



Anti-Bacterial Properties of *Nigella Sativa*

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Abstract : An alarming increase in bacterial strains resistant to existing antimicrobial agents demands renewed efforts to find agents effective against these resistant bacteria. Synergistic combinations of various antimicrobial agents have been introduced as an extra successful strategy to overcome this problem. This study was undertaken to evaluate the antimicrobial property of *Nigella sativa* (Kalonji) essential oil on bacteria isolated from skin and its synergistic effects with ciprofloxacin (CF) and ofloxacin (OF). A total of fourteen (14) bacteria were isolated from skin and were characterized on the basis of Gram's staining and biochemical tests and then tested for their sensitivity to Kalonji oil alone and in combination with ciprofloxacin (CF) and ofloxacin (OF). The oil showed

pronounced antimicrobial activity which was more for Gram positive (+ve) bacteria than Gram negative (-ve) bacteria. A bigger clear zone of inhibition was observed upto 71.98 ± 0.02 and 69.49 ± 0.05 when the oil was combined with ciprofloxacin (CF) and ofloxacin (OF), respectively. The result indicated that *Nigella sativa* oil has inhibitory effect on bacteria. The study provides new insights about synergistic antimicrobial activity. Thus, we can conclude that this essential oil can be used as a bio-control agent to treat bacterial infections apart from its usual traditional uses.

Keywords: *Nigella sativa*; antibacterial activity; synergistic activity; ciprofloxacin; ofloxacin.

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Introduction:

Many developed as well as developing countries are facing problems related to infectious diseases that cause mortality and morbidity (Lewis and Ausubel 2006). In human body bacteria are found on both inner and outer surfaces especially on the skin and mucous membranes. Most of these bacteria are harmless, many of them are beneficial and a few are even necessary. However, there are some bacteria which are categorized as pathogens, which are able to colonize, invade and damage the host and cause illness (Baceiro et al., 2013). Treatment by antibiotics is one of the approaches of modern medicine to treat diseases caused by pathogene. The "Golden Era" of antibiotics from 1930s to 1960s gave rise to many antibiotics

(Nathan and Cars, 2014). Unfortunately, this era ended because researchers were unable to maintain the pace of antibiotic discovery in the face of emerging resistant pathogens. Persistent failure to develop or discover new antibiotics and non-judicious use of antibiotics are the predisposing factors associated with the emergence of antibiotic resistance (Nathan, 2004).

Resistance genes are easily transferred among bacteria especially bacteria on skin and mucous membranes. In dermatological patients resistance problems are found among *Staphylococci*, *Propionibacterium acnes* and to some extent *Streptococci*. *Staphylococcus aureus* strains have developed worldwide resistance to penicillin and now methicillin resistance is a major problem with resistance levels of > 50% in certain areas of the world. All these factors forced researchers to search for antimicrobial substances from different sources, including plants and plant based products (Mandal et al., 2010).

Plants are rich in a wide variety of phytochemical products such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties (Sohn et al., 2004). With better techniques and knowledge, synthesized compounds from natural plants will most likely lead to better results in the field of antimicrobial therapy and control of infectious diseases (Zakaria, 1991; Srinivasam et al., 2001).

Among various medicinal plants, *Nigella sativa* (Kalonji), (Family Ranunculaceae) is emerging as a miracle herb. The seeds of *Nigella sativa* and their oil have been widely used for centuries in the treatment of various ailments throughout the world. It is an important drug in the Indian traditional systems of medicine like 'Unani' and 'Ayurveda' (Goreja, 2003; Sharma et al., 2005). *Nigella sativa* has been extensively studied for its biological activities and therapeutic potential. Pharmacological effects of *Nigella sativa* seeds involve anti-inflammatory, anti-parasitic, antifungal, anticancer, etc. (Khan et al., 2003).

Reports on antimicrobial activity of Kalonji seed and oil on skin bacteria are lacking. Thus, this study was aimed to evaluate the antimicrobial activity of

commercially available black seed and its oil on bacteria isolated from the skin.

Materials and Methods:

In the present study, microbes were collected from the skin. The sterilized cotton swab was dipped in saline and rolled on the overall surface area of both right and left hand. The cotton swab was then rotated on the prepared petri plate of nutrient agar (N.A.) medium. All the petri plates were then incubated for 24-48 hours at 37°C. Different colonies were then isolated on the basis of colour, morphology and consistency by using inoculating loops on the nutrient agar medium plates. The plates were then incubated for 24-48 hours at 37°C. All the skin bacterial isolates were sub-cultured every week.

