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Assessment of antibacterial efficacy of *Moringa oleifera* extracts -A comparative study on mastitic and non-mastitic cultures

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Abstract
The medicinal properties of *Moringa oleifera* are utilised worldwide, especially its antimicrobial activity, based on which several preparations are available in the market. The present study evaluates the antibacterial activity of aqueous extracts of moringa leaf, pods, gum powder, and seed oil against specific microbial cultures. Also, the zone of inhibition was compared among the mastitic and non-mastitic cultures against the different Moringa extracts. The microbial cultures were activated before the antimicrobial activity estimation. The results revealed that the mastitic cultures showed the highest zone of inhibition compared to the non-mastitic cultures for the different concentrations of *M. oleifera* aqueous leaf extracts, pods, and gum powder for different dilutions. The different bacterial cultures showed a significant increase ($p<0.05$) in the mean values of the zone of inhibition of different concentrations of *M. oleifera*, and these cultures differed significantly ($p<0.01$) within the MIC for various dilutions. For different strains of *E. coli* and *Staphylococcus aureus*, the mean values of the zone of inhibition ranged from 2-26 and 2-21.50mm for *M. oleifera* aqueous leaf extract, 9-25 and 8-27mm for *M. oleifera* seed oil extracts, 15-27 and 9-28mm for *M. oleifera* pods powder aqueous extract, 11-24 and 4-17mm for *M. oleifera* gum powder aqueous extract, respectively for various concentrations from 100-300µl. Mastitic cultures were more susceptible to *M. oleifera* extracts and reflected an increase in zone of inhibition with an increase in concentration (100-300µl) and dilution than the non-mastitic cultures. Thus, the present study reveals that the various extracts of *M. oleifera* could be used in the development of prophylactic herbal preparations against mastitis causative agents.

Keywords: *M. Oleifera* seed oil, *M. Oleifera* leaf, *M. Oleifera* pods, *M. Oleifera* gum, mastitis

Introduction
The miracle tree *Moringa oleifera* comes under the family Moringaceae and is distributed worldwide (Anwar *et al.*, 2007) [7] with its vibrancy in the form of 33 species (Daba, 2016) [14]. These highly adaptable plant varieties survive in varied climatic conditions (Anwar *et al.*, 2007) [7]. Moringa is locally referred to as "Sajna"- "Muringa" (India), "Sohnjana" (Pakistan), and in some other countries as "Horse radish" and "Drumstick". Moringa, a perennial plant, can be harvested several times a year. Moringa has several medicinal properties, antioxidant activities (Anwar *et al.*, 2007) [7], and nutritional advantages, and it is highly suggested to be incorporated into the human diet (Haldar and Kosankar, 2017) [24]. It contains several good-quality proteins, vitamins, minerals, and fiber (Fuglie, 1999) [17]. The superiority of Moringa in comparison to other sources is that it has ten times more vitamin A than carrots, 15 times more potassium than bananas (Saini *et al.*, 2016) [53], 9 times more protein than yogurt, 25 times more iron than spinach, 7 times more vitamin C than oranges and 17 times more calcium than milk (Daba, 2016) [14]. Leafy tips of *Moringa oleifera*, per 100 g, contain 78% water, 64 kcal energy, 9.4 g protein, 1.4 g fat, 8.3 g carbohydrates, and 2.0 g dietary fiber (Grubben and Denton, 2004) [22]. Moringa's properties and contents can cure many deficiencies and diseases (Elgamily *et al.*, 2016) [15]. Moringa leaves to aid in treating flu, heartburn, and hyperglycaemia and reduces cholesterol, asthma, diarrhoea, pneumonia, and blood pressure. The plant leaves manifest antimicrobial, anticancer, antioxidant, and antidiabetic properties (Onsare *et al.*, 2013) [39]. Moringa seed powder exhibits its marked potency in treating rheumatism, hyperthyroidism, cramp, Crohn's disease, epilepsy, gout, and sexually transmitted infections, and contains anti-inflammatory, antimicrobial agents (Ighodaro and Akinloye, 2018) [25]. Root bark exhibits anticancer, anti-inflammatory, and cardiac stimulant agents. Moringa flower has hypocholesterolemic and anti-arthritic properties that aid in the cure of diseases of the liver, diarrhoea, spleen problem, and joint pain (Anwar *et al.*, 2007) [7]. *M.*

oleifera has coagulative properties that aid wastewater treatment (Abraham and Okon, 2014) [2]. The plant has a highly superior antimicrobial peptide/protein (4 - 50kDa) that induces a resistance mechanism (Raj *et al.,* 2011) [49] and plays a significant role in innate immune response (Golla *et al.,* 2006) [19]. These antimicrobial peptides/proteins are present in Moringa's flower, seed, leaves, stem, and roots of Moringa and play a vital role in defence against pathogenic parasites, fungi, viruses, bacteria, and neoplastic cells (Raj *et al.,* 2011) [49]. These peptides/ proteins inhibit the growth of fungi through molecular routes such as binding to the chitin or enhancing the permeability of the cell wall (Chuang *et al.,* 2007) [12]. Antimicrobial peptides interact with the membrane via cationic amino acids attracted to the phospholipid head, and hydrophobic anionic peptides interact with the aliphatic fatty acids (Zasloff, 2002; Koczulla and Bals, 2003) [68, 31]. Thus, membrane destabilization is created, and leakage of cytoplasmic contents, loss of membrane potential, change of membrane permeability, lipid distribution, the entry of the peptide and blocking of anionic cell components or the triggering of autolytic enzymes (Zasloff, 2002) [68] kills bacteria. Consequently, every part of the Moringa has an exceptional blend of activities that can utilize for herbal and pharmaceutical preparations.

