a stimulatory effect on the immune system[19] because of which they are of tenuse as nutritional supplement.

Commercial antibiotics and synthetic pesticides used for human and crop protection is harmful to human health, ecosystem and environment. Black seed extracts have also proved to be potent antimicrobial agents against certain pathogenic Gram positive and Gram negative bacteria.[20]

The rise of antibiotic resistance is a global health crisis and governments now recognise it as one of the greatest challenges for public health. It is reaching dangerously high levels in all parts of the world. Antibiotic resistance is compromising the ability of treating infectious diseases and undermining many advances in Medicine.[21]

This present study intends to investigate the antibacterial potentials of the crude Methanolic and diethyl ether extract of *Nigella sativa* seeds on bacterial isolates from wound infections.

MATERIALS AND METHODS

Study Area

Specialist Hospital Sokoto is a government owned Hospital located within the Sokoto metropolis. Sokoto metropolis lies between latitude 13^0 3^1 490N, longitude 5^0 14^1 890E and at an altitude of 272 m above sea level. It is located in the extreme north western part of Sokoto north and Sokoto South Local government areas and also some parts of Kware LGA from the north, Dange-Shuni LGA from south and Wamakko LGA to the west.

Sokoto metropolis was estimated to have a population of 427,760 people[22] with Hausa and Fulani been the dominant ethnic groups followed by Zabaarmawa, Adarawa, Arawa, Kabawa, Nupes with Yorubas, Igbos and others. The major occupations of the inhabitants are; trading, commerce and farming with reasonable proportion of the population working in both public and private sectors.[23]

Specialist Hospital Sokoto is the referral centre of choice by clients from rural areas and many cases of open wounds are mostly seen there than the other tertiary Hospitals. The Hospital serves as referral centre for almost all the Local government areas (LGAs) across the State; it also serves many clients from Niger republic.3

Study Population

The specimens (wound swab and aspirates) were collected from the population of male and female patients with purulent wound seen in surgical outpatient department (SOPD), Female and Male surgical wards of the Specialist Hospital Sokoto after a verbal/written consent of the patients or their relatives was obtained.

Inclusion and Exclusion Criteria

The inclusion criterion covers both male and female patients with purulent wound seen in the surgical out-patient department (SOPD), Male and Female surgical wards of Specialist Hospital Sokoto whose verbal consent were duly obtained. All patients outside the aforementioned category were excluded from this study.

Ethical Issues

Prior to commencement of sample collection an ethical approval was obtained from the ethical committee under the Chairman Medical advisory committee (CMAC), Specialist Hospital Sokoto. Similarly, verbal consents were obtained from patients and their relatives prior to sample collection; this was done via an explanation of the aims and objectives of the study to the patients and their relatives. Patients' privacy and safety are well guarded throughout the period of this study.

Specimen Collection

Specimens (wound swab and aspirates) were collected aseptically using a sterile swab stick and syringes. The wound surfaces were first cleaned with cotton wool soaked in sterile normal saline followed by swabbing the centre of the affected tissues using sterile swab stick. The aspirates were collected by needle aspiration. Prior to the aspiration, the skin around the wound were cleaned with 70% alcohol to get rid of the contaminating microbes and the fluid was collected by inserting the needle deep into the wound changing its angle two to three times to remove fluid from different areas of the wound. The samples were labelled, registered, transported immediately to the Laboratory and processed.

ANALYTICAL METHODS

Bacterial Isolation

Specimens were aseptically cultured onto Blood agar, Chocolate agar and MacConkey agar. Aerobic culture (for the swabs) was carried out on blood and MacConkey agar whereas the anaerobic cultures (for the aspirates) were done on Chocolate agar which was placed inside a candle jar prior to incubation.

The inoculated plates were incubated at 37^0C for 24hours. Cultures were identified on the basis of their physical appearance; Gram's staining reaction and biochemical characteristics using Microgen (Microgen Bio-products) biochemical test system for the Gram negative organisms and Catalase, Coagulase, PYR test (pyrolidonyl arylamidase also called pyrolidonyl aminopeptidase), etc for the Gram positive organisms.

