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Pathogens Identification and Evaluation of Nigella sativa's (Kalonji) Antibacterial Activity

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ABSTRACT

To examine the presence of different pathogens capable of causing diseases in the humans, bacteria were isolated from clinical samples. 40 different clinical samples were collected from which different pathogens were isolated and identified by using conventional methods. In this article we also checked the antibacterial activity of Nigella sativa. The Nigella sativa commonly known as kalonji are use in herbal medicine, throught the world for the prevention and treatment of many diseases. The kalonji oil has many properties like antibacterial, antiparasitic, antioxidant etc. kalonji oil was used to study the antibacterial activity against 5 clinical isolates of bacteria. Disc diffusion technique was applied on inoculated Muellar Hinton agar plates. Among Gram positive bacteria only Staphylococcus aureus was used, Among Gram negative bacteria Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus, and E.coli were used. The oil showed antibacterial activity against Staphylococcus aureus and E.coli and Pseudomonas aeruginosa.

Keywords: Clinical samples, Disc diffusion method, Nigella sativa, N. sativa oil, Thymoquinone.

INTRODUCTION

Pathogens isolation and identification is based on the cultivation of microbes with morphological and physiological characterization lasting 24-48 hours. However, early and accurate identification is always required for fast and targeted antimicrobial treatment. Rapid and fast identification is mostly based on Molecular based techniques but differentiation between closely related species is still difficult. Identification of bacteria based on traditional methods relies on phenotypic identification of the pathogens using Gram staining, Culture and Biochemical methods. (Gobernado, 2003).

Mostly the body fluids are sterile like blood, urine, ear wax, nasal discharge, CSF etc. normally the presence of microbes in these samples may be due to contamination or they may be the normal flora of surrounding area. When the microbes grow in different types of body fluids, they may cause infection in them.

The blood is normally a sterile environment. These are the common pathogens of blood S. aureus, S. epidermidis, S. pyogenes, S. pneumoniae, M. tuberculosis, Legionella, E. coli, K. pneumoniae, Enterobacter, P. aeruginosa, Proteus mirabilis, H.influenzae, and N. meningitidis. (Herbert et al., 2007) Pus is a yellowish viscous substance secreated by wounds in response to body's natural immune system. The common bacterial pathogens were Gram positive cocci like aureus, S. epidermidis and ß-Streptococci, Gram-negative Pseudomonas, K.

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pneumoniae and E. coli. (Bone R et al., 1992) Sputum samples that may have some pathogens are usually used to look for infections by Moraxella catarrhalis, M. tuberculosis, pneumoniae and H. influenzae. (Annane et al., 2005)

Nigella sativa (Klonjii) is used as food preservative and spice. In traditional medicine its oil and seed constituents have important medicinal properties (Riaz et al., 1996). Kalonji is used as food and medicines in many different countries including Pakistan, Iran, India, Saudi Arabia and Egypt. It is used in conventional medicine for treatment or prevention of a variety of respiratory and gastrointestinal infection in the Islamic world (Riaz et al., 1996). It has been also used in many conditions related to kidney and liver function, respiratory, circulatory and immune system support, stomach, intestinal complaints, inflammatory diseases (Malhotra, 2006; Ramadan, 2007). They have insect repellent, antimicrobial, antitumor (Khan et al., 2003) and anti diabetic, anti-H.pylorin, anti asthmatic, anti parasitic, antioxidant, antimicrobial, anti-inflammation, hypoglycemic, antihypertensive, and anticancer activity (Fararh et al., 2002). In nursing mothers seeds of kalonji are utilized for enhancing milk production, also improving digestion and in fighting against parasitic infections, anti-inflammatory antihistaminic, and antihypertensive (Hajhashemi et al., 2004), and for the treatment of chronic colds. Inhalation of its volatile oils is useful. It is very important in curing migraine, paralysis, facial palsy. Its oil is effective in the treating skin infections like eczema, boils, earaches (Khan, 2003; Antuono et al., 2002; Iqbal et al., 2011) and immune stimulation. In pakistan the crop of Kalonji appears to be a potential and multi-purpose crop. Recently, Kalonji seeds have been shown for many biological activities, including: antioxidant, antimicrobial, Immunomodulatory, anti-inflammatory. Its seed or extract inhibits fungal growth in dermatophytes (Aljabre et al., 2005). The extract of Kalonji seed and its derivative, thymoquinone, inhibits some opportunistic fungi including Aspergillum niger, Fusarium solani and Scopulariopsis brevicaulis. Kalonji contains approximately 100 valuable elements. It contains 21% protein, 35% fatty acids, 38% carbohydrates, vitamins such as A, B, B2, C and niacin, minerals like calcium, magnesium, potassium, selenium, iron, zinc and large amount of fixed oils (Ko¨ kdil and Yilmaz, 2005). Kalonji have some active ingredients like fixed oils, nigellone and thymoquinone (Aljabre et al. 2005). The aim of the present study was to evaluate the antibacterial activity of Nigella sativa against different bacterial isolates from clinical samples.