Mastitis is a multi-etiological production disease that creates havoc regarding economic loss among dairy farmers across the globe. The major pathogens causing mastitis are *Staphylococcus aureus, Streptococcus uberis, E. coli, Streptococcus bovis, Streptococcus agalactiae, Streptococcus dysgalactiae, and Klebsiella pneumonia.* The minor pathogens like *Proteus* spp., *Brucella abortus, Klebsiella oxytoca, Mycoplasma* spp., *Pseudomonas aeruginosa, Nocardia* spp., *Pasteurella* spp., *Prototheca zopfii, Corynebacterium bovis, Prototheca wickerhamii* and yeast (Calvinho and Tirante, 2005; Awale *et al.,* 2012) [11, 9] attributes to the transmission of mastitis from subclinical to next stage of mastitis. Among them, *S. aureus* is responsible for causing subclinical mastitis (SCM) (Sakwinska *et al.,* 2011) [54], accounting for 25.64 percent in specific mastitis, 12.24 percent in latent cases, and owing to its adaptational talent in surviving extreme environments (Sharma and Maiti, 2010) [57]. Throughout the world, several studies have been conducted regarding coagulase-negative *Staphylococci* (CNS), the most isolated pathogen from milk (Radostits *et al.,* 2006), and its prevalence rate is highest in crossbred milch cattle (Mir *et al.,* 2014) [36]. CNS is the rising mastitis pathogen, which belongs to normal skin microbiota. Pilipčincová *et al.* (2010) [44] reported that the CNS forms a protective biofilm, which enables them to linger on to the milking machine and the milker's hand, which in turn aids in the spread of infection. Unhygienic circumstances enhance the microbes' favourable condition to flare up as an opportunistic pathogen in the teat, causing mastitis. Another commonly encountered mastitic pathogen is *Corynebacteria* (Pitkala *et al.,* 2004) [45], which persists with routine teat dip usage but could be eliminated using dry cow antibiotic therapy. In New Zealand, the primary mastitis-causing organism is *Streptococcus uberis* (Zadoks *et al.,* 2011) [67]; in the Midwestern United States, it is coliforms (Zadoks and Fitzpatrick, 2009) [66] and in India, it is *Staphylococcus* sp., *Streptococcus* sp., and *E. coli* (Krishnamoorthy *et al.,* 2017) [32]. Scientific studies related to the comparison among the mastitic and non-mastitic cultures against various extracts of different dilutions of *Moringa oleifera* are limited. Thus, the present study focuses on the assessment of the antibacterial efficacy of *Moringa oleifera* leaf aqueous extract, *Moringa oleifera* pods powder aqueous extract, *Moringa oleifera* seed oil extract, *Moringa oleifera* gum powder aqueous extract against different cultures of NCDC (non-mastitic) and Mastitis causative agents obtained from the National Referral Centre for milk quality and Safety lab cultures.

Materials and Methods

The *Moringa oleifera* was harvested from the Artificial Breeding Research Centre (ABRC) garden of ICAR- National Dairy Research Institute (NDRI), Karnal, Haryana. A single source of plant material was used throughout the experiment. The National Collection of Dairy Cultures (NCDC) cultures used were NCDC-208 (*Streptococcus agalactiae*), NCDC-138 (*Klebsiella pneumoniae*), NCDC-133 (*Staphylococcus aureus*), and NCDC-135 (*Escherichia coli*). The current study also used the National Referral Centre for milk quality and Safety (NRCMQS) cultures of *Escherichia coli* and *Staphylococcus aureus* organisms isolated from mastitis milk. The cultures were activated using brain heart infusion broth before the MIC estimation.

Preparation, standardization, and MIC estimation of:

a. *Moringa oleifera* leaf aqueous extract

The hot water *Moringa oleifera* leaf aqueous (MOLA) extract was prepared according to the procedure developed at ABRC, ICAR-NDRI, Karnal, Haryana. *Moringa oleifera* trees within the ABRC garden were identified, and mature fresh leaves were harvested. The leaves were weighed, washed with fresh water, and then air-dried for 24 hours at room temperature. The dried leaves were kept in a hot-air oven at 45°C to remove moisture. After drying, moisture loss was determined before blending and storing at 4°C. The whole experiment was conducted using a single source of dried leaf powder. For MOLA extract preparation, fifty grams of dried *Moringa oleifera* leaf powder was soaked in 250ml of autoclaved distilled water (1:5 ratio) and incubated at 55 °C overnight in a hot air oven. After incubation, filtrate and leaf residue was separated using cheesecloth. Further, the filtrate was filtered using HiMedia® paper grade-40 filter paper, and till further use, the aqueous extract was aliquoted and stored at -20 °C.

Further, the obtained *Moringa oleifera* leaf aqueous extract was standardized, and the efficacy was analyzed on selected bacterial cultures. The antimicrobial activity of the *Moringa oleifera* leaf aqueous extract against the selected non-mastitic cultures obtained from NCDC and mastitis organisms isolated during the previous experiments from milk obtained from NRCMQS was performed according to the method standardized by Wang *et al.* (2012) [64]. The plates containing 15ml nutrient agar medium were poured into Petri plates. The culture was activated before the start of the experiment. Soft agar containing 10^5 target cultures was overlaid. The wells were made with the help of a well borer. After that, MOLA extract was added at different selected concentrations to other wells. The plates were kept aside for proper diffusion. Bacterial plates were incubated at 37 ^{O}C for 24 hours. The MIC formed was measured in mm at the end of the experiment. The obtained data were further processed and analyzed using suitable statistical software.

