ANTIBACTERIAL ACTIVITY OF BLACK SEED (*NIGELLA SATIVA*) CRUDE OIL AGAINST STREPTOCOCCI ORGANISMS ISOLATED FROM MASTITIS COWS

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ABSTRACT

Mastitis of cattle causes a massive economic loss worldwide. The treatment of mastitis is generally considered to be limited because of low cure rates and the cost of milk discarded during the withdrawal period. The criticism of use of antibiotics to treat mastitis is that it leads to development of resistant bacteria and presence of residual effect of antibiotic in milk. Therefore, now many researchers are turning to find non-antibiotic approaches in order to reduce the use of antibiotics. In the traditional veterinary medicine, medicinal herbs are used to treat animal diseases. This is believed to be natural and safe therapeutic methods. In this *in vitro* study, the Black seed (*Nigella sativa*) oil was examined for its antibacterial activity against one of the most common mastitis pathogen, Streptococci, in order to develop an herbal remedy for Streptococcus mastitis as a substitute for antibiotics. This is the first report demonstrating this oil on mastitis causing Streptococci. In this study, Streptococci organisms isolated from 40 clinically infected milk samples were investigated. All the organisms tested were susceptible and the antibacterial effect of Black seed oil on Streptococci organisms was very significant as evidenced by the inhibition zone sizes obtained. In conclusion, the antibacterial effect of this seed on Streptococci, which causing mastitis was statistically significant and this study suggesting that Black seed oil can be used as antibacterial agent targeting on Streptococcus mastitis.

Keywords : Black seed, *Nigella sativa*, Mastitis, Streptococcus, Antibacterial.

INTRODUCTION

Mastitis, Inflammation of mammary gland caused usually by bacteria is the most prevalence infectious disease of adult dairy cattle. Mastitis causes a massive economic loss worldwide[1]. The data on the prevalence of pathogen demonstrate the significant differences between organisms [2]. A study identified a marked difference in the pathogens isolated from clinical mastitis cases between the dry and lactating period. *Streptococci* were isolated from 42.8% of dry period cases but from only 20.7% of cases occurring in lactation [2].

Conventional treatment of mastitis is the intramammary infusion of antibiotics, although alternatives including herbal and homoeopathic approaches assume importance [3]. The criticism of use of antibiotics to treat mastitis is that it leads to development of resistant strains of bacteria that can be transferred to man and strictly should adapt to withdrawal period in order to minimize antibiotic residue [4]; [5]; [6]. Due to the extensive use of antibiotics in dairy herds, contamination of milk has become a subject of public concern. And also the organic regulations state that alternative remedies should be used in preference to chemically synthesised allopathic veterinary medicinal preparations, that they are effective and safe for the condition to be treated [2]. Therefore, an increasing number of veterinarians are turning to non-antibiotic approaches in order to reduce the use of antibiotics. In the traditional veterinary medicine, medicinal herbs and acupuncture are used to treat animal diseases. These are believed to be natural and safe therapeutic methods.

Black seed (English name) is a small aromatic black color seed (slightly smaller than the sesame seed) of a medicinal plant, namely *Nigella sativa,* a member of buttercup family, Ranunculaceae. The plant is also known by other names e.g. Black cumin / Fennel flower (English), Karun jiragum (Tamil), Kaluduru (Sinhala), Kalounji (Hindi) and Habbah Sauda (Arabic). This Black seed (Seeds of *Nigella sativa*) and Black seed oil is a common drug used in Ayurvedic medicine in Sri Lanka and widely available in Sri Lankan market in the form of seeds, its oil and varieties of commercial pharmaceutical products.

Antibacterial effect of Black seed was first reported almost four decades ago [7] in which the oil was shown to inhibit the growth of both Gram-positive and Gram-

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negative organisms except certain strains of *Pseudomonas aeruginosa.* Recently, crude extracts of Black seed were reported to have a promising effect on multi-resistance organism including Gram-positive and Gram-negative bacteria [8]. The filter paper disc impregnated with the extract of Black seed was examined and was exhibited inhibition of Gram-positive bacteria represented by *Staphylococcus aureus.* Gram-negative bacteria represented by *Pseudomonas aeruginosa* and *E. coli* [9]. As for all the benefits packed into this tiny seed, it is waiting to be discovered, ongoing researchers will have to judge.