Characterization and identification of each colony isolates was achieved by initial morphological examination of the colonies in the plate (aseptically) for colonial appearance, size, form, texture consistency, colour, odour, opacity and the result was recorded. Gram's staining provided a preliminary identification of the bacteria and the biochemical tests provided the characterization and secondary identification of the bacteria.

Antibiotic susceptibility test was performed to detect the susceptibility and resistance of the isolated bacterial strains against a panel of different antibiotics by 'disk diffusion method' (Bauer et al., 1966). The liquid culture of the isolates was poured on the MHA plate (100ml) using micropipette and was evenly spread using spreader under aseptic conditions. Seven different antibiotic discs namely Streptomycin (10mcg/disc), Gentamycin (10mcg/disc), Kanamycin (30mcg/disc), Ciprofloxacin (5mcg/disc), Ofloxacin (5mcg/disc), Ampicillin (10mcg/disc), Norfloxacin (10mcg/disc) were evenly placed around the periphery of the MHA plate at regular intervals using sterile forceps. The plates were then incubated at 37°C for 24 hours and the zone of inhibition was then measured (in mm). Among the seven different antibiotics used, two (Ciprofloxacin and Ofloxacin) were selected based on the maximum zone of inhibition of the discs. The susceptibility of bacteria was also tested against the Kalonji seed and oil separately.

Synergistic effect of the two selected antibiotics (Ciprofloxacin and Ofloxacin) with Kalonji oil was studied. For this, pastes of the selected antibiotics were prepared with Kalonji oil. The liquid culture of the isolates (100µml) was poured on the MHA plate using micropipette and was evenly spread. The discs loaded with the prepared paste (antibiotic + Kalonji oil) was impregnated on the MHA plate. The plates were incubated for 24-48 hours at 37°C and the zone of inhibition was measured. This experiment was repeated two more times and the mean along with standard error was calculated.

Results and Discussion:

A total of 14 strains were isolated from the skin. Preliminary identification of the bacteria was done on the basis of Gram's staining and colony morphology (Table 1) and further identification was done on the basis of biochemical tests (Table 2). Seven (7) out of fourteen (14) isolated strains of the bacteria were selected for further study. Among the seven isolated strains four were identified which were named as Sample 'A' = *E. coli*, Sample 'B' = *Bacillus spp.*, Sample 'C' = *Staphylococcus spp.*, Sample 'D' = *Staphylococcus spp.* In this study, it was found that the seven isolated strains which were tested against the different antibiotics showed maximum zone of inhibition (in mm) against ciprofloxacin and ofloxacin. The gram positive bacteria showed better results as compared to gram negative bacteria (Table 3). Among the different concentrations of Kalonji seed extracts tested against the skin isolates, crude extract showed the best result (Table 4). It was also found that Kalonji oil showed better result as compared to Kalonji seed extract against the skin bacterial isolates when combined with the antibiotic ciprofloxacin and ofloxacin, respectively (Table 4 and 5). The antimicrobial effect of Kalonji oil was enhanced when combined with ciprofloxacin and ofloxacin (Table 6 and 7).

In the experiments conducted with Kalonji seed extracts, the crude aqueous extract showed very little zone of inhibition while the serially diluted concentrations showed no significant result against the 14 bacteria isolated from skin. This difference from earlier studies in antimicrobial property of aqueous

extract might be due to number of strains tested, origin of strains tested or due to variation in active ingredients in Kalonji seeds used in the study (Mehta and Gupta, 2016). The difference might be due to the fact that in the reported study (Enany et al, 2009) bacteria were tested against 100% of Kalonji seed extract, which does not appear to be a practical approach.

While the aqueous extract of Kalonji seed did not show any antimicrobial Property, Kalonji oil was found to be more effective on Gram (+) ve Bacteria, with maximum zone of inhibition of 24.80 ± 0.05 (Table 5) which is a conformity with the earlier studies (Agarwal et al., 1979; Ali et al., 2001) . A number of compounds derived from plants often show considerable activity against Gram (+) ve but not against Gram (-) ve Species. Effective permeability barrier comprising the outer membrane restricts the penetration of Amphipathic compounds and Multi Drug Resistance Pumps that extrudes the toxins across this barrier are present in Gram (-) ve Bacteria. The apparent ineffectiveness of plant antimicrobials may be due to the permeability barrier (Tegos et al., 2002).

