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Research Article

Antimicrobial Activity of N-Hexane Extract of *Nigella Sativa* against Some Pathogenic Bacteria

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Abstract

The therapeutic properties and the constant availability of medicinal plants makes them an indispensable asset in the healthcare sector of developing countries especially in rural areas where modern healthcare systems are not readily available. This study evaluated the phytochemical compositions and antibacterial efficacy of *Nigella sativa* extract. The qualitative analysis of phytochemical properties was carried out using standard methods. The antimicrobial study was performed against four pathogenic bacteria (E. coli, S. aureus, Salmonella typhi, and Streptococcus pyogenes) using the agar well diffusion technics, the diameter of the zones of inhibition was read in mm, and MIC values were obtained. Results revealed the presence of saponins, anthraquinones, tannins coumarins, phenols and cardiac glycosides. The extract produced dose dependent increase inhibition *S. aureus* (8.35±0.35 - 18.35±0.53 mm), *E. coli* (5.43±0.15 - 11.33±0.85 mm) and *Streptococcus pyogenes* (5.43±0.02 - 15.35±0.56 mm) but was completely in active against *Salmonella typhi* at all concentrations (80-200 mg/mL) tested. The extract had MIC of 32, 1.28 and 1.28 mg/mL against *Escherichia coli, Staphylococcus aureus* and *Streptococcus pyogenes* respectively. In conclusion, N-hexane extract of *N. sativa* showed inhibitory effect on *S. aureus, S. pyogenes* and *E. coli* and thus may be useful in treating infections of which these organisms are the etiological agent.

Keywords: Antimicrobial; Phytochemicals; Nigella sativa; Microorganism

Introduction

The increasing resistance of microorganism to numbers of standard antibiotic therapies are of global problem causing enormous public health concerns [1]. Current drug's effectiveness is getting limited due to a large number of multi-drug resistant bacterial strains such as pneumococci resistant to penicillin and macrolides, methicillin-resistant staphylococci, vancomycin-resistant enterococci as well as multidrug resistant gram-negative organisms [2]. The efficacy of these synthetic chemotherapeutic agents is decreasing, they are often worsened by various side effects associated with these drugs [3]. There is, therefore, an unmet medical need to find an alternative for the treatment of various diseases caused by various microbial agents [4].

Human have relied on plants for food and medicinal purposes since ancient times and some of the chemotherapeutic agents being used today for curing diseases are of plant origin [5]. In developing countries, plants still play important role in the food, construction and health sector. The therapeutic properties and the constant availability of medicinal plants makes them and indispensable assets in the healthcare of developing countries especially in rural areas where modern healthcare system are unavailable [6]. Essential oil is part of the secondary metabolites found in higher plants. Generally, aromatic oils are useful ingredients in the cosmetics and pharmaceutical industry, they are also crucial components of soap, detergents and toothpaste [7,8].

Nigella sativa is an aromatic oil producing plant belonging to the Ranunculaceae family. *N. sativa* is native to the Mediterranean, however it is now being cultivated in various part of the world and known by names which defer based on geographical locations.

Among its popular names are coriander seed, black caraway seed, Love-in-a-mist, Black seed, Black Cumin, etc. [9].

N. sativa is believed to have high therapeutic potentials and has been reported to be effective in treating various ailments [10]. Like some medicinal plants, Black seed has been reported to possess a modulating effect on biological pathways and systems [11]. A clinical experiment carried out by [12,13] concluded that *N. sativa* has a short-term effect on systolic and diastolic blood pressure, [14], reported that extracts of *N. sativa* was able to reduce triglycerides, cholesterol in clinical trials. The plant has also proven to have a stabilizing effect on man's health, boost the functionality of the immune system in fighting infectious diseases [15]. Various literatures have shown that *N. sativa* lin seeds have a lot of pharmacological activity such as bronchodilator effect, anti-inflammatory, anticancer, neuroprotective, antihistamine, hepato-protective, hypoglycemic and antiulcer activities [16-19]. The present study evaluated the antimicrobial activity of *N. sativa* against some pathogenic organism.