Plant Collection and Identification

The *N. sativa* seed was purchased from herbal drug shop in the Sokoto main Market. The seed was identified and authenticated in the herbarium of Biological Sciences Department,

Usmanu Danfodiyo University Sokoto by Abdulazeez Salihu where voucher specimen (UDUH/ANS/0106) of the *N.sativa* seed was deposited.

Preparation and Extraction

The seeds were milled to powder using mortar and pestle in the department of Pharmacognosy, Usmanu Danfodiyo University, Sokoto and stored at room temperature with plastic packaging until use.

Extraction of the Seed

Maceration method of extraction was employed in this study for the extraction of the plant's seed.

For the methanol extraction of the seed, 500g of the seeds powder was dissolved in 1 litre (1L) of absolute Methanol. Similarly, 500g of the same powder was dissolved in 1 litre of diethyl ether for the diethyl ether extraction.

The mixtures were allowed to stand for 24hrs at room temperature and filtered using Whatman number 1 filter paper. The filtrates were evaporated to dryness at 50^0C in a water bath. However, after the complete dryness of the solvent, the diethyl ether extract was found to be 100% oil. The resultant extracts were measured and expressed as a % extract yield of the original sample using the formular below.

Percentage (%) yield= E/OX100

Where: E = Weight of the extract and

O = Weight of the original sample

The extracts were placed inside a screw capped sterile containers and kept in a refrigerator at 2-8^0C until use.

Preparation of paper Discs

Paper discs of 6mm in diameters were punched from Whatman no. 1 filter paper. The paper discs for the diethyl ether extract were labelled E while that of methanol extract were labelled M.

The prepared paper discs were sterilized with ultraviolet light at room temperature.

Determination of the Antibacterial activity of the Crude Methanol and Diethyl ether Extracts of *N. sativa* seeds

Kirby Bauer disc diffusion method (devised by[23] Bauer *et al*., 1966) was used to determine the antibacterial activities of the crude methanol and diethyl ether extracts of *N. sativa* seeds.

Ten microliters (10µL) of the prepared concentrations (1000 mg/mL, 500 mg/mL, 300 mg/mL and 100 mg/mL) of the methanol extracts of *N. sativa* seeds were impregnated to each of the prepared and labelled paper discs to make an extract containing discs of 10mg/disc, 5mg/disc, 3mg/disc and 1mg/disc.

However, different dilutions of the diethyl ether extracts (which was 100% oil) was prepared using dimethylsulphoxide (DMSO) as a diluent and 100%, 50%, 30% and 10% v/v dilutions of the oil to DMSO ratio were made. Ten microliters (10µl) of the prepared concentrations were impregnated to each of the prepared and labelled paper discs to make an extract containing discs of 100%, 50%, 30% and 10% v/v of oil/DMSO ratio.

The extracts impregnated discs were placed in an incubator (at 25^0C) for 24 hours to dry. After drying, the discs were placed in an appropriate, well labelled screw capped containers and store in refrigerator at 2-8^0C.

Preparation of the Inoculum

Direct colony suspension method was the technique employed in the preparation of the inoculum in this study as recommended by.[24]

Selected colonies from the identified isolates were picked with sterile wire loop and placed in a test tube containing 5mL of sterile normal saline to make a suspension. The turbidity of the inoculum suspension was adjusted to that of 0.5 McFarland standard against a card with a white background and contrasting black lines under an illuminated surface.

Inoculation of Tests Plate

The carefully adjusted inoculum suspension was allowed to stand for 15 minutes and a sterile cotton swab was dipped into the adjusted suspension, rotated several times and press firmly on the inside wall of the tube above the fluid to remove the excess fluid from the swab.[24] There after the swab was streaked over the entire sterile surface of the dried Mueller Hinton agar plate. This procedure was repeated twice by rotating the plate at approximately 60° each time to ensure an even distribution of the inoculum.[24]

Application of Discs to Inoculated Agar Plates

The extract impregnated discs, controls and the standard antibiotics were aseptically placed onto the surface of the inoculated plates. The discs were gently pressed on the agar using a sterile forcep to provide uniform contact with the surface. The discs were distributed at least 22mm away from each other and 14mm away from the edge of the plate. The plates were inverted and placed in an incubator set at 35 ± 2^0C within 15 minutes after the application of the discs for 18-24 hours.[24] Antibacterial activity was recorded as a zone of clearing around the discs and was recorded once the zone was greater than 6mm.[25]

The zones were measured to the nearest millimetre using ruler held at the back of the inverted petri plate. The petri plates were held a few inches above a black background illuminated with reflected light.