b. *Moringa oleifera* seed oil (MOSO) preparation: The

MOSO was prepared based on the procedure developed by Saad (2015) [52]. *Moringa oleifera* pods were plucked from the ABRC garden and cleaned. The pods were sun-dried, and the seeds were removed using a knife manually. The seeds thus obtained were cleaned thoroughly. After cleaning, the seeds were put inside the electric oven to reduce the moisture content further. Then, the seeds were undergone size reduction by grinding using a domestic blender. The ground seeds were sieved using a 250µm sieve and weighed using an electronic balance. Oil extraction was carried out using the Soxhlet apparatus with n-hexane as solvent. 20g of the ground sample was poured into the thimble. Two third volume of the round bottom flask was filled with the solvent. The heating mantle was adjusted to about (60- 70) ºC, and heating commenced. As the solvent was heated continuously, it evaporated and condensed back into the sample in the thimble. The oil extracted, containing some portion of the solvent, was then recycled back to the round bottom flask as it refluxed. The total reflux process continued until complete oil extraction was observed. A rotary evaporator was used to separate the oil from the solvent at the temperature of 65ºC. The obtained oil was stored at room temperature for further use. The MIC estimation was carried out similarly to the MOLA extract.

c. *Moringa oleifera* pod powder aqueous (MOPPA) extract:

The preparation of *Moringa oleifera* pods was carried out based on the procedure developed by Gull *et al.* (2016). *Moringa oleifera* pods were plucked from the ABRC garden and cleaned. The pods (500g) were thoroughly air-dried and pulverized mechanically into powder using an electric grinder. The powder (20g) was extracted by soaking in 100mL of distilled water in screw-capped 250mL conical flasks for 24 hours with shaking at 160rpm in an orbital shaker at ambient temperature. The extracts were filtered through eight layers of muslin cloth, and then the filtrate was subjected to filtration through filter paper grade 40. The aqueous extract was aliquoted and stored at -20 °C till further use. The standardization and MIC estimation were carried out similarly to the MOLA extract.

d. *Moringa oleifera* gum powder aqueous (MOGPA) extract

The preparation for *Moringa oleifera* gum was carried out based on the procedure developed by Panda *et al.* (2006) [41]. The gum was collected from the trees (injured site) of the ABRC garden. It was dried, ground, and passed through sieve no 80. Dried gum (10g) was stirred in distilled water (250 ml) for 6-8h at room temperature. The supernatant was obtained by centrifugation. The residue was washed with water, and the washings were added to the separated supernatant. The procedure was repeated four more times. Finally, the supernatant was made up to 500ml and treated with twice the volume of acetone by continuous stirring. The precipitated material was washed with distilled water and dried at 50-60° under a vacuum. 20g of MOGP- precipitated material was added to 100 ml distilled water in a conical flask and incubated in an orbital shaker for 24 hours at 55 °C. The obtained extract was filtered by using a Watman filter paper grade 42. The aqueous extract was aliquoted and stored at -20 °C till further use. The standardization and MIC estimation were carried out similarly to the MOLA extract.

Statistical analysis

The data was analyzed using SPSS 26.0 software (IBM Corporation, Armonk, New York, USA). One-way ANOVA was used to estimate the antibacterial activity level of different extracts concentrations against the selected cultures with a significance level of $p<0.01$. The post hoc used was Duncan's multiple range test. A univariate linear model was used to estimate the significance level at $p<0.05$ of different extract concentrations in each culture under consideration.

Results

a. *Moringa oleifera* leaf aqueous extract

The *Moringa oleifera* leaf aqueous extract (hot) was prepared according to the standard procedure. Two sets of dilutions, *i.e.*, 1:4 (1g MOL powder in 4ml hot water) and 1:5 (1g MOL powder in 5ml hot water) of different concentrations (50, 100, 150, 200, 250µl) were used against the selected organisms to find out the MIC of hot water extract prepared. The control used was 50 µl of antibiotic (oxytetracycline). The MIC was calculated according to standard procedure and is depicted in Table 1 for 1:5 and Table 2 for 1:4 dilutions.

MIC estimation of MOLAE for 1:5 dilution

The values of MIC (zone of inhibition-2mm) were comparable among the different concentrations of MOLAE and were significant ($p<0.05$) to control (OTC) (zone of inhibition-32mm) for NCDC 208. For NCDC 135, 133, and 138, the mean values of the zone of inhibition showed a significant increase ($p<0.05$) as the concentration of MOLAE increased from 50- 250µl. A similar increase was observed for NRCMQS cultures also. For 50µl concentration of MOLAE, NRCMQS *S. aureus* showed the highest mean value of zone of inhibition (9.50m). But, for 100 µl concentration of MOLAE, NRCMQS *E. coli* showed the highest mean value of zone of inhibition of 16.00mm. The highest mean values zone of inhibition for 150, 200, and 250 µl concentrations of MOLAE were observed in NCDC 135 with values of 21.00mm, 22.00mm, and 24.00mm, respectively. Thus, all the cultures showed a significant increase in MIC values of different concentrations of MOLAE to the control ($p<0.05$), and other cultures differ significantly within the concentrations of MOLAE ($p<0.01$).

MIC estimation of MOLAE for 1:4 dilution

The values of MIC among NCDC 135, 133, 138, and NRCMQS *E. coli* and *S. aureus* showed a significant increase ($p<0.05$) as the concentration increased from 50- 100 µl. Within a concentration, different cultures varied significantly in the mean values of the zone of inhibition ($p<0.01$). The highest mean values of the zone of inhibition for 50, 100, 150, 200, and 250 µl concentration was observed in NCDC 138 (12.00mm), NRCMQS *S. aureus* (17.50mm), NCDC 138 (20.00mm), NCDC 138 (21.00mm) and NCDC 138 (27.00mm), respectively. All values of MIC showed a significant difference compared to the control group of MOLAE (Table 2).

