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In-vitro Antimicrobial activity of Moringa Oleifera and Ocimum Sanctum leaves against subclinical mastitis-causing pathogens

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ABSTRACT

Among the 164 culturally positive samples, *In vitro* drug sensitivity test was conducted on 20 samples and among these 19, 17, 15, 13, 9 and 7 samples showed sensitivity of 95.00, 85.00, 75.00, 65.00, 45.00 and 35.00 percent to Enrofloxacin, Ceftriaxone, Amoxicillin + Clavulanic acid, Tetracycline, Gentamicin and Streptomycin, respectively. These samples also showed maximum sensitivity to leaf extract of *Moringa oleifera* and *Ocimum sanctum* at 30 mg and 50 mg concentration with highest zone of inhibition as 16mm and 20mm, respectively.

Keywords: *In vitro*, Antibiotic activity, *Moringa oleifera* and *Ocimum sanctum*

1. INTRODUCTION

Ocimum sanctum (Tulasi) is a valuable herbal medicine being used in a wide spectrum of animal diseases. The positive effects of these remedies may be related to the presence of lipophilic constituents that have antibacterial, anti-inflammatory and immune modulation potential as substantiated by elimination of intra mammary infections (Shafi *et al.* 2016). Similarly, every part of *Moringa oleifera* (Drum stick) is used for certain nutritional and medicinal purpose. Besides being a good source of protein, vitamins, oils, fatty acids, micro-macro minerals elements and various phenolics, *Moringa* is also reported as anti-inflammatory, antimicrobial, antioxidant, anticancer, cardiovascular protective, hepatoprotective, anti-ulcer, diuretic, antiurolithiatic, and antihelminthic properties (Fozia *et al.* 2012).

2. REVIEW OF LITERATURE

Akinjogunla *et al.* (2011) conducted *in vitro* drug sensitivity and reported that the discs containing 25.0mg/ml of aqueous leaf extract of *Ocimum gratissimum* showed highest Mean \pm SD zones of inhibition against both clinical and environmental Moxifloxacin resistant *E coli*, while the discs containing 6.25mg/ml showed the lowest inhibitory zones.

Gajendrasinh *et al.* (2012) concluded that *Escherichia coli* are found to be highly susceptible to ethanol extract of Tulasi leaves at 250 μ g concentration.

Moyo *et al.* (2012) reported that the acetone extract of *Moringa oleifera* leaves at a concentration of 5 mg/ml showed antibacterial activities against *Escherichia coli*, *Enterobacter cloacae*, *Proteus vulgaris*, *Staphylococcus aureus* and *Micrococcus kristinae*.

Sayeed *et al.* (2012) analyzed the fruit extract of *Moringa oleifera* for antibiotic sensitivity and found broad-spectrum antibacterial and antifungal activity and recorded highest zone of inhibition at the concentration of 200 μ g/ disc for *Pseudomonas aeruginosa* (22 mm) and *Colletotrichum* Spp. (14 mm).

Vikash *et al.* (2012) conducted research to investigate the *in vitro* antimicrobial and antioxidant activity of aqueous, ethanolic and methanolic extract of *Moringa oleifera* leaf extract and got MIC at 2.4 mg/ml for *B.subtilis*, *E.coli* and 7.4 mg/ml for *S.aureus*.

Khanitta and Angelika (2015) conducted antibiotic sensitivity of *Moringa oleifera* seeds and residues against *Staphylococcus aureus* and reported maximum inhibition zone of 20.67 and 24.67 mm, respectively, at 100 mg/ml concentration.

2.1 Conflict of Interest

To evaluate the anti-microbial activity of herbs against mastitis causing pathogen and implement the herbal therapy in sub clinical mastitis to rule out the most hazardous problem that is Anti-Microbial Resistance.

3. MATERIALS AND METHODS

Among the culturally positive milk samples 20 samples were selected for antibiotic sensitivity test. The antibiotic sensitivity of the whole milk sample cultures was done *in vitro* by disc diffusion method on Muller Hinton agar (MHA) plates.