Determination of the Minimum Inhibitory Concentrations (MIC) of the Methanolic seed extracts of *N. sativa* seeds.

The MIC of the Methanolic seed extract of *N.sativa* was determined using[24] standard. Thioglycollate broth was prepared and sterilised using autoclave. Five percent (5%) serum was added after allowing the broth to cool to about 45^0C to form a 5% serum enriched Thioglycollate (Thio-S).

One millilitre (1mL) of the prepared broth was dispensed into tubes 1-9, 11 and 12 in a series of 12 test tubes. 2mL of the broth was dispensed into tube 10 to serve as broth control.

The solution of the Methanolic seed extract of *N. sativa* was prepared by dissolving 500mg of the dried extract in 5ml of sterile distilled water to make a stock solution of 100mg/ml.

One millilitre (1mL) of the stock solutions (100mg/mL) of the extracts was dispensed into tubes 1 and 2.

Subsequently, from tube 2, doubling dilution was carried out in which 1mL from tube 2 was transferred up to tube 9 and 1mL was discarded. The working inoculum was prepared from an overnight cultures using serum enriched Thioglycollate broth (Thio- S). The broth-cultures were diluted 1:200 by mixing 0.1mL of the inocula and 19.9 mLs of the broth.[25] From this dilution, 1mL of the inoculum was transferred into each tube from tube 1-12 with the exception of tube 10. The final concentrations of the Methanolic seed extracts of *N. sativa* in each of the test tubes numbered 1-9 after dilutions were; 100, 50, 25, 12.5, 6.25, 3.13, 1.56,

0.78 and 0.39mg/ml respectively. Ciprofloxacin (30µg/ml) was used as a positive control (tube 11) and distilled water as negative control (tube 12). The tubes were incubated at 35±2^{0}C for 18-24hrs. At the end of the incubation, the lowest concentration of the extracts showing no growth was taken as the MIC.

Determination of the Minimum Bactericidal Concentrations (MBC) of the Methanolic seed extract of *N. sativa*.

The MBC was determined by sub-culturing (on solid media) 0.01ml (10µL) of the highest concentrations of the dilutions which showed visible growth and all the tubes showing no visible sign of growth from the MIC tube dilution test[25] (Och. MBC was the lowest concentration that results in killing 99.9% of the test organisms.[26]

Phytochemical screening of the crude Methanol extracts of *N. sativa*

The crude methanol extract of *N. sativa* seed was tested for the presence of phenols, tannins, terpenoids, alkaloids, saponin, carbohydrate, fixed oil, volatile oil, flavonoid, anthraquinones, cardiac glycosides and protein using the method of.[27]

Determination of the Acute toxicity of the Crude Methanol extract of *N. sativa* seed.

Twelve (12) albino rats weighing between 123 aand 193 were purchased from the Pharmacology Department, Faculty of Pharmacy, Usmanu Danfodiyo University Sokoto. They were certified healthy by a Veterinary doctor. The animals were kept in the Animal house of the Pharmacology Department, UDUS in wire mesh cages. They were maintained under Veterinay supervision and were fed with pellet made from growers mash and water ad libitum.The LD50 of the extracts was determined by the method of.[28]

Phase I

In this phase, three groups of three animals each were given the following doses of the extracts; 10, 100 and 1000mg/kg body weight of the extracts.Observation on the adverse effects of the extracts such as tremor, salivation, off feed, time of death were made at regular interval for 24 hours.3

Phase II

In this phase, three groups of one animal each were given higher doses of the extracts; 1600, 2900 and 5000 mg/kg body weight of the animals to the groups; I, II and III respectively. Toxic symptoms were observed for 24 hours.

Data Analysis

Data was presented in the form of mean ± SEM. The mean inhibitory zone diametres, MICs and MBCs of the crude extracts of *N. sativa* seed were compared to that of the standard antibiotics by one way ANOVA followed by tukey's test. Mean differences were considered significant when $p < 0.05$. All the statistical analysis were carried out using the Statistical Packages for Social Sciences (SPSS) version 20.0 (California Inc., USA).3.