This *in vitro* study is aimed at detecting the antibacterial activity of Black seed oil against Streptococci, one of the most common mastitis causing bacteria isolated from mastitis milk, that can be direct to develop an herbal remedy as a substitute for antibiotic treatment and this is the first study of its kind to demonstrate this seed for mastitis.

Materials and methods

All the clinical strains of bacteria were isolated from series of mastitis positive milk samples submitted to the clinical microbiology laboratory of Faculty of Veterinary Medicine and Animal Science, University of Peradeniya for isolation and identification of organisms.

Isolation of organisms

The organisms were isolated from milk samples through cultivating the organisms to obtain discrete colonies and sub culturing the organisms to obtain pure cultures. For the first step, milk was streaked on blood agar medium with an inoculating swab and those inoculated plates were placed in the incubator at 37ºC for 24-hr period to obtain discrete colonies. For the sub culturing, the discrete colonies obtained from previous primary culture were inoculated separately on the blood agar plate and incubated at 37ºC for 24-hr period to obtain pure cultures.

Identification of organisms

Initially the microorganisms were identified by using the Gram's stain. The bacteria which retain the aniline dye under the microscopic view are termed Gram positive (purple in color). For further identification of Streptococci, microscopic appearance of bacteria was considered and it was confirmed by catalase test. Microscopically the Streptococci appear as cocci in pairs or short chains. For the catalase test, the Streptococci do not produce bubbles when it is mixed with a drop of hydrogen peroxide on a clean glass slide.

Preparation of plant material and discs for assays

The Black seed seeds collected by Sri Lankan herbalists were purchased from local herbal stores in Colombo and its oil was extracted mechanically through physical compression without the use of any chemicals in order to use for microbial assays. Coconut oil and distilled water were used as control. For the preparation of impregnated disc, 6 mm diameter paper discs were used.

Antimicrobial assay

For the detection of antibacterial activity, the disc-diffusion assay [10] was used. The pure bacterial suspension in normal saline was prepared and the turbidity was adjusted by comparison with a 0.5 McFarland turbidity standard. The sterile Muller-Hinton agar plates were inoculated with sterile, non-toxic swab which was dipped into the standardized pure bacterial suspension in order to obtain a uniform inoculation. The three discs were placed and gently pressed on the agar surface and each of the three discs were impregnated separately with 15 µl of plant extract, coconut oil and distilled water.All the plates were then incubated at 37ºC in ambient air for 18-24 hours. Following appropriate temperature and incubation atmosphere, the diameters of the zones of inhibitions in all the plates were measured across the center of the disc to the nearest mm using a caliper. The inhibition zone size of e" 10 mm in diameter was considered to be susceptible to Black seed crude oil based on literature review[11].

Results

This prospective study included 40 clinical isolates from clinically infected milk samples. Black seed crude oil exhibited a strong antibacterial activity. All the Streptococci organisms tested were susceptible and it was very impressive as evidenced by the inhibition zone sizes obtained (Table-1). The mean zones of inhibition produced by Black seed oil against Streptococci were 32.6 ± 5.0. The coconut oil and distilled water used as a control had no inhibitory effect on Streptococci.

Table 1: Diameter of Inhibitory zone (mm) and the susceptibility of each Streptococci organismisolated frommilk of clinically infected cows. S=Susceptible.