Table 1: Mean ± S. E. of MIC of different concentrations of MOLAE (hot water) at 1:5 dilution against the selected microorganism

Zone of inhibition (mm)	Concentrations of MOLAE (hot water) -MIC (µl) for 1:5 dilution					
Microorganisms	**50**	**100**	**150**	**200**	**250**	**Control**
NCDC 208	2.00^{Ap} ±0.07	2.00^{Ap} ±0.06	2.00^{Ap} ±0.07	2.00^{Ap} ±0.05	2.00^{Ap} ±0.07	32.00^{Dq} ±0.51
NCDC 135	2.00^{Ap} ±0.06	11.00^{Cq} ±0.17	21.00^{Er} ±0.16	22.00^{Fs} ±0.14	24.00^{Ft} ±0.26	29.04^{Au} ±0.67
NCDC133	2.00^{Ap} ±0.08	10.00^{Bq} ±0.09	13.00^{Br} ±0.18	13.50^{Bs} ±0.16	14.00^{Bt} ±0.27	32.06^{Du} ±0.55
NCDC 138	2.00^{Ap} ±0.07	16.00^{Eq} ±0.16	19.00^{Dr} ±0.13	21.00^{Es} ±0.29	23.00^{Et} ±0.14	31.00^{Cu} ±0.68
NRCMQS *E. coli*	8.00^{Bp} ±0.15	16.00^{Fq} ±0.13	18.00^{Dr} ±0.18	20.00^{Ds} ±0.15	21.00^{Dt} ±0.11	30.50^{BCu} ±0.64
NRCMQS *S. aureus*	9.50^{Cp} ±0.11	14.50^{Dq} ±0.24	17.50^{Cr} ±0.22	18.50^{Cs} ±0.21	20.50^{Ct} ±0.24	30.50^{Bu} ±0.53

Means bearing different superscripts differ significantly ($^{p, q, r, s, t, u}p<0.05$) between rows and ($^{A, B, C, D, E, F}p<0.01$) within each concentration.

Table 2: Mean ± S. E. of MIC of different concentrations of MOLAE (hot water) at 1:4 dilution against the selected microorganism

Zone of inhibition (mm)	Concentrations of MOLAE (hot water) -MIC (µl) for 1:4 dilution					
Microorganisms	**50**	**100**	**150**	**200**	**250**	**Control**
NCDC 208	2.00^{Ap} ±0.08	2.00^{Ap} ±0.07	3.00^{Aq} ±0.08	3.00^{Aq} ±0.05	4.00^{Ar} ±0.07	32.00^{Cs} ±0.61
NCDC 135	8.00^{Cp} ±0.07	13.00^{Dq} ±0.18	17.00^{Cr} ±0.16	21.00^{Ds} ±0.24	26.00^{Et} ±0.36	29.00^{Au} ±0.77
NCDC133	8.00^{Cp} ±0.08	10.00^{Bq} ±0.19	12.00^{Br} ±0.28	15.00^{Bs} ±0.16	17.00^{Bt} ±0.27	32.00^{Cu} ±0.55
NCDC 138	12.00^{Ep} ±0.16	16.00^{Eq} ±0.36	20.00^{Er} ±0.13	21.00^{Ds} ±0.29	27.00^{Ft} ±0.24	31.00^{Bu} ±0.78
NRCMQS *E. coli*	4.50^{Bp} ±0.11	11.00^{Cq} ±0.13	16.50^{Cr} ±0.18	18.00^{Cs} ±0.15	23.00^{Dt} ±0.31	30.50^{Bu} ±0.64
NRCMQS *S. aureus*	9.50^{Dp} ±0.22	17.50^{Fq} ±0.34	19.50^{Dr} ±0.23	20.50^{Ds} ±0.21	21.50^{Ct} ±0.31	30.50^{Bu} ±0.43

Means bearing different superscripts differ significantly ($^{p, q, r, s, t, u}p<0.05$) between rows and ($^{A, B, C, D, E, F}p<0.01$) between columns

b. *Moringa oleifera* seed oil extract

MOSO was extracted using the standard procedure. Methanolic and phenolic extraction was carried out to find out the best way of extraction. The MIC values obtained for phenolic MOSO extract are depicted in Table 3 and for methanolic extract in Table 4.

MIC estimation of MOSO for phenolic extract

The MOSOE at different concentrations showed a significant difference ($p<0.05$), and the mean values of the zone of inhibition increased ($p<0.05$) as the concentration increased from 100-30µl (Table 3). All the values of MIC were significant ($p<0.05$) in comparison to the control (50 µl of OTC- antibiotic). Within a concentration, different cultures depicted a significant difference in their mean inhibition zone values (p<0.01). The highest mean values of the zone of inhibition for 100, 150, 200, 250, and 300µl concentration was observed in NRCMQS *E. coli* (13.00mm), NRCMQS *S. aureus* (19.00mm), NRCMQS *S. aureus* (21.00mm), NRCMQS *S. aureus* (24.00mm), NRCMQS *S. aureus* (27.00mm), respectively. For NCDC 208, the concentration of 300 µl revealed the highest mean value of MIC (20.00mm). A similar trend was observed for all other NCDC and NRCMQS cultures.

MIC estimation of MOSO for methanolic extract

The MOSOE at different concentrations showed a significant difference ($p<0.05$), and the mean values of the zone of inhibition increased ($p<0.05$) as the concentration increased from 100-30µl (Table 4). All the values of MIC were significant ($p<0.05$) in comparison to the control (50 µl of OTC- antibiotic). Within a concentration, different cultures depicted a significant difference in their mean inhibition zone values (p<0.01). The highest mean values of the zone of inhibition for 100, 150, 200, 250, and 300µl concentrations were observed in NRCMQS *S. aureus* with 13.00, 19.00, 21.00, 24.00, and 27.00 mm, respectively. For NCDC 208, the concentration of 300 µl revealed the highest mean value of the zone of inhibition (26.00mm). A similar trend was observed for all other NCDC and NRCMQS cultures.