RESULT

The Identified Bacterial Isolates

Seventy four (74) organisms were isolated from the 101 specimens analysed. Out of these, 30 (37.8%) were *Pseudomonas aeruginosa*, 24(32.4%) were *S. aureus*, 10(13.5%) were *E.coli*, 4(5.4%) were *S. liquefaciens*, 4(5.4%) were coagulase negative *Staph*, 2(2.7%) were *Proteus mirabilis* and 2(2.7%) were *S. pyogenes* (Table 1.0).

Table 1.0: The identified Bacterial isolates and their source.

			N = 74
Bacteria No.	Isolated (N)	Source Percentage	(%)
Staphylococcus aureus	24	Wound swab/pus/asp.	32.4
Streptococcus pyogenes	2	Wound aspirate	2.7
Serratia liquefaciens	4	Wound swab	5.4
Proteus mirabilis	2	Wound swab	2.7
Coag. negative *Staph.*	4	Wound swab	5.4
Escherichia coli	10	Wound swab/pus	13.5
Pseudomonas aeruginosa	28	Wound swab/pus/asp	37.8
	74		100

Comparison of the inhibitory zone diametres of the crude Methanol and Diethyl ether extracts of *N. sativa* with the Standard antibiotics on bacterial isolates.

A significant difference ($p < 0.05$) was observed between the mean inhibitory zone diametres of the methanol extract of *N. sativa* when compared with the standard antibiotics (Table 2.1). Highly significant difference was observed on Coagulase negative *Staph* [29.0±1.87, 25.3±2.14 and 19.8±4.17mm at 10, 5 and 3mg/disc respectively, in contrast to Ciprofloxacin (23.5±1.89) and Pefloxacin (18.8±4.27) $F = 12.4$, $P = 0.001$] and the lowest was observed on *S. aureus* [25.1±1.51 at 10mg/disc, in comparison to Pefloxacin (22.1±1.24), $F = 1.95$, $P = 0.046$]. However, no significant difference ($p > 0.05$) was observed on *P. mirabilis* at all concentrations of the extract ($F = 0.21$, $P = 1.022$). No activity was recorded with methanol (Table 2.1).

No significant difference ($p > 0.05$) was observed between the inhibitory zone diametres of the diethyl ether extracts of *N. sativa* when compared with the standard antibiotics against all the bacterial isolates. The most highly insignificant difference was observed on *P. aeruginosa* [7.0±0.55 at 10mg/disc, in contrast to Ciprofloxacin (21.0±2.04) and Pefloxacin (18.4±1.89), F = 0.074, P = 2.10]. No activity was recorded with DMSO (Table 2.2).

Table 2.1: Comparison of the inhibitory zone diametres of the crude Methanol extracts of N. sativa seed with standard antibiotics on bacterial isolates.

	Zones of inbhibition (mm)								
	ME extracts (mg/disc)				Neg. Control	Std drug (mg/disc)			
Isolates	10	5	3	1	M.	Cipro (0.03).	Pef (0.03).	F	P
S. pyogenes	27.0±1.50**	22.5±0.50	18.0 ±0.00	10.0 ±1.00	6.0 ± 0.00	26.0±2.00	25.0±5.00	4.30	0.011
P. mirabilis	26.0±2.00	21.5±1.50	17.5±1.50	14.0±0.00	6.0 ±0.00	29.0±1.00	29.5± 1.50	0.21	1.022
CN Staph	29.0±1.87**	25.3±2.14**	19.8±4.17*	15.8±3.90	6.0±0.00	23.5±1.89	18.8±4.27	12.4	0.001
S. liquefaciens	27.3±0.48*	23.3±0.48	18.5±1.50	12.3±1.93	6.0±0.00	28.0±2.12	25.5±2.50	2.10	0.04
E. coli	24.3±2.64**	18.4±2.69	12.6±2.60	7.9±1.59	6.0±0.00	22.2±2.91	23.1±3.17	4.56	0.008
S. aureus	25.1±1.51*	20.5±1.33	15.9±1.19	9.8±0.93	6.0±0.00	25.1±1.54	22.1±1.24	1.95	0.046
Pseudomonas	23.0±1.51**	17.8±1.46	11.9±1.33	9.0±0.93	6.0±0.00	21.0±2.04	18.4±1.89	5.8	0.0061

Data are presented as mean ±SEM. Values with the superscript (**) are significantly greater than the two standard antibiotics on the right while values with superscript (*) are significantly greater than one of the antibiotics on the right by using ANOVA (at $p < 0.05$)

Values > 6 ± SEM indicate some activity.