3.1 Inoculation of plates

The MHA plates were inoculated with 6-8-hour pure culture grown in nutrient broth to test the sensitivity. The culture was applied onto the surface of the MHA plates using a sterile swab. The plates then were kept covered for 15 minutes at room temperature for drying the inoculums.

3.2 Antimicrobial discs

The antibiotic discs were placed at least 20 mm apart using flamed forceps and the plates were kept for 15 minutes in the refrigerator for pre diffusion of antibiotic. Final incubation was done at 37°C for 18 to 24 hours before reading the sensitivity. The following discs were used for antimicrobial sensitivity testing (Table 1).

Table 1: Antibiotic discs used for ABST

S. No.	Antimicrobial agent	Symbol	Concentration
1.	Amoxyclav	AMC	30 mcg
2.	Ceftriaxone	CRT	30 mcg
3.	Enrofloxacin	EN	10mcg
4.	Gentamicin	GEN	10 mcg
5.	Streptomycin	S	10 mcg
6.	Tetracycline	TE	30 mcg

3.3 Preparation of *Moringa oleifera* leaves extract for ABST

Collected fresh *Moringa oleifera* leaves and sun dried for 3 days. The dried leaves grinded to make fine powder and measured as 5mg, 10mg, 20mg, 30mg, 40mg and 50mg quantity. Measured powder was soaked in 1ml of Acetone for 72 hours separately to prepare 5mg, 10mg, 20mg, 30mg, 40mg and 50mg concentrated solutions and kept at room temperature undisturbed. Supernatant was collected as extract after 72 hours (Figure1).

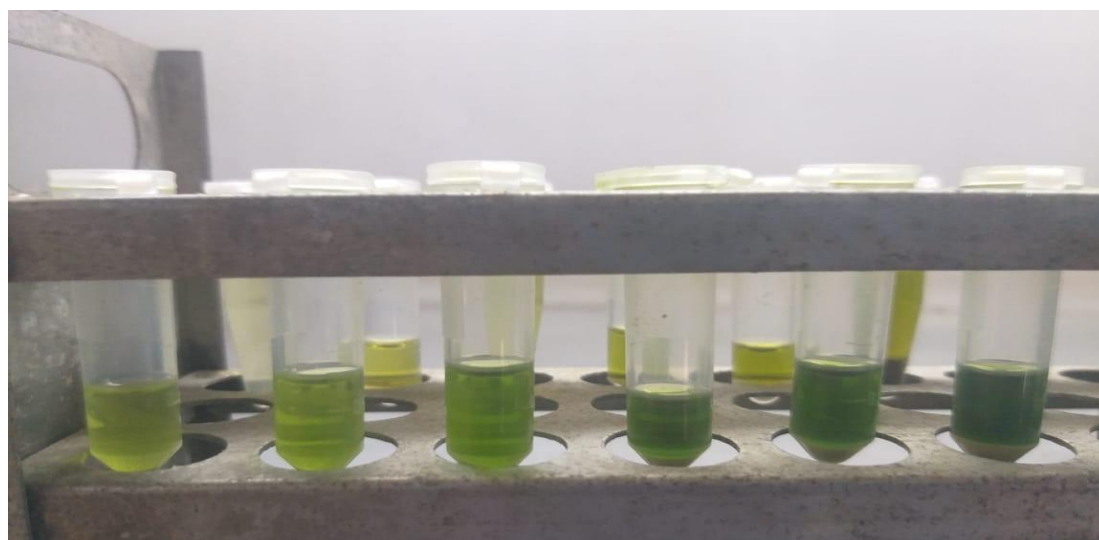


Figure 1: Different concentrated Extracts of *Moringa oleifera* leaves

3.4 Preparation of *Ocimum sanctum* leaves extract for ABST

Collected fresh *Ocimum sanctum* leaves and sun dried for 3 days. The dried leaves grinded to make fine powder and measured as 5mg, 10mg, 20mg, 30mg, 40mg and 50mg quantity. Measured powder was soaked in 1ml of Acetone for 72 hours separately to prepare 5mg, 10mg, 20mg, 30mg, 40mg and 50mg concentrated solutions and kept at room temperature undisturbed. Supernatant was collected as extract after 72 hours (Figure2).