Sample No.	Diameter of Inhibitory Zone (mm)	Susceptibility (S) / Resistance(R)
01	36.2	S
02	35.5	S
03	34.7	S
04	28.4	S
05	30.6	S
06	29.5	S
07	32.3	S
08	30.6	S
09	34.3	S
10	36.2	S
11	35.6	S
12	27.8	S
13	31.0	S
14	33.4	S
15	36.5	S
16	29.0	S
17	30.2	S
18	29.6	S
19	31.1	S
20	27.8	S
21	34.2	S
22	35.6	S
23	37.3	S
24	34.4	S
25	35.2	S
26	31.0	S
27	33.5	S
28	32.3	S
29	35.8	S
30	34.7	S
31	31.5	S
32	29.1	S
33	35.2	S
34	34.2	S
35	35.3	S
36	30.4	S
37	36.1	S
38	30.8	S
39	29.3	S
40	30.5	S

DISCUSSION

In this study, Black seed crude oil was highly effective against Streptococci. This finding is similar to the reports of Toppozada *et al.,* [5] and Bilal *et al.,* [11] who reported the antibacterial activity of *Nigella sativa* crude oil. Toppozada *et al.,* [7] reported that the antimicrobial activity is confined solely to the essential oil which was named *Nigellone*.

Black seed oil has been reported to have several other potent therapeutic values that could act on mastitis. Particularly, Mutabagani and El-Mahdy [12] and Peter Schleicher and Mohamed Saleh [13] who reported on anti-inflammatory and analgesic activity of *Nigella sativa* extract and this finding suggest that this oil can be used as analgesic and anti-inflammatory agent for pain and acute inflammation occurring in mastitis. Agarwal *et al.,* [14] reported the Galactagogue action of *Nigella sativa* which significantly increase the milk production in mammary gland comparatively than estrogen therapy. It has also been found that the Black seed extract modulates the immune system by increasing the number and activity of immune competent cells [15]; [16]. The antibacterial properties of the volatile oil of Black seeds have also been further examined [17]; [18]; [19]; [20]; [21]; [22]; [23] and suggested that the volatile oil would be a good substitute for common antibiotics [18]; [20].

In the present study, it was mainly concentrated only on Streptococci, one of the most common mastitis causing organisms and to our knowledge, this is the first report demonstrating this oil on Streptococci organism causing mastitis, in which all the Streptococcus tested were susceptible to this Black seed crude oil suggestive of the use of this oil as a good herbal substitute. The data on the prevalence of pathogens in clinical mastitis demonstrate the significant differences between dry and lactating period. Streptococci were isolated from 42.8% of dry period cases but from only 20.7% of cases occurring in lactation [2] and if this data represent the true population, it can be suggested that Black seed oil can be used to control significant portion of clinical mastitis cases.

In conclusion, the effective antibacterial activity of Black seed oil could provide additive effects on Streptococcus mastitis in addition to its other collective therapeutic values. However, this seeds should be further examined and through proper pharmacological and clinical investigations should be made available for general use as new alternative therapy for Streptococcus mastitis.

REFERENCES:

Andrews A.H., Blowey R.W., Boyd H, Eddy R.G. (1992): Bovine Medicine: *Disease and Husbendry of cattle*, Blackwell Science Ltd., pp.289 - 300.

Hayton, A.J. and Bradley, A.J. (2004): *The control of mastitis on organic units.* 2004.

Hillerton, J.E. and Berry, E.A. (2005): Treating mastitis in the cow – a tradition or an archaism. *J. Applied Microbiology*., Volume **98** (6), pp. 1250.

Wilson, D.J., Gonzalez, R.N., Case, K.L., Garrison, L.L. and Grohn, Y.T. (1999), Comparison of seven antibiotic treatments with no treatment for bacteriological efficacy against bovine mastitis pathogens. *J. Dairy Sci.* **82**: 1664 - 1670.

Hillerton, J.E. and Kliem, K.E. (2002), Effective treatment of clinical mastitis to minimize the use of antibiotics. *J. Dairy Sci.* **85**, 1009 - 1014.

Craven, N. (1987) Efficacy and financial value of antibiotic treatment of bovine clinical mastitis during lactation – A review. *Br. Vet. J.* **143**: 410 - 422.