Materials and Methods

Bacteria Strains

Clinical isolates used for the study were obtained from Niger state General hospital, Minna. They were collected as pure isolates on agar slants and transported to Microbiology laboratory of Federal University of Technology, Minna where the experiment was performed. The bacterial pathogens used in the study are *E. coli, S. aureus, Salmonella typhi, and Streptococcus pyogenes*.

Sample Collection and Authentication

Dried seeds of *N. sativa* were purchased at a local market in Minna, Niger state. The seed was identified at the department of Plant Biology as Federal University of Technology Minna.

Extraction of Plant Material

Procedure described by [20] was used for the N-Hexane extraction of the *N. sativa*. The dried *N. sativa* seeds were grinded into powder using an electric blender. The Powered seed was then subjected to N-Hexane extraction. 10 g of the powder was submerged into 100 ml of N-Hexane and covered with a paper foil. The set up was placed in a shaker for 48 h at room temperature, after which it was subjected to centrifuging for 15 minutes at 2000 rpm. The supernatant was filtered off using a Whatmann filter paper 1. Rotary evaporator set at 55^0 C was used for drying the extracts and for removing the N-Hexane solvent. The crude extract was weighed and preserved in sterile air-tight universal bottle and stored at 4^oc.

Phytochemical Screening

Qualitative phytochemical screening procedures previously described by Harbone [21,22] were used to screen the crude extract of *N. sativa* for the presence of saponin, terpenoids, steroids, anthraquinones, tannin, flavonoid, anthraquinones and coumarins.

Culture and Standardization of the Bacteria Strain

The clinical isolates of the test organism were plated out on nutrient agar by streaking method, a loopful of the test microorganisms were then transferred into 5ml of nutrient broth; this was later incubated for 24 hours at 37^oC. After incubation, 0.2 ml of the culture was transferred into 20ml of nutrient brought and incubated for 3-5 hours to standardize the culture to 106 cfu/ml [23].

Antibacterial Assay

The following bacteria: *S. pyogenes, Salmonella typhi, Staphylococcus aureus and Escherichia coli* were the species used for the experiments. Organisms were isolated by standard methods, maintained on agar plates and refrigerated until further use. The antibacterial activity of the N-hexane extract of *N. sativa* at various concentrations (80, 120, 160 and 200 mg/ml) was carried out using agar-well diffusion method according to the method of CLSI [24] as described by [25]. For comparison, Ampicillin and tween 80 oil were used as positive and negative control respectively. Zones of inhibition obtained were measured with meter rule in millimeter, 5 mm which is the diameter of the used corn borer was subtracted from each measured inhibition zones, the final result is taken as the zones of inhibition. A broth micro-dilution method [26] was used to determine the minimum inhibitory concentration (MIC) of the extracts.

Statistical Analysis

Values were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± SE of the mean. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $P < 0.05$.

Result

Phytochemical composition of the N-Hexane extracts of N. sativa

Table 1: phytochemical constituent of *Nigella sativa*.

Phytochemical	Inference
Tannin	+
Flavonoid	_
Phlobatannin	_
Saponin	+

Alkaloid	_
Anthraquinones	+
Phenol	+
Cardiac glycosides	+
Coumarins	+
Steroids	_
Terpenoids	_

The qualitative analysis of phytochemicals in N-Hexane extract of *N. sativa* revealed the presence of saponins, anthraquinones, tannins coumarins, phenols and cardiac glycosides while flavonoids, alkaloids, steroids, phlobatannin and terpenoids were not detected (Table 1).

Antibacterial activity

Zone of Inhibition: The zone of inhibition of the organism caused by N-Hexane extracts of *N. sativa* is shown in Table 2. The extract was completely in active against *Salmonella typhi* at the concentrations (80-200 mg/mL) tested. The extract produced dose dependent increase inhibition *S. aureus* (8.35±0.35 - 18.35±0.53 mm), *E. coli* (5.43±0.15 - 11.33±0.85 mm) and *Streptococcus pyogenes* (5.43±0.02 - 15.35±0.56 mm). The standard drug (Ampicillin cause inhibition of 26.24±0.54 mm, 30.06±0.32 mm, 23.35±0.49mm and 28.92±±0.56 mm *against E. coli, Staphylococcus aureus, Salmonella typhi* and *Streptococcus pyogenes* respectively.