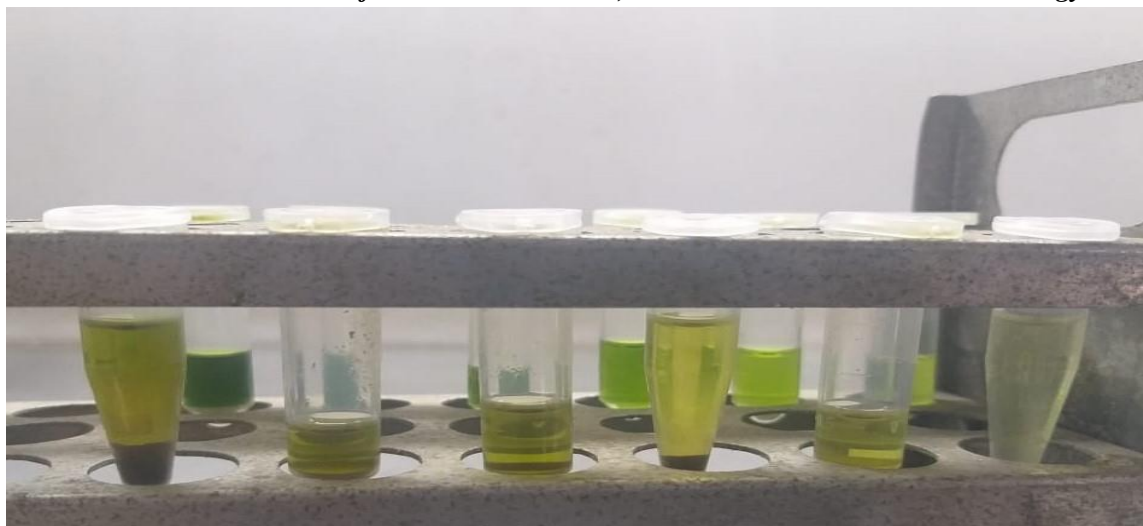


Figure 2: Different concentrated Extract of *Ocimum sanctum* leaves

***In vitro* drug sensitivity test of *Moringa oleifera* and *Ocimum sanctum* leaves**

The whole milk culture of SCM affected cows inoculated on fresh MHA plate and allowed to dry for few seconds in laminar air flow. The sterile plane discs were placed at least 20 mm apart using flamed forceps on MHA plates. 20 µL of different concentrated extract solution (5mg, 10mg, 20mg, 30mg, 40mg and 50mg) placed on each disc along with one sterile plane disc as control and allowed to diffuse for 15 minutes. Final incubation was done at 37°C for 24 hours before reading the sensitivity.

4. RESULTS

In the present investigation, a total 20 milk samples were subjected to antibiotic sensitivity test and out which 19, 17, 15, 13, 9 and 7 samples were found sensitive and 1, 3, 5, 7, 11 and 13 samples were resistant for Enrofloxacin (10mcg), Ceftriaxone (30mcg), Amoxicillin + Clavulanic acid (30mcg), Tetracycline (30mcg), Gentamicin (10mcg), Streptomycin (10mcg), respectively (Table 2, Figure 3 &4). These 20 samples also tested for antibiotic sensitivity (ABST) with *Ocimum sanctum* and *Moringa oleifera* leaves extract and revealed antibiotic activity against pathogenic bacteria. *Moringa oleifera* extract showed highest zone of inhibition at 30mg (16mm), followed by at 5mg (15 mm), 10mg (15 mm), 50mg (14mm), 20mg (13mm) and least at 40mg (12mm) concentrations (Table 3, Figure 5&6). Similarly, *Ocimum sanctum* extract showed highest zone of inhibition at 50mg (20mm) followed by at 40mg (15mm), 30mg (15mm), 10mg (13mm), and least at 20mg (12mm) and 5mg (12mm) concentrations (Table 4, Figure 7&8).

