



Antimicrobial Activity of *Nigella sativa* (Black Seed) Combined with Honey Wax Against Selected Clinical Isolated

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ABSTRACT

The study “Antimicrobial activity of *Nigella sativa seed* (Black seed) combined with honey wax against selected clinical isolated” was carried out to investigate the antimicrobial activity of the black seed separately and in combination with honey on *Klebsiella pneumonia*, *Escherichia coli*, *Salmonella species* and to determine the minimum inhibition concentration using disc diffusion assay and dilution method. *Nigella sativa seed* (Black seed) was extracted using three solvents (petroleum ether, Methanol, Aqueous solution) and honey was diluted at different concentration (25%, 50%, 75% and 100%) the combined extract of *Nigella sativa seed* (Black seed) and honey using ratio 1:1 v/w. The zones of inhibition were measured. The combined extracts of *Nigella sativa seed* (Black seed) with honey showed more effective at 50%, 75% and 100% concentrations against the test organisms with the zone of inhibition of 11.5mm, 12.7mm and 14mm respectively. The control antibiotic (Ciprofloxacin) shows the zone of inhibition of 12.5mm against *Salmonella species*. The negative control which is distilled water showed no zone of inhibition. The minimum inhibitory concentration (MIC) showed 12.0mg/ml of the petroleum ether extract. The result of this study demonstrated the Antimicrobial activity of *Nigella sativa seed* (Black seed) combined with honey which can be used as an alternative source of drug against enteric infection.

Keywords: Honey wax, *Nigella sativa*, *Klebsiella pneumonia*, *Escherichia coli* and *Salmonella species*

INTRODUCTION

Infectious diseases are the second major courses of diseases worldwide and constitute the third leading cause of death. Bacterial pathogens are responsible for several serious diseases (Singh and Pandeya, 2011). A number of general the family *Entero bacteriaceae* (Graham *et al.*, 2007) are human intestinal pathogens. These include *E.coli*, *klebsiella Pnenmoniae*, *salmonella Speaice*. *Sallamonella* are group of motile rod-shaped bacteria living in the intestinal tracts of host and causes *Salmonellosis* in human and are usually acquired through ingestion of contaminated foods or drinks (Omololn *et al.*, 2009). They are potential enteric pathogens and transmission of salmonella to a susceptible host usually occurs through consumption of contaminated foods. The most common sources of salmonella include beef, poultry and eggs, dairy products, vegetables fruits and shell fish have also been implicated as sources of salmonella (Alena and Mark 2009). The genus salmonella consist of over 2668 different sero-types that are responsible for various forms of diseases in both human and animals.

Some strains of certain bacteria have developed resistance to antibiotics in clinical use (Anun.2000; Chouhan *et al.*, 2010). The resistance to antibiotics from antimicrobial agents has already become a serious issue in many areas of the world especially in developing countries (Shears, 2009). The ability of bacteria to deceive any kind of conventional therapy has become apparent and pathogens resistant to one or more antibiotics are emerging and spreading worldwide.

Additionally, unnecessary use of antibiotics has further stimulated this problem (Omololn *et al.*, 2009) of bacteria resistance towards antibiotics cause significant increase in the occurrence of infectious diseases. Other adverse effect associated with the use of antibiotics on the host include hyper sensivity, immune-suppression and allergic reactions. The need to develop alternative antibacterial drugs from medicinal plants and other natural extracts for the treatment of infection diseases cannot be over emphasized.

Natural products and natural extracts have been used as major sources for the development of new drugs (Dash *et al.*, 2011). A wide range of medicinal natural extracts are used as raw material, collected in larger quantities and traded in markets as stock for herbal industries. While some of these raw drugs are collected in smaller quantities by local communities and folk healers for local use (Uniyal *et al.*, 2006). Plants used for traditional medicines a wide range of substances that can be used to treat infectious diseases coursed by various pathogenic bacteria.

Clinical microbiologist have great interest in screening various medicinal natural extracts for new therapeutics development (Periyasamy *et al.*, 2010) keeping in mind the adverse side effect of antibiotics used for the treatment of infectious diseases, *salmonella species* from diverse sourced where isolated.

N.sativa is an indigenous herbaceous plant, belongs to the *rannunculaceae* family that is more commonly known as the fennel flower plant. This plant has finely divided foliage and blue flowers, which produce black seeds and it grows to a maximum height of about 60cm. The plant is known by many other names e.g kaloji (urdu), habba-tussawda (Arabic), black cumin (English). It is cultivated extensively in Pakistan and India, and also grows in the modern countries. In Islamic medicine the use of the black seed is recommended in daily use because it is regarded as one of the greatest form of healing medicine available.