Key: ME = Methanol extracts, Std drug= Standard antibiotics, M. = Methanol.

Cipro. = Ciprofloxacin, Pef. = Pefloxacin, CN Staph = Coagulase negative Staph., Pseudomonas = P. aeruginosa.

Table 2.2: Comparison of the inhibitory zone diametres of the crude Diethyl ether extracts of *N. sativa* seed with standard antibiotic on Bacterial isolates.

	Zones of inhibition (mm)								
	DE extracts (v/v)				Neg. Control	Std drug (mg/disc)			
Isolates	**100%**	**50%**	**30%**	**10%**	**D.**	**Cipro (0.03).**	**Pef (0.03).**	**F**	**P**
S. pyogenes	8.0 ±2.00	6.0±0.00	6.0± 0.00	6.0±0.00	6.0±0.00	26.0±2.00	25.0±5.00	0.32	1.00
P. mirabilis	11.0±5.00	6.0±0.00	6.0±0.00	6.0 ±0.00	6.0±0.00	29.0±1.00	29.5± 1.50	0.96	0.082
CN *Staph*	13.3±5.19	10.3±4.3	9.5±3.50	9.0±3.00	6.0±0.00	23.5±1.89	18.8±4.27	1.02	0.075
S. liquefacien	8.5±2.50	7.0±1.00	6.0±0.00	6.0±0.00	6.0±0.00	28.0±2.12	25.5±2.50	0.53	0.97
E. coli	7.9±1.90	7.6±1.60	7.5±1.50	7.0±1.00	6.0±0.00	22.2±2.91	23.1±3.17	0.09	1.24
S. aureus	9.1±1.21	7.1±0.65	6.5±0.45	6.0±0.00	6.0±0.00	25.1±1.54	22.1±1.24	0.71	0.094
Pseudomonas	7.0±0.55	6.0±0.00	6.0±0.00	6.0±0.00	6.0±0.00	21.0±2.04	18.4±1.89	0.074	2.10

Data are presented as mean ± SEM. No significant difference ($p > 0.05$) was observed between the inhibitory zone diametres of the diethyl ether extract of *N. sativa* when compared with the standard drug by using ANOVA. Values greater than 6±SEM indicate some activity.

Key: DE = Diethyl ether extracts, Std drug= Standard antibiotics, D. = Dimethyl sulphoxide, Cipro. = Ciprofloxacin, Pef. = Pefloxacin, CN Staph = Coagulase negative *Staph*., *Pseudomonas* = *P. aeruginosa*

Comparison of the MICs and MBCs of the crude Methanol extracts of *N. sativa* with Ciprofloxacin

No any significant difference ($p > 0.05$) was observed between the MICs and MBCs of the extracts when compared with that of ciprofloxacin (Table 3.0).The most highly insignificant difference was observed on *P. aeruginosa* [MIC (3.43±3.44 in contrast to Ciprofloxacin (0.040±3.37) and MBC (7.45 ±5.77 in contrast to Ciprofloxacin (0.085±7.05), F = 0.14, P = 2.32].

Table 3.0: Comparison of the MICs and MBCs of the crude Methanol extracts of *N. sativa* seed with Ciprofloxacin against the Bacterial isolates.