Table 2: *In vitro* drug sensitivity of bacteria from whole milk cultures of SCM affected cows (n=20)

S. no	Name of the antimicrobial agent	Symbol	Concentration (mcg)	No. of milk samples tested	No. of samples sensitive	Percentage	No. of samples resistant	Percentage
1.	Enrofloxacin	EN	10	20	19	95.00	1	5.00
2.	Ceftriaxone	CRT	30	20	17	85.00	3	15.00
3.	Amoxicillin + Clavulanic acid	AMC	30	20	15	75.00	5	25.00
4.	Tetracycline	TE	30	20	13	65.00	7	35.00
5.	Gentamicin	GEN	10	20	9	45.00	11	55.00
6.	Streptomycin	S	10	20	7	35.00	13	65.00

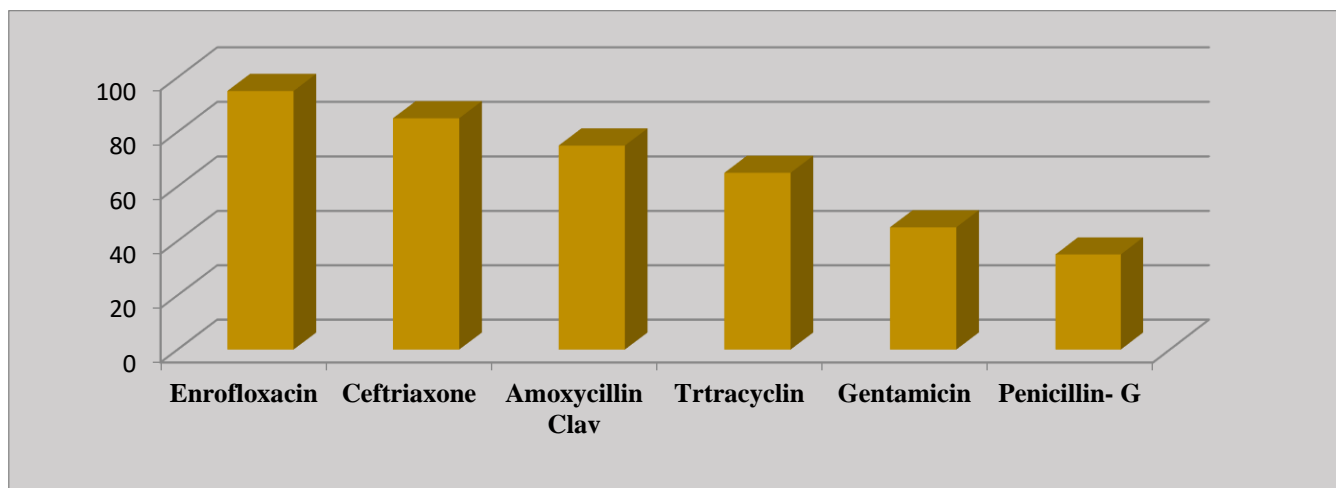


Figure 3: *In vitro* drug sensitivity of bacteria from whole milk cultures of SCM affected cows



Figure 4: *In vitro* drug sensitivity of bacteria from whole milk cultures of SCM affected cows

Table 3: *In vitro* drug sensitivity of *Moringa oleifera* leaves extract on whole milk cultures of SCM affected cows

S.no.	Concentration	Zone of inhibition
1.	5 mg	15
2.	10 mg	15
3.	20 mg	13
4.	30 mg	16
5.	40mg	12
6.	50mg	14