Honey has a very long history of human consumption as the oldest sweetener and health food. As far back as 5500bc, honey was mentioned in the writing of Egypt, India and China. The importance of honey for human use is described in several classical text of ancient Greece, such as humer's llaid and the odyssey, and in philosophical text plato, Aristotte, and others. The use of honey in therapy is described in 5000-old Egyptian writings; papyrus eber's is full of praises of the culture properties of honey. Honey have used in ayurvedic medicine in India for at least 4000 years. The Therapeutic use of honey in wound healing is recoded on a Sumerian day tablet. However, the Talmud mentioned the use of honey in nutrition and treatment of wounds and several pathologies

STATEMENT OF PROBLEM

Major reasons for decline in the population of antibiotics are the presence of resistance among various pathogenic bacteria. In addition, over prescription and mis-use of traditional antibiotics favor the survival rate of pathogen. Many people preferred to have more autonomy over their medical care so there is the need of alternative source of remedy that would allowed cheap and way to cope with infectious diseases.

AIM

The aim of the study was to determine the antimicrobial activities of *Nigella sativa* (black seed) combine with honey against *klebsiella*, *pnemoniae*, *E.coli*, *Salmonella species*

OBJECTIVES are;

1. To measure the zone of inhibition of *klebsiella*, *pnemoniae*, *E.coli* *Salmonella species* to a *nigella sativa*[black seed] combined with honey extracts.

2. To determine the minimum inhibitory concentration.

MATERIALS AND METHODS

COLLECTION OF MATERIALS

The seed of *nigella sativa* and honey were purchased from a medicine store in Bauchi metropolis.

TEST MICRO ORGANISMS

The test of microorganisms used in this study (bacteria; *Klebsiella pneumoniae*, *Escherichia coli*) were obtained from specialist hospital Bauchi at bacteriology department.

PREPARATION OF MEDIA

Nutrient agar were prepared base on manufacture's instruction, after autoclaving it was allowed to cool at 45°C to 50°C water bath, the freshly prepared medium was poured into a petri dishes on a level, the agar medium was allowed to cooled to room temperature.

PREPARATION OF SAMPLES

The seed of *nigella sativa* was grounded into uniform powder using a grinder and it was store in a clean dry container, honey was dissolved to concentration of 5%,15%,25% were made by diluting the concentrated honey(100 percent) with distilled water (Bandej et al; 2007;durairay et al; 2009-,dubey et-, 2009).

STANDARDISATION OF THE TEST ORGANISMS

All the isolated bacteria were re-identified by biochemical procedures. These biochemical tests include indole hydrogen sulphide gas from D glucose lactose tests.

PREPARATION OF EXTRACTS

About 15 gram powdered samples of *nigella sativa* seed was extracted with petroleum ether, methanol and aqueous by cold extraction method and kept 24 hours, and then filtrated with what man no.1 filter paper, and evaporated on water bath and store at 4 °C.

PREPARATION OF ANTIBIOTIC DISC

Whatman filter paper no1 used to prepare disc approximately 6mm in diameter, which are placed in a petri dish and sterilized in a hot air oven.

The loop full used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2mm this delivers 0.005ml of antibiotics to each disk.

PREPARATION OF INNOCULUM

Inoculums was prepared from a bacterial culture 4-5 isolated colonies was picked and subculture to a tube containing 1ml of nutrient broth. This was kept in incubator for 24hours at 37c for growth of bacteria. The turbidity of the growing nutrient broth was adjusted with 0.5 McFarland standard,0.5 McFarland was prepared by adding 0.6ml of 1%(v/v)barium chloride(BaCl₂) to 99.4ml of 1% sulphuric acid (H₂SO₄) with constant stirring to maintain a suspension.

SUSCEPTIBILITY TEST

Antibiotics susceptibility test was carried out by Kirby-Bauer method. Kirby-Bauer antibiotic testing (KB testing or disk diffusion antibiotic sensitivity testing).

Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab is rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removes excess inoculums from the swab. The dried surface of a nutrient agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60 each time to ensure an even distribution of inoculums. As a final step, the rim of the agar is swabbed. The lid is left ajar for 3 to 5 minutes that allows proper setting of the inoculums. The plates were incubated overnight at 37°C. Anti-bacterial activity was evaluated by measuring the zone of inhibition against the test organism. The zone of inhibition diameter is noted down and then compared with the standard antibiotics and results are interpreted.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by micro dilution method using serially diluted plant extracts according to the NCCLS protocol (NCCLS, 2000). The aqueous, methanol and petroleum ether extracts were diluted to get series of concentrations from 6.25mg/ml to 100mg/ml in sterile nutrient broth. The microorganism suspension of 50µl was added to the broth dilutions. These were incubated for 18 hours at 37°C. MIC of each extract was taken as the lowest concentration that did not give any visible bacterial growth.

RESULT

Table 1: Average zone of Inhibition of petroleum ether extract

Isolates	Zone of inhibition (mm)	M I C (mg/ml)	Ciprofloxacin 30ug
<i>Klebsiella pneumonia</i>	9.5	12.3	9.5
<i>Escherichia coli</i>	10.3	12.6	13.2
<i>Salmonella species</i>	9.0	12.0	11.5

Key

MIC=Minimum Inhibition Concentration

Table 2: Average zone of Inhibition of methanol extract

Isolates	Zone of inhibition (mm)	M I C (mg/ml)	Ciprofloxacin 30ug
<i>Klebsiella pneumonia</i>	10.0	15.3	9.5
<i>Escherichia coli</i>	9.5	14.6	13.2
<i>Salmonella species</i>	8.0	13.0	11.5

Key

MIC=Minimum Inhibition Concentration

Table 3: Average zone of Inhibition of Aqueous extract

Isolates	Zone of Inhibition(mm)	Ciprofloxacin 30ug (mm)
<i>Klebsiella Pneumonia</i>	2.0	9.5
<i>Escherichia coli</i>	5.5	13.2
<i>Salmonella species</i>	1.0	11.5

Table 4: Average zone of Inhibition of *Nigella sativa* combine with honey extract

Isolates	Zone of Inhibition(mm)	Ciprofloxacin 30ug (mm)	Control
<i>Klebsiella pneumonia</i>	10.0	9.5	-
<i>Escherichia coli</i>	11.5	13.2	-
<i>Salmonella species</i>	9.0	11.5	-

Table 5: Average zone of Inhibition of honey extract

Isolate	Zone of Inhibition (mm)			Ciprofloxacin 30ug (mm)
	25%	50%	75%	
<i>Klebsiella pneumonia</i>	1.5	6.2	8.0	9.5
<i>Escherichia coli</i>	2.0	8.5	9.6	13.2
<i>Salmonella species</i>	1.0	2.0	4.0	11.5

DISCUSSION

The antimicrobial activity of the extracts of *Nigella sativa* seed combined with honey was evaluated against the different clinical strains of bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Salmonella specie*). In the present study three different extracts (petroleum ether, methanol and aqueous) of the combined extract of *Nigella sativa* and honey extract showed pronounced activity against the test organisms Similarly, in a study conducted on the extract of *Nigella sativa* it was found to be effective on Gram negative bacteria. The Gram positive bacterial strains were resistant due to the fact that they possess an outer membrane which acts as a barrier to many environmental substances including antibiotics (Jigna *et al.*, 2007). And also study conducted by (Abdelmalek *et al.*, 2013) shows the antibacterial activity of honey alone and in combination with *Nigella sativa* seeds against *Pseudomonas aeruginosa*, the results of the antimicrobial activity of the investigated extract are shown in which the honey extracts showed less inhibition against all the bacteria tested at lower concentrations. Generally, the petroleum ether and methanol extract of *Nigella sativa* exhibited higher antibacterial effect compared with aqueous extracts this may be due to lower accessibility of the extraction of anti-bacterial agents into the extract as reported by morsi,2000.

CONCLUSION

Honey and *Nigella sativa* have been found to possess active biochemical constituents that acts as antimicrobial agents against the selected organism. The results of this study show that adding honey to *Nigella sativa* increases the antibacterial effect against all the test organism. The combination of honey with *Nigella sativa* displayed valued potency on the test organisms than when used in single form. This emphasized that combination of two or more substances with medicinal values could be better if their components will not cause a reaction that could cause health disaster (NCCLS). Performance standards for antimicrobial disk and the healing mechanism of combination between medicinal plants and honey requires further investigation. It is therefore concluded that honey and *Nigella sativa* combinations due to their synergistic effect have potential to be used in the treatment of enteric infections.