Table 3: Mean ± S. E. of MIC of different concentrations of *Moringa oleifera* seed oil phenolic extract against the selected microorganism

Zone of inhibition (mm)	Concentrations of MOSO- Phenolic extract- MIC (µl)					
Microorganisms	**100**	**150**	**200**	**250**	**300**	**Control**
NCDC 208	9.00^{Bp} ±0.08	16.00^{Dq} ±0.17	17.00^{Cr} ±0.18	19.00^{Ds} ±0.16	20.00^{Bt} ±0.27	31.00^{Bu} ±0.38
NCDC 135	10.00^{Cp} ±0.07	12.00^{Cq} ±0.11	13.00^{Br} ±0.17	17.00^{Cs} ±0.18	22.00^{Dt} ±0.26	30.00^{Au} ±0.57
NCDC133	10.00^{Cp} ±0.08	11.00^{Bq} ±0.16	12.00^{Ar} ±0.18	15.00^{As} ±0.17	21.00^{Ct} ±0.28	32.50^{Cu} ±0.66
NCDC 138	8.00^{Ap} ±0.14	10.00^{Aq} ±0.12	13.00^{Br} ±0.23	16.00^{Bs} ±0.14	19.00^{At} ±0.24	30.00^{Au} ±0.51
NRCMQS *E. coli*	13.00^{Ep} ±0.10	16.00^{Dq} ±0.22	18.00^{Dr} ±0.31	20.00^{Es} ±0.24	23.00^{Et} ±0.33	31.00^{Bu} ±0.42
NRCMQS *S. aureus*	11.00^{Dp} ±0.09	19.00^{Eq} ±0.18	21.00^{Er} ±0.19	24.00^{Fs} ±0.27	27.00^{Ft} ±0.28	31.00^{Bu} ±0.59

Means bearing different superscripts differ significantly ($^{p, q, r, s, t, u}p<0.05$) between rows and ($^{A, B, C, D, E, F}p<0.01$) between columns.

Table 4: Mean ± S. E. of MIC of different concentrations of *Moringa oleifera* seed oil methanolic extract against the selected microorganism

Zone of inhibition (mm)	Concentrations of MOSO- methanolic extract -MIC (µl)					
Microorganisms	**100**	**150**	**200**	**250**	**300**	**Control**
NCDC 208	8.00^{Ap}±0.08	13.50^{Bq} ±0.17	14.00^{Br} ±0.18	18.00^{As} ±0.16	26.00^{Dt} ±0.27	31.00^{Bu} ±0.48
NCDC 135	9.00^{Bp}±0.07	16.00^{Dq} ±0.38	20.00^{Er} ±0.27	21.00^{Cs}±0.38	23.00^{Bt} ±0.26	30.00^{Au} ±0.57
NCDC133	8.00^{Ap}±0.08	10.00^{Aq} ±0.06	12.00^{Ar} ±0.28	20.00^{Bs} ±0.37	25.00^{Ct} ±0.38	32.50^{Cu} ±0.36
NCDC 138	11.00^{Cp}±0.14	15.00^{Cq} ±0.12	17.00^{Cr} ±0.23	20.00^{Bs} ±0.32	22.00^{At} ±0.24	30.00^{Au} ±0.34
NRCMQS *E. coli*	11.00^{Cp}±0.10	18.00^{Eq} ±0.16	19.00^{Dr} ±0.21	23.00^{Ds} ±0.20	25.00^{Ct} ±0.33	31.00^{Bu} ±0.52
NRCMQS *S. aureus*	13.00^{Dp}±0.19	19.00^{Fq}± 0.28	21.00^{Fr} ±0.29	24.00^{Es} ±0.27	27.00^{Et} ±0.28	31.00^{Bu} ±0.59

Means bearing different superscripts differ significantly ($^{p, q, r, s, t, u}p<0.05$) between rows and ($^{A, B, C, D, E, F}p<0.01$) between columns.

c. *Moringa oleifera* pod powder aqueous (MOPPA) extract

The MOPPAE was prepared according to the standard procedure. Two sets of dilution, 1:5 and 1:4, of different concentration was used against the selected set of organisms. The MIC values obtained for 1: 5 and 1:4 dilution is depicted in Tables 5 and 6, respectively.

MIC estimation of MOPPA extract for 1:5 dilution

The MOPPAE at different concentrations showed a significant difference ($p<0.05$), and the mean values of the zone of inhibition increased ($p<0.05$) as the concentration increased from 100-30µl (Table 5). All the values of MIC were significant ($p<0.05$) in comparison to the control (50µl of OTC- antibiotic). Within a concentration, the different cultures showed a significant difference in their mean values of the zone of inhibition (p<0.01). The highest mean values of the zone of inhibition for 100, 150, 200, 250, and 300µl concentration were observed in NCDC 133 (18.00mm), NCDC 135 (20.00mm), NCDC 135 (22.00mm), NRCMQS *S. aureus* (26.00mm) and NRCMQS *S. aureus* (27.00mm), respectively. For NCDC 208, the concentration of 300µl revealed the highest mean value of zone of inhibition (25.00mm). A similar trend was observed for all other NCDC and NRCMQS cultures.

MIC estimation of MOPA extract for 1:4 dilution

The MOPPAE at different concentrations showed a significant difference ($p<0.05$), and the mean values of the zone of inhibition increased ($p<0.05$) as the concentration increased from 100-30µl (Table 6). All the values of MIC were significant ($p<0.05$) in comparison to the control (50µl of OTC- antibiotic). Within a concentration, different cultures depicted a significant difference in their mean inhibition zone values (p<0.01). The highest mean values of the zone of inhibition for 100, 150, 200, 250, and 300µl concentrations were observed in NCDC135 (18.00mm), NRCMQS *S. aureus* (20.00mm), NRCMQS *S. aureus* (22.00mm), NRCMQS *S. aureus* (27.00mm), NRCMQS *S. aureus* (28.00mm), respectively. For NCDC 208, the concentration of 300µl revealed the highest mean value of zone of inhibition (20.00mm). A similar trend was observed for all other NCDC and NRCMQS cultures.