	MIC (mg/ml)		MBC (mg/ml)			
Isolate	***N. sativa***	**Cipro**	***N. sativa***	**Cipro**	**F**	**P .**
S.pyogenes	3.13±0.00	0.023±7.84	6.25±0.00	0.047 ±15.60	0. 20	2.01
P. Mirabilis	1.56±0.00	0.009 ±0.00	6.25±0.00	0.016±0.00	0.65	1.09
CN *Staph*	1.57±5.54	0.014±1.96	4.69±9.01	0.047 ± 9.01	0.56	1.24
S. liquefaciens	1.07±1.96	0.019 ±3.92	3.90 ±7.83	0.039 ±7.68	0.84	0.92
E. coli	1.91±2.30	0.026±2.61	5.55±10.53	0.052±5.20	0.42	1.50
S. aureus	2.09 ±1.65	0.025±1.73	6.40±5.04	0.054±4.89	0.32	1.70
Pseudomonas	3.43±3.44	0.040±3.37	7.45 ±5.77	0.085±7.05	0.14	2.32

Data are presented as mean ± SEM. No significant difference ($p > 0.05$) was observed between the MICs and MBCs of the crude extracts when compared with ciprofloxacin by using ANOVA.

Key:

Cipro = Ciprofloxacin.

CN staph = Coagulase negative *Staphylococcus*.

Results of the preliminary phytochemical screening of the crude extracts of *N. sativa* seeds.

The results of the preliminary phytochemical screening of the crude extracts of *N. sativa* are shown in Table 4.0. The diethyl ether extract of revealed the presence of tannins, cardiac glycoside, carbohydrate, terpenoids and fixed oil and the methanolic extract revealed the presence of alkaloids, tannins, saponins anthraquinones, flavonoid, carbohydrate, terpenoids, fixed oil and protein.

Table 4.0: Preliminary phytochemical screening results of Diethyl ether and Methanol extracts of *N. sativa*.

Constituents	Type of test	ME of NS	DE of NS
Alkaloids	Meyer's test	+++	-
	Wagner's test	++	-
Tannins	$FeCl_3$ test	++	-
	Strong lead sub metals test	++	+
Saponins	Froathing test	+	-
Cardiac glycoside	Kella-Killiani's test	++	-
Anthraquinones	Borntrager's test	++	-
Flavonoids	NaOH test	+++	-
	Schinoda's test	++	-
Carbohydrate	Mollisch's test	+	+
	Fehling's test	+	+
Terpenoids	Liebermann-Buchard's test	+++	+
Fixed oil	Paper test	+++	+++
Protein	Xanthoproteic test	-	++

Key:

+++ and ++ = Present, + = trace, - = Not detected, ME = Methanol extract, DE = Diethyl ether extract, NS = *N. sativa*.

Determination of the LD_{50} of crude Methanolic seed extracts of *N. sativa*

The oral acute toxicity studies showed an LD_{50} of the crude Methanolic seed extracts of *N. sativa* to be greater than 5000mg/kg body weight of the wistar albino rats (Table 5.0).

Table 5.0: Results of the oral acute toxicity of Methanolic seed extract of *N. sativa.*

Phase	Groups	no. of animals	Weight (g)	Dose (mg/kg)	O.P	B.C	Mortality
I	I	3	i.133 ii.123 iii.130	10	24hrs	No	No
	II	3	i.167 ii.165 iii.164	100	24hrs	No	No
	III	3	i. 190 ii. 181 iii. 185	1000	24hrs	No	No
II	I	1	159	1600	24hrs	No	No
	II	1	154	2900	24hrs	Excited	No
	III	1	130	5000	24hrs	Calm	No

The oral LD_{50} of the Methanolic seed extract of *N. sativa* was found to be > 5000mg/kg.

Key:

O.P = Observation period

B.C = Behavioural changes

DISCUSSIONS

In this study, the antibacterial activity of the crude extracts of *N. sativa* are tested against seven wound associated bacteria viz; *S. aureus*, *P. aeruginosa*, *E.coli*, *S. liquefaciens*, *S. pyogenes*, coagulase negative *Staph* and *P. mirabilis* (Table 1.0). The antibacterial activity of the extracts was recorded when the inhibition zone was greater than 6mm.

This study revealed a significant antibacterial activity of the crude methanol extract of *N. sativa* to all the tested bacteria at varying concentrations (Table 2.1). A highly significant difference was observed between the inhibitory zone diametres of the crude extracts when compared with the standard antibiotics on Coagulase negative *Staph* (F = 0.001, F = 12.4) and the lowest was observed on *S. aureus* (P = 0.046, F = 1.95).