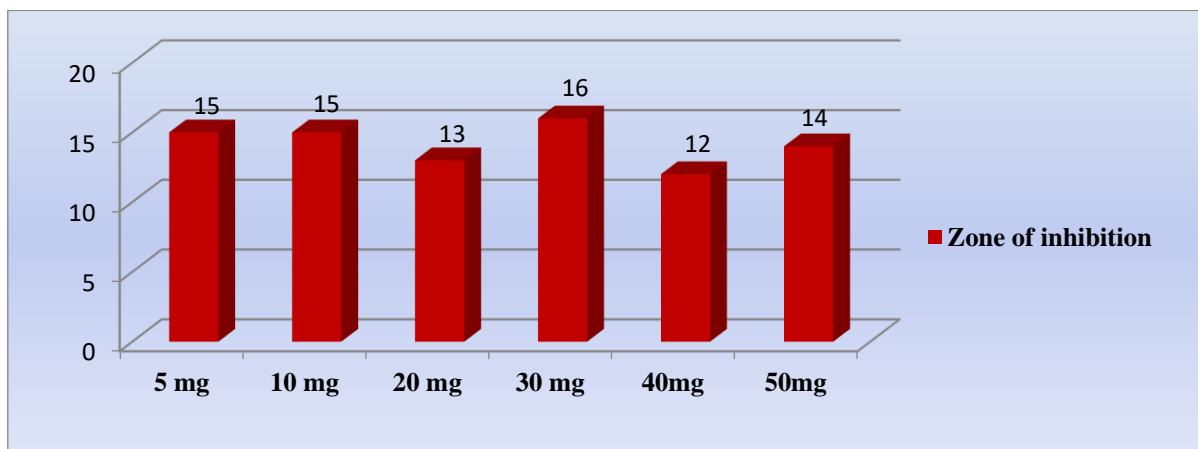


Figure 5: *In vitro* drug sensitivity of *Moringa oleifera* leaves extract on whole milk cultures of SCM affected cows.



Figure 6: Zone of inhibition at different concentrations by *Moringa oleifera* leaves extract on whole milk culture.

Table 4: *In vitro* drug sensitivity of *Ocimum sanctum* leaves extract on whole milk cultures of SCM affected cows.

S. No.	Concentration	Zone of inhibition
1.	5 mg	12
2.	10 mg	13
3.	20 mg	12
4.	30 mg	15
5.	40mg	15
6.	50mg	20

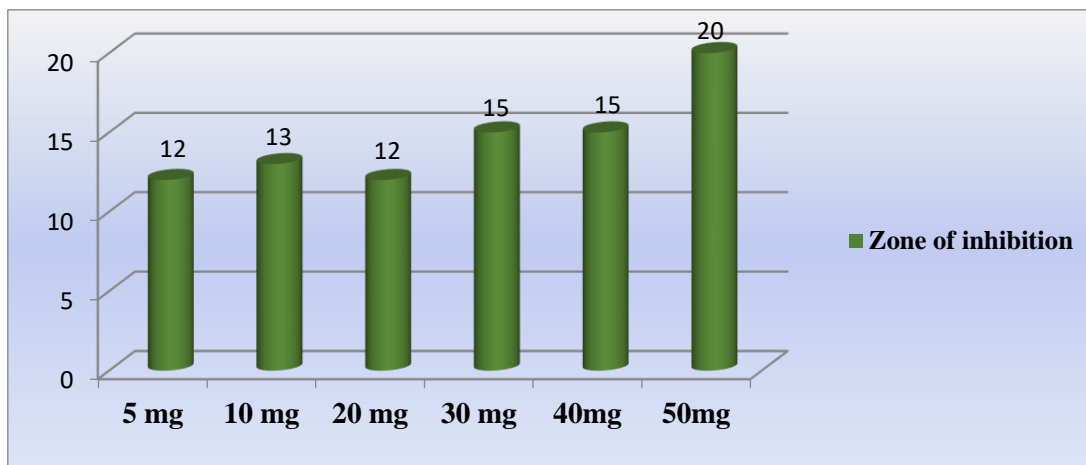


Figure 7: *In vitro* drug sensitivity of *Ocimum sanctum* leaves extract on whole milk cultures of SCM affected cows.