Table 2: Zone of inhibition (mm) of the organism caused by N-Hexane extracts of *N. sativa*.

	Concentrations(mg/ml)					
	80	120	160	200	Ampicillin	Control
E. coli	-	5.43±0.15	9.35±0.34	11.33±0.85	26.24±0.54	0.00±0.00[a]
Staphylococcus aureus	8.35±0.35	11.25±0.28	11.95±0.84	18.35±0.53	30.06±0.32	0.00±0.00[a]
Salmonella typhi	-	-	-	-	23.35±0.49	0.00±0.00[a]
Streptococcus pyogenes	5.43±0.02	8.95±0.38	12.14±0.67	15.35±0.56	28.92±±0.56	0.00±0.00[a]

Minimum Inhibitory Concentration: Minimum Inhibitory Concentration of N-Hexane extract of *N. sativa* are shown in Table 3. The extract had MIC of 32, 1.28 and 1.28 mg/mL against Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes respectively.

Table 3: Minimum Inhibitory Concentration of N-Hexane extract of *N. sativa*.

Organism	MIC (mg/mL)
Escherichia coli	32
Staphylococcus aureus	1.28
Salmonella typhi	_
Streptococcus pyogenes	1.28

Discussion

Generally, natural products are known to contain diverse bioactive secondary metabolites that confer to them a diverse pharmacological property [27]. The phytochemical screening of the crude extract of N-Hexane extract of *N. sativa* indicated the presence of saponins, tannins, anthraquinones, phenols coumarins, and cardiac glycosides while flavonoids, alkaloids, steroids, phlobatannin, and terpenoids were absent. This result is similar to the report of [20]. These phytochemicals have been reported for different biological activities. Therefore, the presence of these phytochemicals in *N. sativa* is an indication that these plants if properly screened would yield a drug template [28]. The dose-dependent microbial inhibition observed in this study may be due to the presence of these bioactive compounds or a synergy between members of the phytochemical compounds detected [29].

The result showed that *Salmonella typhi* is not inhibited by the N-Hexane extract of *N. sativa* at any of the concentration tested, indicating that the N-Hexane extract has no effect on the organism. Also, *E. coli* was fairly susceptible at a high concentration of the N-Hexane extract. In general, the N-Hexane extract was found to have higher inhibitory effect on the Gram-positive bacteria tested than the Gram-negative bacteria tested. This result is similar to the study of [4] who reported that all of the N-Hexane extract of black seed was inhibitory to Gram positive bacteria than it was to Gram negative bacteria. The lack of conspicuous activity on Gram negative bacteria may be due to the structure of their cell wall which

has permeability barrier that can reduce the active penetration of amphipathic substances into the Gram negative cell wall. Certain Gram-negative bacteria have also been associated with efflux pumps through which antibiotics are extrude from within the cell [30].

Minimum inhibitory concentration (MIC) is the lowest concentration of an extract that inhibit the visible growth of the test organism after 24hrs incubation [31-33]. The low MIC value (1.28) obtained for both *S. aureus* and *S. pyogenes* indicate that small quantity of the extract is needed to inhibit both organisms. Thus, *N. sativa* is very potent against both pathogens. However, despite the fact that the individual components of the oil, such as carvacrol, thymol, and terpenoids, have been recognized as potential antimicrobial agents, their precise mechanism of action has not been fully clarified [34].

Conclusion

The N-Hexane extract of *N. sativa* showed inhibitory effect on *S. aureus, S. pyogenes* and *E. coli* while *Salmonella typhi* was not inhibited at all the concentration tested, indicating that the extract is not effective against the organism. *N. sativa* may be useful in treating infections of which *S. aureus and S. pyogenes* are the etiological agent.

Conflicts of Interest

The authors declare that they have no competing interests.

Consent for publication

Not applicable

Authors Contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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