MATERIALS AND METHODS

Sample size: 40 different clinical samples were collected from Tahir Shamsi lab, Karachi which include 18 pus samples, 14 blood samples, 7 sputum samples and 1 pleuritic fluid sample.

Isolation of organisms: All samples were first inoculated on nutrient agar plates with the help of sterile wire loop for primary isolation and incubated at 370C for 24 hour. From the last day incubated plates, performed gram staining, biochemical tests that include sugar tubes (glucose, lactose, and sucrose), TSI agar, IMVIC and citrate. Enzyme test were also performed that includes catalase and coagulase, to identify organism. For further confirmation some selective agar were also used.

Antimicrobial Sensitivity Testing: Kalonji oil was purchase from local market of Karachi, Pakistan. Disc (6mm in diameter) were punched out from Whatman's filter paper that placed in petri dish on a distance of 2-4mm. Discs were sterilized in a hot air oven at 1600C for 1hour. 20µL of oil was pipette out on to each disc in order to soak the oil on disc properly and incubated at 370C for 1 hour. Discs were then placed in air tight container and refrigerate at 40C until used.

For antimicrobial testing of kalonji, Kirby Bauer Disc Diffusion method was followed by making lawn of bacterial culture on Mueller Hinton's agar (MHA) and placed oil soaked disc on it. Incubated

the plate at 370C for 24.

RESULTS AND DISCUSSION

Forty different clinical samples were used for the isolation of pathogens. In pus samples out of 18, 6 Staphylococcus, 3 Klebsiella, 2 E.coli, 2 Enterobacter, 2 Bacillus, 2 Proteus and 1 Micrococcus were isolated. In blood samples out of 14, 5 Salmonella typhi, 3 Streptococcus, 2 Proteus, 2 Klebsiella, 1 Pseudomonas and 1 E.coli were isolated. In sputum samples out of 7, 3 Streptococcus, 2 Pseudomonas, 1 Staphylococcus aureus and 1 Bacillus were identified (Figure 1-3).

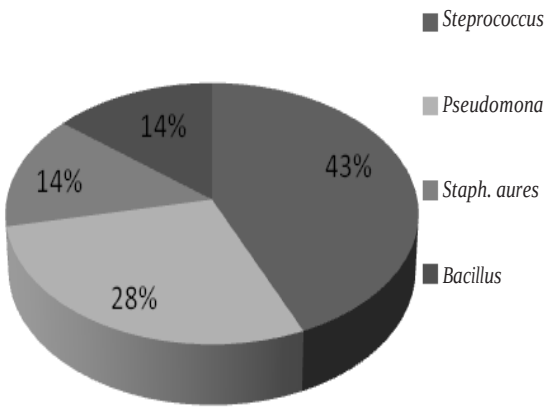

Figure 1: Distribution of isolated organisms in sputum samples.

In antimicrobial sensitivity testing, E.coli, aureus and Pseudomonas were sensitive were found to be sensitive to Nigella sativa and the zone of inhibition is about 120 mm, 150 mm and 210 mm respectively, While Klebsiella and Proteus were resistant to Nigella sativa.

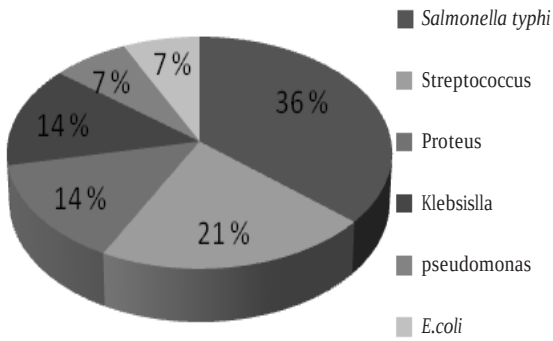

Figure 2: Distribution of isolated organisms in blood samples.

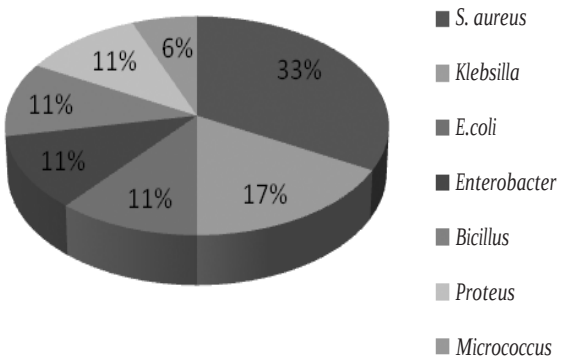

Figure 3: Distribution of isolated organisms in pus samples.