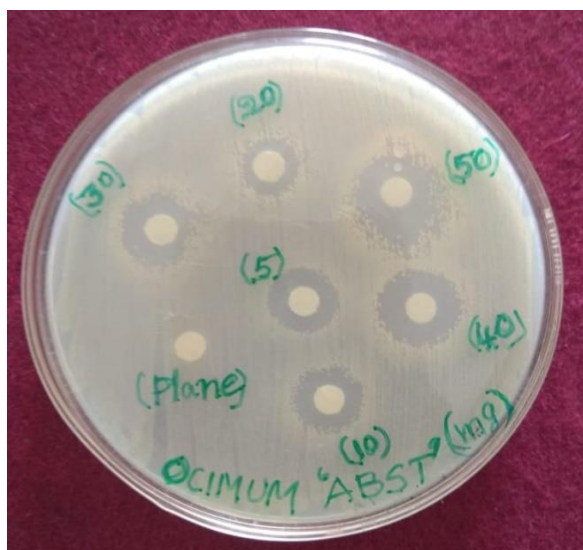


Figure 8: Zone of inhibition at different concentrations by *Ocimum sanctum* leaves extract on whole milk culture.

7. DISCUSSION

The pathogenic bacteria which are sensitive to synthetic antibiotics were also showing sensitivity to *Moringa oleifera* and *Ocimum sanctum* leaves. Antibiotic sensitivity test was under taken with acetone extract of *Moringa oleifera* and *Ocimum sanctum* leaves to know the antibacterial activity and measured the zone of inhibition of 15mm, 15mm, 13mm, 16mm, 12mm and 14mm at concentrations of 5mg, 10mg, 20mg, 30mg, 40mg and 50mg, respectively with *Moringa oleifera* extract against mixed culture of *Staphylococcus* spp., and *Escherichia coli*. These findings are in close relation to Moyo *et al.* (2012) who reported antibacterial activities against *Escherichia coli*, *Enterobacteriaceae*, *Proteus vulgaris*, *Staphylococcus aureus* and *Micrococcus kristinae* with the acetone extract of *Moringa oleifera* leaves at a concentration of 5 mg/ml. Similarly, Vikash *et al.* (2012) investigated the *in vitro* antimicrobial and antioxidant activity of aqueous, ethanolic and methanolic extract of leaf *Moringa oleifera* and recorded MIC at 2.4 mg/ml for *Bacillus subtilis* and *E. coli* and 7.4 mg/ml for *Staphylococcus aureus*. Sayeed *et al.* (2012) reported broad-spectrum antibacterial and antifungal activity with the fruit extract of *Moringa oleifera* and found highest zone of inhibition at the concentration of 200 µg/ disc for *Pseudomonas aeruginosa* (22 mm) and *Colletotrichum Spp.* (14 mm). Khanitta and Angelika (2015) tested extracts of *Moringa oleifera* seed and its residue against *Staphylococcus aureus* isolates at 100 mg/ml concentration and reported the maximum inhibition zone of 20.67 and 24.67 mm, respectively.

Antibiotic sensitivity test with acetone extract of *Ocimum sanctum* leaves showed zone of inhibition of 12mm, 13mm, 12mm, 15mm, 15mm and 20mm at concentrations of 5mg, 10mg, 20mg, 30mg, 40mg and 50mg, respectively with highest inhibition at 50mg/ml and lowest at 5 and 20mg/ml. Similarly, Akinjogunla *et al.* (2011) also recorded highest zone of inhibition at 25.0mg/ml and low at 6.25mg/ml. with *Ocimum* extract. Gajendrasinh *et al.* (2012) reported highest susceptibility of *E. coli* to ethanol extract of *Ocimum*

leaves at 250 µg concentration. As per Veena and Sudeep (2016) the zone of inhibition was greatest at concentration of 1000 µg/ml for both cold and hot water extracts against all test organisms and the maximum inhibition zone of 14mm recorded for *E. coli*.

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ABBREVIATIONS

ABST	-	Anibiotic Sensitivity Tesy
Mcg	-	Micro Gram
Mg	-	Milli Gram
Mg/MI	-	Milli Gram Per Milli Litre
MHA	-	Muller Hinton Agar
MIC	-	Minimum Inhibitory Concentration
Mm	-	Milli Metre
SCM	-	Sub Clinical Mastitis