Table 5: Mean ± S. E. of MIC of different concentrations of *Moringa oleifera* pods powder aqueous (MOPPA) extract at 1:5 dilution against the selected microorganism

Zone of inhibition (mm)	Concentrations of MOPPAE- MIC (µl) for 1:5 dilution					
Microorganisms	**100**	**150**	**200**	**250**	**300**	**Control**
NCDC 208	$15.00^{Bp} \pm 0.18$	$19.00^{Cq} \pm 0.17$	$20.00^{Br} \pm 0.18$	$21.00^{Cs} \pm 0.26$	$25.00^{Ct} \pm 0.37$	$32.00^{u} \pm 0.58$
NCDC 135	$15.00^{Bp} \pm 0.17$	$20.00^{Dq} \pm 0.18$	$22.00^{Dr} \pm 0.17$	$23.00^{Es} \pm 0.28$	$26.00^{Dt} \pm 0.21$	$32.00^{u} \pm 0.67$
NCDC133	$16.00^{Cp} \pm 0.11$	$17.00^{Bq} \pm 0.16$	$18.00^{Ar} \pm 0.18$	$19.00^{As} \pm 0.27$	$20.00^{At} \pm 0.18$	$32.00^{u} \pm 0.56$
NCDC 138	$17.00^{Dp} \pm 0.14$	$19.00^{Cq} \pm 0.12$	$21.00^{Cr} \pm 0.23$	$22.00^{Ds} \pm 0.34$	$25.00^{Ct} \pm 0.24$	$32.00^{u} \pm 0.51$
NRCMQS *E. coli*	$15.00^{Bp} \pm 0.10$	$17.00^{Bq} \pm 0.12$	$18.00^{Ar} \pm 0.21$	$20.00^{Bs} \pm 0.13$	$21.00^{Bt} \pm 0.23$	$32.00^{u} \pm 0.62$
NRCMQS *S. aureus*	$9.00^{Ap} \pm 0.09$	$16.00^{Aq} \pm 0.18$	$18.00^{Ar} \pm 0.19$	$26.00^{Fs} \pm 0.27$	$27.00^{Et} \pm 0.38$	$32.00^{u} \pm 0.69$

Means bearing different superscripts differ significantly ($^{p, q, r, s, t, u}p<0.05$) between rows and ($^{A, B, C, D, E, F}p<0.01$) between columns

Table 6: Mean ± S. E. of MIC (mm) of different concentrations of *Moringa oleifera* pods powder aqueous (MOPPA) extract at 1:4 dilution against the selected microorganism

Zone of inhibition (mm)	Concentrations of MOPPAE- MIC (µl) for 1:4 dilution					
Microorganisms	**100**	**150**	**200**	**250**	**300**	**Control**
NCDC 208	$14.00^{Bp} \pm 0.18$	$15.00^{Aq} \pm 0.17$	$16.00^{Ar} \pm 0.18$	$19.00^{As} \pm 0.16$	$20.00^{At} \pm 0.27$	$32.00^{u} \pm 0.58$
NCDC 135	$18.00^{Fp} \pm 0.17$	$20.00^{Cq} \pm 0.18$	$21.00^{Er} \pm 0.17$	$25.00^{Ds} \pm 0.18$	$27.00^{Dt} \pm 0.16$	$32.00^{u} \pm 0.67$
NCDC133	$9.00^{Ap} \pm 0.08$	$15.00^{Aq} \pm 0.16$	$17.00^{Br} \pm 0.18$	$21.00^{Bs} \pm 0.27$	$24.00^{Ct} \pm 0.18$	$32.00^{u} \pm 0.56$
NCDC 138	$16.00^{Dp} \pm 0.14$	$18.00^{Bq} \pm 0.12$	$20.00^{Dr} \pm 0.13$	$23.00^{Cs} \pm 0.24$	$24.00^{Ct} \pm 0.32$	$32.00^{u} \pm 0.51$
NRCMQS *E. coli*	$17.00^{Ep} \pm 0.14$	$18.00^{Bq} \pm 0.12$	$19.00^{Cr} \pm 0.11$	$21.00^{Bs} \pm 0.23$	$22.00^{Bt} \pm 0.13$	$32.00^{u} \pm 0.52$
NRCMQS *S. aureus*	$15.00^{Cp} \pm 0.19$	$20.00^{Cq} \pm 0.18$	$22.00^{Fr} \pm 0.29$	$27.00^{Es} \pm 0.17$	$28.00^{Et} \pm 0.28$	$32.00^{u} \pm 0.69$

Means bearing different superscripts differ significantly ($^{p, q, r, s, t, u}p<0.05$) between rows and ($^{A, B, C, D, E, F}p<0.01$) between columns

d. *Moringa oleifera* gum powder aqueous (MOGPA) extract

The MOGPAE was prepared according to the standard procedure. Two sets of dilution, 1:5 and 1:4, of different concentration was used against the selected set of organisms. The MIC values obtained for 1: 5 dilution and 1:4 dilution is depicted in Table 7 and Table 8, respectively.

MIC estimation of MOGPA extract for 1:5 dilution

The MOGAE at different concentrations showed a significant difference ($p<0.05$), and the mean values of the zone of inhibition increased ($p<0.05$) as the concentration increased from 100-30µl (Table 7). All the values of MIC were significant ($p<0.05$) in comparison to the control (50µl of OTC- antibiotic). Within a concentration, the different cultures showed a significant difference in their mean values of the zone of inhibition (p<0.01). The highest mean values of the zone of inhibition for 100, 150, 200, 250, and 300µl concentrations were observed in NCDC 135 (14.00mm), NCDC 135 (16.00mm), NRCMQS *E. coli* (17.00mm), NRCMQS *E. coli* (18.00mm) and NRCMQS *E. coli* (21.00mm), respectively. For NCDC 208, the concentration of 300µl revealed the highest mean value of zone of inhibition (10.00mm). A similar trend was observed for all other NCDC and NRCMQS cultures.