Drug Synergism between known antibiotics and bioactive plant extracts is a novel concept and could be beneficial (Synergistic or additive interaction) or deleterious (antagonistic). In the present study, significant results were obtained by the combination of oil with commercially available Antibiotics (Ciprofloxacin and Ofloxacin) with maximum zone of inhibition with Ciprofloxacin of 28.68 ± 0.07 (Table 6) and with Ofloxacin of 69.49 ± 0.05 (Table 7). These results were in similar to those of Esimone et al. (2006) according to which combination of two agents exhibit significant synergism only if test organism is resistant to at least one of the agents. As an application of the present study, herbal band-aid was prepared to fight against skin acne problem and a significant result was obtained (Fig. 1). The observation of the present study may be an important step in planning for development of an effective therapeutic preparation from Kalonji at least for topical infections. Kalonji oil in combination with antibiotics might be a source of an antimicrobial component for topical application to inhibit the growth of microbes causing skin infections.

Table 1. Cultural and morphological characteristics of isolated bacteria

Sl. No.	Sample name (no. of strains)	Source	Media	Colony morphology	Gram's reaction	Shape
1.	A(2)	Skin flora	Nutrient agar	Size- Large; Shape- Circular; Margin- Entire; Colour- White; Texture- Smooth & Moist; Light Transmission- Opaque	Negative	Rod
2.	B(2)	Skin flora	Nutrient agar	Size-Medium to Large; Shape- Circular; Margin- Irregular; Colour-Grey-White; Texture- Smooth & Dry; Light Transmission-	Positive	Rod
3.	C(4)	Skin flora	Nutrient agar	Size- Large; Shape- Round; Margin- Entire; Colour- Golden-Yellow; Texture- Smooth & Moist; Light Transmission- Opaque	Positive	Cocci
4.	D(2)	Skin flora	Nutrient agar	Size- Small; Shape- Round; Margin- Entire; Colour- White; Texture- Smooth & Moist; Light Transmission- Opaque	Positive	Cocci
5.	E(1)	Skin flora	Nutrient agar	Size-Medium to Large; Shape- Circular; Margin- Irregular; Colour- White; Texture- Smooth & Moist; Light Transmission- Opaque	Negative	Rod
6.	F(1)	Skin flora	Nutrient agar	Size- Large; Shape- Circular; Margin- Irregular; Colour- Pink; Texture- Smooth & Moist; Light Transmission- Opaque	Negative	Rod
7.	G(2)	Skin flora	Nutrient agar	Size-Medium to Large; Shape- Circular; Margin- Entire; Colour- Orange; Texture- Smooth & Moist; Light Transmission- Opaque	Positive	Rod

Table 2. Biochemical characterization of isolated bacteria

Sample name (no. of strains)									
Sl. No.	Biochemical test	Media used	Sample 'A' (2)	Sample 'B' (2)	Sample 'C' (4)	Sample 'D' (2)	Sample 'E' (1)	Sample 'F' (1)	Sample
1.	Indole Production Test	Tryptophan broth	Positive	Negative	Negative	Negative	Negative	Positive	Negative
2.	Methyl-Red Test	MR-VP broth	Positive	Negative	Positive	Negative	Negative	Positive	Negative
3.	Voge's-Proskauer Test	MR-VP broth	Negative	Positive	Positive	Positive	Positive	Negative	Negative
4.	Citrate Utilization Test	Simmon's Citrate Agar	Negative	Positive	Positive	Negative	Positive	Negative	Positive
5.	Oxidase test	No media used	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6.	Catalase test	No media used	Negative	Positive	Positive	Positive	Positive	Positive	Positive
7.	Coagulase Test	Blood plasma	Negative	Negative	Positive	Negative	Negative	Negative	Negative
8.	Nitrate reduction test	Nitrate broth	Positive	Positive	Positive	Positive	Positive	Negative	Positive
9.	Urease test	Urease broth	Negative	Negative	Positive	Positive	Negative	Positive	Negative

Table 3. Average zone of inhibition of the seven antibiotic discs against skin isolates