Antibiotic sensitivity testing was performed on five selected bacterial strains. Proteus from pus sample was found to be resistant against cefactor, gentamycin and was sensitive to polymaxin B(18 mm), ceftrioxon (25 mm). S.aureus was sensitive to amikacin (13 mm) & gentamycin (14mm) while resistant to cefactor & penicillin G. E.coli was sensitive to Polymaxin B (13 mm) gentamycin(14 mm) and resistant to cefactor & gentamycin. Pseudomonas was sensitive to to ciprofloxacin (20 mm) and gentamycin (20mm) while resistant to ceftrioxon and ofloxacin. Klebsiella was sensitive to gentamycin (13mm) while resistant to, polymaxin B, gentamycin and ceftrioxon. Some studies (Khan and Ather, 2006) shows the oil was more effective on gram positive than gram negative bacteria because of gram negative have outer membrane that act as effective permeability barrier, which restricts the penetration of unnecessary compounds. Kalonji oil showed pronounced activity against S. aureus, P. aeruginosa and E. coli, while dosen't shows activity against Klebsiella and Proteus (Figure 4-5).

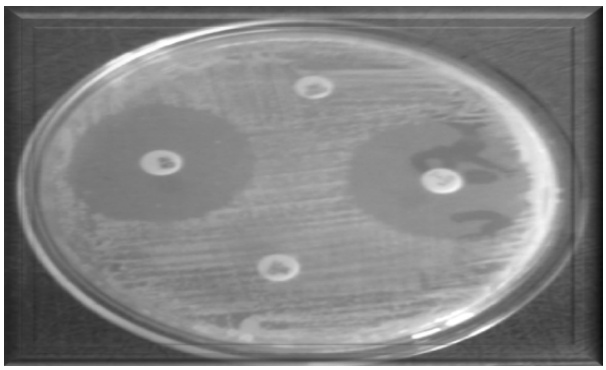

Figure 4: Inhibition of *S.aureus* by Antibiotics

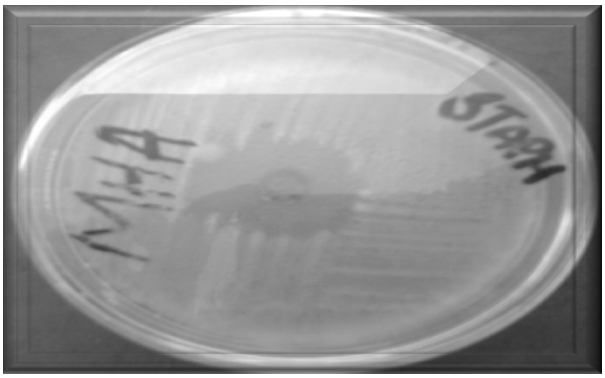

Figure 5: Inhibition of *S.aureus* by *N. sativa.*

The out coming results may be due to the reason that kalonji oil was obtained from different commercial sources, that may contain different amount of Thymoquinone that has antibacterial activity.

Table I: Susceptibility pattern of bacteria against *Nigella sativa*

Organism	Sensitive	Resistant
E.coli	120 mm	-
Staphylococcus aureus	150 mm	-
Klebsiella	210 mm	-
Proteus	-	Resistant
Pseudomonas	-	Resistant

Table II: Susceptibility pattern of bacteria against antibiotics

P.aeruoginosa		
Antibiotics	**Resistant**	**Sensitive**
Ciprofloxacin (CIP5)	-	20mm
Gentamycin (CN10)	-	20mm
Ceftrioxone (CRO30)	resistant	-
Ofloxacin (OFX5)	resistant	-

E.coli			***Klebsiella***		
Antibiotic	**Resistant**	**Sensitive**	**Antibiotic**	**Resistant**	**Sensitive**
Polymyxin (PB3OO)	–	13mm	Polymyxin (PB3OO)	Resistant	–
Gentamycin (CN10)	–	14mm	Gentamycin (CN10)	–	13mm
Cefaclor (CEC30)	resistant	–	Cefaclor (CEC30)	Resistant	–
Penicillin G	resistant	–	Ceftrioxon (CRO30)	–	–

Proteus			***Staphylococcus***		
Antibiotics	**Resistant**	**Sensitive**	**Antibiotics**	**Resistan**	**Sensitive**
Polymyxin (PB3OO)	–	170 mm	Ofloxacin (OFX5)	–	300 mm
Ceftrioxon (CRO30)	–	250 mm	Cefaclor (CEC30)	Resistant	–
Cefaclor (CEC30)	resistant	–	Gentamycin (CN10)	–	280 mm
Ceftrioxon (CRO30)	resistant	–	Ceftrioxon (CRO30)	Resistant	–

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