MIC estimation of MOGPA extract for 1:4 dilution

The MOGPAE at different concentrations showed a significant difference ($p<0.05$), and the mean values of the zone of inhibition increased ($p<0.05$) as the concentration increased from 100-30µl (Table 8). All the values of MIC were significant ($p<0.05$) in comparison to the control (50µl of OTC- antibiotic). Within a concentration, different cultures depicted a significant difference in their mean inhibition zone values (p<0.01). The highest mean values of the zone of inhibition for 100, 150, 200, 250, and 300µl concentrations

were observed in NRCMQS *E. coli*, and the values are 16.00, 18.00, 20.00, 22.00, and 24.00mm, respectively. For NCDC 208, the concentration of 300µl revealed the highest mean value of zone of inhibition (12.00mm). A similar trend was observed for all other NCDC and NRCMQS cultures.

Table 7: Mean ± S. E. of MIC of different concentrations of *Moringa oleifera* gum powder aqueous extract (hot water) at 1:5 dilution against the selected microorganism

Zone of inhibition (mm)	Concentrations of MOGPAE (hot water)- MIC (µl) for 1:5 dilution					
Microorganisms	**100**	**150**	**200**	**250**	**300**	**Control**
NCDC 208	5.00^{Bp} ±0.08	6.00^{Bq} ±0.07	8.00^{Br} ±0.08	9.00^{Bs} ±0.06	10.00^{Bt} ±0.07	31.00^{Bu} ±0.58
NCDC 135	14.00^{Fp} ±0.17	16.00^{Fq} ±0.18	17.00^{Er} ±0.17	18.00^{Es}±0.18	20.00^{Et} ±0.16	30.00^{Au} ±0.57
NCDC133	8.00^{Cp} ±0.08	11.00^{Cq} ±0.06	13.00^{Cr} ±0.18	14.00^{Cs} ±0.17	15.00^{Ct} ±0.18	30.00^{Au} ±0.56
NCDC 138	12.00^{Ep} ±0.14	13.00^{Dq} ±0.12	14.00^{Dr} ±0.13	15.00^{Ds} ±0.14	16.00^{Dt} ±0.13	30.00^{Au} ±0.51
NRCMQS *E. coli*	11.00^{Dp} ±0.10	14.00^{Eq} ±0.12	17.00^{Er} ±0.11	18.00^{Es} ±0.10	21.00^{Ft} ±0.13	32.00^{Cu} ±0.62
NRCMQS *S. aureus*	4.00^{Ap} ±0.09	5.00^{Aq} ±0.08	6.00^{Ar} ±0.09	7.00^{As} ±0.07	9.00^{At} ±0.08	30.00^{Au} ±0.59

Means bearing different superscripts differ significantly ($^{p, q, r, s, t, u}p<0.05$) between rows and ($^{A, B, C, D, E, F}p<0.01$) between columns

Table 8: Mean ± S. E. of MIC of different concentrations of *Moringa oleifera* gum powder aqueous extract (hot water) at 1:4 dilution against the selected microorganism

Zone of inhibition (mm)	Concentrations of MOGPAE (hot water) -MIC (µl) for 1:4 dilution					
Microorganisms	**100**	**150**	**200**	**250**	**300**	**Control**
NCDC 208	7.00^{Ap} ±0.08	7.00^{Ap} ±0.07	8.00^{Aq} ±0.08	10.00^{Ar} ±0.06	12.00^{As} ±0.17	31.00^{Bt} ±0.58
NCDC 135	12.00^{Dp} ±0.10	14.00^{Dq} ±0.13	15.00^{Dr} ±0.12	17.00^{Ds} ±0.11	21.00^{Et} ±0.16	30.00^{Au} ±0.57
NCDC133	10.00^{Cp} ±0.08	12.00^{Cq} ±0.06	14.00^{Cr} ±0.12	16.00^{Cs} ±0.14	17.00^{Ct} ±0.16	30.00^{Au} ±0.66
NCDC 138	13.00^{Ep} ±0.14	16.00^{Eq} ±0.12	17.00^{Er} ±0.13	19.00^{Es} ±0.14	20.00^{Dt} ±0.16	31.00^{Bu} ±0.51
NRCMQS *E. coli*	16.00^{Fp} ±0.11	18.00^{Fq} ±0.12	20.00^{Fr} ±0.18	22.00^{Fs} ±0.20	24.00^{Ft} ±0.21	32.00^{Cu} ±0.52
NRCMQS *S. aureus*	8.00^{Bp} ±0.09	10.00^{Bq} ± 0.08	11.00^{Br} ±0.09	13.00^{Bs} ±0.15	14.00^{Bt} ±0.11	30.00^{Au} ±0.59

Means bearing different superscripts differ significantly ($^{p, q, r, s, t, u}p<0.05$) between rows and ($^{A, B, C, D, E, F}p<0.01$) between columns

