



PRELIMINARY ANTIMICROBIAL SCREENING OF BLACK SEED EXTRACT AGAINST SOME SELECTED BACTERIA

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Abstract

Antibiotic resistance is one of the challenging issues in the field medical sciences however; plants played a vital role in the traditional treatment of certain infectious diseases without any trace of resistance. In this study, the antibacterial effects of methanolic and chloroformic extracts of black seed (*Nigella sativa* family: *Ranunculaceae*) have been investigated. The organisms were collected from a clinical case at the University of Maiduguri Teaching Hospital, Nigeria. Similarly, the plant was collected from Maiduguri Monday Market, Nigeria. The aim of this study was to assess the in vitro antibacterial activity of black seeds extracts having different concentrations against some selected micro-organisms. The inhibitory effects of extracts were assessed using disc diffusion method at four different concentrations viz: 20mg/ml, 50mg/ml, 100mg/ml, and 200mg/ml. Results showed that the methanolic and chloroformic extracts have inhibitory effects against all the bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*) tested, except *Shigella dysenteriae* which was resistant at all concentrations. The diameter of zone of inhibition in methanolic extracts was higher (13mm) than chloroformic extract (12mm) at same concentrations. At concentration of 100mg/ml, the highest antibacterial activity of 13mm was recorded in *Strep. pyogenes* and *k. pneumonia*. Chloroformic extract of black seed had a remarkable sensitivity against *K. pneumonia* and *P. aeruginosa* with inhibition zones of 12mm and 13mm at 100mg/ml respectively. Antimicrobial activity was also observed in methanolic extract of black seed in *E. coli*, *S. dysenteriae*, *Staph. aureus* and *P. aeruginosa* with an inhibition zone of 10 mm, 11mm, 12 mm and 13 mm respectively.. However, the results also revealed a significant scope to develop a broad spectrum antimicrobial formulation and can be used as food preservative.

Keywords: Black seed, Antibacterial, Methanolic, Chloroformic, Extract, Inhibition

Introduction

One of the accomplishments in human therapeutics in our present generation was the discovery and development of antibiotics against infections. A huge group of antibacterial agents has been launched and used effectively to treat major infectious diseases (Wood, 1990).

However, the usage of antibiotics and antibacterial chemotherapeutics is becoming restricted day in day out because bacteria are capable of developing resistance to antibiotics shortly after their introduction (Okeke *et al.*, 2005), most antibiotics have side effects. Therefore, it is essential to search for other drugs with lesser rate of resistance development and lesser toxicity to certain extent (Neu, 1992).

Synthetic drugs are not only expensive and inadequate but also often subjected to adulterations and side effects. Therefore, with the advancement of technology, scientists are challenged to come out with new ideas of alternative and novel to become the usage of microbial resistant drugs (Nor *et al.*, 2013).

Pharmacological industries have fashioned a member of new antibiotics in recent times, but resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic capability to acquire and transmit resistance to drugs, used as therapeutic agents (Cohen, 1992). Such a fact is caused by concerned, because of the number of patients in hospital who are immune compromised and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals set up resulting in high mortality (Molli *et al.*, 1991).

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example to control the use of antibiotic, develop

research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural (Gislene *et al.*, 2000).

According to World Health Organization (WHO) (Santos *et al.*, 1995), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developing countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties safety and efficiency (Ellof *et al.*, 1998).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. The last few years, a number of studies have been conducted in different countries to prove such efficiency (Alonso-Paz *et al.*, 1995). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oil as well as tannin (Saxena *et al.*, 1994).

Nigella sativa is herbaceous plant which is better known as black seed, a habitat of Southeast Asia and Mediterranean countries. Indian people used this plant as a food preservative as well as a protective and treatment for several disorders (Merfort *et al.*, 1997).