Toppozada H.H., Mazloum H.A. and Al-Dakhakhny M. (1965): The antibacterial properties of *Nigella sativa* seeds. *J. Egypt. Med. Assoc.*, **48** suppl. 187 - 202.

Koneman E.W., Allen S. D., Janda W. M., Schreckenberger P. C. and Winn W. C. (1997), Mycology: *In color atlas and text book of Diagnostic microbiology, Lippincott*, USA; P: 1001 - 1008.

Hanafy, M.S.M. and Hatem, M.E. (1991): Studies on the antimicrobial activity of *Nigella sativa* seed (Black cumin). *J. Ethno. Pharmacol.* Sept., **34** (2 and3), 275:278.

Lennette E.H. (1985): *Manual of Clinical Microbiology* (4th Ed.) American Association for Microbiology, Washington, D.C., pp. 978 - 987.

Bilal, N.E., Batouk, A., Abu-Eshy, S., Al-Gamdi, B. and Al-wabel, A.A. (1996): Antibacterial effect of *Nigella sativa* on selected microorganisms. *J. Hepatology, Gastroenterology and Infectious diseases.* **4** (4), 105 - 111.

Mutabagani, A. and El-Mahdy, S.A.M. (1997): A study of anti-inflammatory activity of *Nigella sativa* L. and thymoquinone in rats, *Saudi Pharm. J.* **5**, (2-3), 110 - 113.

Peter Schleicher, M.D. and Mohamed Saleh, M.D. (2000): *Black Cumin: The Magical Egyptian Herb for Allergies, Asthma, and Immune Disorders* (Inner Traditions).

Agarwal IP, Achar MVS and Tamankar BP. (1970): Galactagogue action of *Nigella sativa*. *Indian J. Med. Science*., pp. 535 - 537.

Medenica, R., Mukerjee, S., Huschart, T., Koffskey, J. and Corbitt, W. (1993). *Nigella sativa* plant extract increases number and activity of immune competent cells in humans, *Expt. Hematol.* **21**, 1186.

Kandil, O., Radwan, N.M., Hassan, A.B., Amer, A.M., El-Banna, H.A. and Amer, W.M. (1994): d fraction of *Nigella sativa (N-sativa*) exhbit antimicrobial and immunological activities, *FASEB Journal* **8**(5), A951.

El-Kamali, H.H., Ahmed, A.H., Mohammed, A.S., Yahia, A.A.M., El-Tayeb. I.H. and Ali, A.A. (1998): Antibacterial properties of essential oils from *Nigella sativa* seeds, Cymbopogen citrates leaves, and Pulicaria undulate aerial parts, *Fititerapia* **69** (1), 77 - 78.

Hasan, C.M., Ahsan. M. and Islam, S.N. (1989): *In vitro* antibacterial screening of the oils of *Nigella sativa* seeds, *Bangladesh J. Botany* **18**, 171 - 174.

Karawya, M.S., Hashim, F.M., El-Wahab, S.M., Abd, El-Deeb, K.S., Soliman, S.N., Salam, I.A., Mokhtar. N. and El-Hossiny, S. (1994): Essential oil and lipids of *Nigella sativa* seeds and their biological activity, *Zagazig J. Pharm. Sci.* **3**(2), 49 - 57.

Rathee, P.S., Mishra, S.H. and Kaushal, R. (1982): Antimicrobial activity of essential oil, fixed oil and unsaponified matter of *Nigella sativa* L. *Indian J. Pharmac.* **44**, 8 - 10.

Sexena, A.P. and Vyas, K.M. (1986): Antimicrobial activity of seeds of some ethnomedicinal plants, *J. Econ. Tax. Bot.* **8** (2), 291 - 299.

Agarwal R, Kharya M.D. and Shrivastava R. (1979): Antimicrobial and Anthelminthic activities of essential oil of *Nigella sativa* L. *Indian J. Exp. Biol.,* **17**, 1264 - 1265.

El-Alfy, T.S., El-Fatary, H.M. and Toama, M.A. (1975): Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa* L. seeds, *Pharmazie* **30**, 109 - 111.