Sl. No.	Gram positive/negative	Sample name	Antibiotics used (zone of inhibition in millimetre)						
			Streptomycin (10mcg/disc)	Gentamycin (10 mcg/disc)	Kanamycin (30 mcg/disc)	Ciprofloxacin (5 mcg/disc)	Ofloxacin (5 mcg/disc)	Ampicillin (10 mcg/disc)	Norfloxacin (10 mcg/disc)
1.	Gram +VE	B	11.95±0.08	17.9±0.1	11.83±0.08	24.91±0.06	28.5±0.4	1.8±0.16	18.72±0.05
2.		C	19.93±0.06	10.88±0.2	11.35±0.04	26.85±0.04	23.38±0.06	0±0	0±0
3.		D	21.45±0.05	16.61±0.04	13.08±0.03	27.84±0.02	28.72±0.02	10.04±0.06	15.61±0.07
4.		G	12.17±0.02	16.03±0.06	22.34±0.03	28.68±0.06	29.60±0.04	16.50±0.04	20.66±0.04
5.	Gram -VE	A	11.96±0.06	17.92±0.12	11.58±0.05	21.12±0.08	22.34±0.04	0±0	18.90±0.08
6.		E	11.82±0.04	18.97±0.06	15.65±0.04	20.88±0.08	21.66±0.04	14.39±0.0	12.96±0.0
7.		F	10.84±0.01	13.95±0.0	12.86±0.0	18.60±0.05	23.04±0.04	11.36±0.01	14.94±0.01

Table 4. Average zone of inhibition with serially diluted Kalonji seed extract against skin isolates

Sl. No.	Gram positive/negative	Sample Name	Zone of inhibition (in milimetre) of different concentrations (in microlitre)					
			CRUDE	10-1	10-2	10-3	10-4	10-5
1	GRAM+VE	B	3.23±0.25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2		C	4.66±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
3		D	1.76±0.15	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
4		G	2.26±0.05	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
5	GRAM -VE	A	1.76±0.15	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
6		E	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.2	0.0±0.3	0.0±0.4
7		F	0.7±0.62	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Table 5. Zone of inhibition (in mm) of Kalonji oil against the skin isolates

Sl. No.	Gram positive/negative	Sample name (no.of colonies)	Reading 1 (in mm)	Reading 2 (in mm)	Reading 3 (in mm)	Mean ± SEM
1.	Gram +VE	B(2)	20.25	20.20	20.30	20.25 ± 0.05
2.		C(4)	22.06	22.00	22.10	22.05 ± 0.05
3.		D(2)	24.08	24.00	24.05	24.04 ± 0.04
4.		G(2)	24.80	24.85	24.75	24.8 ± 0.05
5.	Gram -VE	A(2)	11.50	12.00	11.00	11.5 ± 0.5
6.		E(1)	16.90	16.85	16.85	16.86 ± 0.02
7		F(1)	15.02	15.00	15.05	15.02 ± 0.02

Table 6. Zone of inhibition of Kalonji oil and ciprofloxacin against the skin isolates

Sl. No.	Gram positive/negative	Sample name (no.of colonies)	Reading 1 (in mm)	Reading 2 (in mm)	Reading 3 (in mm)	Mean ± SEM
1	Gram+VE	B(2)	71.99	71.95	72.00	71.98 ± 0.02
2		C(4)	58.01	58.10	58.15	58.08 ± 0.07
3		D(2)	63.93	63.90	70.00	65.94 ± 3.51
4		G(2)	63.08	63.00	63.10	63.06 ± 0.05
5	Gram -VE	A(2)	49.01	49.05	48.95	49 ± 0.05
6		E(1)	36.97	36.90	37.00	36.95 ± 0.05
7		F(1)	56.02	56.00	56.10	56.04 ± 0.05

Table 7. Zone of inhibition of Kalonji oil and ofloxacin against the skin isolates

Sl. No.	Gram positive/negative	Sample name (no.of colonies)	Reading 1 (in mm)	Reading 2 (in mm)	Reading 3 (in mm)	Mean ± SEM
1	Gram+VE	B(2)	63.02	63.00	63.10	63.04 ± 0.05
2		C(4)	69.49	69.55	69.45	69.49 ± 0.05
3		D(2)	57.90	57.80	57.85	57.85 ± 0.05
4		G(2)	57.38	57.34	57.30	57.34 ± 0.04
5	Gram -VE	A(2)	48.19	48.15	48.22	48.18 ± 0.03
6		E(1)	36.97	37.00	36.90	36.95 ± 0.05
7		F(1)	44.70	44.65	44.72	44.69 ± 0.03



Fig. 1. Depicting significant decrease in acne after treatment with herbal band-aid loaded with Kalonji oil

Conclusion:

It is concluded that *Nigella sativa* (Kalonji) is antimicrobial in nature. Large zone of inhibition obtained by Kalonji oil against bacteria isolated from skin, when used in combination with antibiotics (synergistic) may reduce the bacterial resistance power and enhance their sensitivity towards commonly used antibiotics.

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