These findings are in agreement with the work of[29] who reported a dose dependent antimicrobial activity of *N. sativa* that is more on Gram positive than Gram negative bacteria. It is also in agreement with the work of[30], who reported an antimicrobial activity of methanol extract of *N. sativa* against a wide range of micro-organisms.[31] and[32] have also reported significant antibacterial activity of the methanol extract of *N.* sativa.[33] also reported that the methanol extract of *N. sativa* seed had a strong antibacterial activity against strains of *L. monocytogenes* yielding a significant greater inhibition zone than that of Gentamicin.[34]

also reported strains of MRSA as sensitive to the ethanolic extract of *N. sativa* at concentration of 4mg/disc.

These activities may be due to the phytochemical constituents present in the Methanolic seed extract of *N. sativa* (tannins, flavonoids, alkaloids, saponins, anthraquinones, terpenoids and fixed oil) (Table 4.0). Studies have reported the antibacterial activities of these constituents[35]; [36]; [37]; [38] and.[39] However,[40] attributed the antibacterial activity to thymoquinone and melanin.

No significant antibacterial activity was observed between the inhibitory zone diametres of the methanol extract when compared with the standard antibiotics on *P. mirabilis* (F = 1.022, P = 0.21). This finding is in agreement with the work of[29] who reported *P. mirabilis* to be insensitive to *N. sativa* extracts at varying concentrations. With 1mg/disc of the methanol extracts of *N. sativa*, no significant differences were observed between the inhibitory zone diametres of the extracts when compared with the standard antibiotics on all the clinical bacterial isolates (Table 2.1).

An explanation to this may be due to the low quantity of the bioactive components at this concentration possibly due to the decrease in quantity of the extract.

No any significant difference was observed between the mean inhibitory zone diametres of the diethyl ether extracts of *N. sativa* when compared with the standard antibiotics on all the clinical bacterial isolates (Table 2.2). The most highly insignificant difference was observed on *P. aeruginosa* (F = 0.074, P = 2.10) and *E. coli* (F = 0.09, P = 1.24) (Table 2.2). These findings are in conformity with the work of[41] who reported diethyl ether, hexane, chloroform and ethanol extract of *N. sativa* as less effective in comparison with methanol extract.

The low antibacterial activity observed with the crude diethyl ether extract of *N. sativa* in comparison with the crude methanol extract may be due to the differences in the polarity of the active compounds.

No any significant difference was observed between the MICs and MBCs of the crude methanolic seed extract of *N. sativa* when compared with Ciprofloxacin (Table 3.0). The most highly insignificant difference was observed on *P. aeruginosa* [MIC (3.43±3.44 in

contrast to Ciprofloxacin (0.040±3.37) and MBC (7.45 ±5.77 in contrast to Ciprofloxacin (0.085±7.05), F = 0.14, P = 2.32].

The non significant findings between the MICs and MBCs of the crude extracts in contrast to that of the standard antibiotic may be due to the differences in the purity of the active compounds in the extract and the Ciprofloxacin.

Studies by[42], [34] and[43] on the MIC and MBC of crude extract of *N. sativa* have reported values lower than the ones obtained in this study, contrary to these however,[30] reported higher values.

The disparities in the MICs and MBCs of *N. sativa* obtained from this study in contrast to the ones reported from the previews studies may be explained by the different extraction methods and solvent system used.

The acute toxicity of the crude Methanolic seed extract of *N. sativa* was found to be greater than 5000mg/kg body weight of the wistar albino rats (Table 5.0).

These findings are in conformity with the work of[44] Zaoui *et al*., (2002) who reported low toxicity, high LD_{50} values, stabilized hepatic enzymes and organ integrity associated with *N. sativa* extract.

The low toxicity observed suggests a wide margin of safety for therapeutic doses of this extract, which means that they may be relatively safe even at higher doses.

CONCLUSIONS

Findings from this study suggests that the crude methanol extracts of *N. sativa* seeds possessed an antibacterial activity. Significant activity was observed with the extracts against some wound associated bacteria despite their multi drug resistance antecedents.

Due to its antibacterial activity coupled with less or non toxic effects observed, this plant material may become an alternative source of antibacterial agents that would complement the effort of the existing antibiotics or provide a novel or lead compound that may be employed to forestall the antibiotic resistance menace.

Further studies are recommended to isolate and characterize the respective bioactive components of this extract and correlate their action to specific phytoconstituents which would enhance possible drug development.

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