Nigella Sativa is a herbal plant which is properly called black cumen, black seed and the seed of blessing (Habbatul-barakah in Arabic countries). The seeds have traditionally been used for thousands of years in the Middle East, Far East and Asia as a food stabilizer and medicine for some illness (Randhaura *et al.*, 2002). Furthermore, both antibiotic sensitive and multi-drugs resistant gram-positive and gram-negative bacteria isolates were susceptible to *N. Sativa* extracts (Hattem *et al.*, 1991). The black seed also contain 36-

38% fixed oil, proteins, alkaloids, saponins and essential oils in more proportion (Burtis & Bucar 2000). Black seed extract or oil has been reported to have an antimicrobial activity against some bacteria (Morsi 2000). There for, the aim of this work is to determine the antimicrobial activity of *Nigella sativa* (Black seed) extract against some selected bacteria.

Materials and Methods

Sample Collection and preparation

The sample (Black seed) was brought from Maiduguri Monday market. It was grinded into a fine powder using pestle and mortar and immediately was taken to the laboratory. Soxhlet extraction was performed according to (Udaya Sankar, 1989). The organisms were collected from a clinical case at the University of Maiduguri Teaching Hospital, Nigeria.

Tabulation of Result

Table 1 : Antimicrobial activity of black seed against some selected microorganisms.

Organisms	Zone of inhibition Methanol	chloroform
<i>Escherichia coli</i>	10	10
<i>Shigella dysenteriae</i>	R	R
<i>Klebsiella Pneumoniae</i>	12	11
<i>Pseudomonas aeruginosa</i>	R	13
<i>Staphylococcus aureus</i>	11	10
<i>Streptococcus pyogenes</i>	13	10

Table 2 : MIC (mg/ml)

Organisms	Inhibition Zone (Methanol)	(Chloroform)	MIC
<i>Escherichia coli</i>	7mm	7mm	200mg/ml
<i>Staph. aureus</i>	8mm	7mm	200mg/ml
<i>P. aeruginosa</i>	R	9mm	100mg/ml
<i>Klebsiella</i>	8mm	8mm	100mg/ml
<i>Strept. Pyogene</i>	9mm	7mm	100mg/ml

Result and Discussion

This study reports the antimicrobial activity of different concentrations of black seed extract against *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus pyogenes* and *Klebsiella pneumonia*. The inhibitory effects of extracts were assessed using disc diffusion method at four difference concentration that is 20 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml. The investigation of both methanolic and chloroformic extracts showed no inhibition against all the bacteria tested at lower concentration (<50mg/ml). According to Parek *et al.*, 2006; methanol, ethanol and water are the most commonly used solvents for determining the antimicrobial activity of plants.

Similarly, the diameter of inhibition zone in methanolic extracts are higher (13mm) than chloroformic extract (12mm) on the contrary, this is because different sources of extracts, agro-climate factor, handling of experiment as well as phytochemical ingredients in the extract can greatly contribute to the differences of result obtained. (Erdman *et al.*, 2007).

Positive result was observed in methanol extract of black seed at concentration of 100mg/ml, the highest antibacterial activity of 13mm was recorded in *Streptococcus*

Determination of Antimicrobial Activity

A disc of blotting 6mm paper is impregnated with a known volume of Black seed extract and placed on a plate of sensitivity testing agar uniformly inoculated with the test organisms, and placed in an incubator for 24 hours. The extract diffuses from the disc into the test organism is inhibited at a distance from the disc that is related (among other factors) to the sensitivity of the organisms. Organisms sensitive to the extract are inhibited at a distance from the disc where as resistant strains have smaller zones of inhibition or grow up to edge of the disc (Monica, 2004).

Determination of MIC

MIC (Minimum Inhibitory Concentration) was determined by disc diffusion method using dilution method. The stock samples were diluted to have 100mg/ml, 200 mg/ml, 1000mg/ml the lowest concentrations that shows activity was taken as the MIC. (Monica, 2004)

pyogenes and activity was also recorded in *Klebsiella pneumonia*, *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with an inhibition zone of 10 mm, 11 mm, 12 mm and 13 mm respectively. Chloroform extract of black seed had a remarkable sensitivity toward *Klebsiella pneumonia* and *Pseudomonas aeruginosa* with inhibition zones of 12 mm and 13 mm at 100 mg/ml.

Conclusion

From the result obtained on the antimicrobial activity of black seed and hence the minimum inhibitory concentration, black seed extract were found to be effective against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. Therefore, further investigation can be conducted to analyze its possible potentials in antibiotic use and food preservative.

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