Discussion

a. MOLAE

For dilution 1:5, the mean values of the zone of inhibition for *Streptococcus agalactiae* were 2 mm in all the concentrations, and for 1:4 dilution, the MIC ranged from 2-4 mm. For different strains of *E. coli*, the mean values of the zone of inhibition ranged from 2-24 mm for 1:5 dilution and 4.5 to 26 mm for 1:4 dilution. Similarly, for the different strains of *Staphylococcus aureus*, the mean values of the zone of inhibition ranged from 2 to 20.50mm for 1:5 dilution and 8 to 21.50mm for 1:4 dilution. The mean values of the zone of inhibition range for *Klebsiella pneumonia* were 2- 23 mm for 1:5 dilution and 12 to 27 mm for 1:4 dilution. The Zone of inhibition reflected for the NRCMQS *S. aureus* culture increased with an increase in concentration compared to NCDC 133 culture, indicating the effectiveness of MOLAE towards the mastitis-causing pathogens in 1:5 dilution. But, a similar pattern of inhibition was not observed with NRCMQS *E. coli* and NCDC135 cultures. Still, at lower concentrations, the NRCMQS *E. coli* showed a comparatively higher zone of inhibition than the NCDC 133 culture in 1:5 dilution. For 1:4 dilution, the NRCMQS cultures depicted the highest zone of inhibition values compared to the NCDC cultures at different concentrations of MOLAE. The present study's findings were in line with Abalaka *et al.* (2012) [1]; Anthonia (2011) [6]; Bukar *et al.* (2010) [10]; Thilza *et al.* (2010) [61]; Nikkon *et al.* (2003) [38]. Ashok and Pari (2003) [8] reported the antibacterial activity of the aqueous extracts of *Moringa oleifera* had inhibition against *S. aureus* (10 mm) and *E. coli* (12 mm), and the values corroborate with the present study. Thus, several studies pointed out that aqueous extract has antibacterial activity against *Escherichia coli* (Anthonia, 2011 [6]; Bukar *et al.*, 2010 [10]; Thilza *et al.*, 2010) [61] with an inhibition zone of 12 mm (Gomashe *et al.*, 2014) [20]; *Staphylococcus aureus* (Nikkon *et al.*, 2003; Vinoth *et al.*, 2012) [38, 62] and other bacterial species (Priya *et al.*, 2011; Abalaka *et al.*, 2012) [47, 1] which were in line with the present study. Paray *et al.* (2018) [43] reported that the antibacterial activity measured as a zone of inhibition (in mm) for *Moringa oleifera* extract against *E. coli*, *Proteus* spp., *E. faecium*, *E. faecalis*, *S. aureus*, and *S. agalactiae* was 7.50, 12.75, 10.25, 10.25, 8.75, and 8.50, respectively, against bacterial species which were in line with the present study except for the mean values of the zone of inhibition observed for *S. agalactiae*. The variation in the mean values of the zone of inhibition obtained in the present study may be due to the differences in the strains of microorganisms used compared to other studies. The antibacterial activity of the *Moringa oleifera* leaf extract has been studied worldwide. Different concentrations, extraction techniques, and solvents were used to illustrate the potency of the extracts obtained in each case. Pandit and Vyas (2020) [42] conducted a study to identify the antibacterial activity of *M. oleifera* aqueous leaf extract. The leaf extract showed the most potent antibacterial activity against *P. aeruginosa* (20 mm) at its highest 200 mg/ml concentration. In addition, *M. oleifera* leaf extract showed an intermediate antibacterial effect against *S. aureus*, Coagulase-negative *Staphylococci*, *Enterobacter* spp., and *Streptococcus* spp. A similar study was conducted by Akpor *et al.* (2020) [4] to evaluate the antibacterial activity of ethanolic leaf extracts of *Moringa oleifera* and found that extracts inhibit the growth of *Escherichia coli*, *Staphylococcus aureus* (MIC- 500 mg/L), *Pseudomonas aeruginosa* (MIC- 1000 mg/L), and *Bacillus subtilis* (MIC- 500 mg/L). The phytochemical constituents in the ethanolic extract include alkaloids, cardiac glycosides, terpenoids, tannins, saponins, and phlorotannins. *Klebsiella* spp. was inhibited in all the concentrations of Moringa ethanolic extract in the study. On the other hand, Singh and Tafida (2013) [59] reported that the aqueous, ethanol, and methanol extracts of the moringa leaves have an inhibitory ability against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. They also pointed out that the

inhibitory effects of the extracts were significantly higher on *Escherichia coli* than on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Kalpana *et al.* (2013) [28] reported that Moringas' petroleum ether, ethanol, aqueous, and chloroform extracts had maximum antibacterial activity against *Staphylococcus aureus.* Ajayi and Fadeyi (2015) [3] observed that petroleum ether extract of Moringa leaf has more inhibitory activity against *Streptococcus* spp. than *Staphylococcus aureus*. Thus, the present study aligned with the above findings about the antimicrobial activity of moringa leaf extract.

b. MOSO

In the current study, the phenolic extract exhibited mean values of the zone of inhibition of 9 to 20 mm, and for methanolic extract, it was 8 to 26 mm for *Streptococcus agalactiae.* The mean values of the zone of inhibition ranged from 10 to 23 mm for phenolic extract and 9 to 25 mm for methanolic extract for *E. coli* strains used in the present study. The mean values of the zone of inhibition ranged between 10-27 mm for a phenolic extract and 8- 27 mm for a methanolic extract for various concentrations used against *S. aureus.* For *Klebsiella pneumonia,* the mean values of the zone of inhibition ranged from 8 to 19 mm and 11 to 22 mm for phenolic and methanolic extract, respectively. The mean values of the zone of inhibition of antibiotic solution against the different strains of microorganisms used in the present study were the highest and ranged from 30 to 32.50 mm. For phenolic and methanolic extracts, the NRCMQS cultures depicted the highest zone of inhibition values compared to the NCDC cultures at different concentrations of MOSO. Several studies have reported the antibacterial activity of MOSO (Ramachandran *et al.,* 1980; Chuang *et al.,* 2007; Salaheldeen *et al.,* 2014) [50, 12, 55]. Amina *et al.* (2019) [5] determined the MIC of MOSO against strains of *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Shigella flexneri*, and *Candida albicans* at 50 mg mL^{-1} concentrations 8, 5, 10, 8, 4, 7, 12 mm, respectively. The inhibition zone against bacteria ranges from 10-16 mm at 50 mg mL^{-1} concentration. The observations of the present study were higher than the reported as the concentration of MOSO used was from 100-300 µl. Similarly, Farhan *et al.* (2021) [16] reported the antibacterial effect of MOSO against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Klebsiella pneumoniae* in all concentrations tested except for *E. coli* and *K. pneumoniae*, which at a concentration of 125 µl/ml in petroleum ether solvent extraction of oil. The MIC and inhibition zone for *Staphylococcus aureus*, *Bacillus cereus*, *E. coli*, and *Klebsiella pneumonia* were 64, 128, 256, 256µl/ml and 18.50, 14.16, 11.83, and 11.33 mm in concentration 500 µl/ml, respectively, which were in line with the present study even though the extraction procedure of oil was different. Moringa seed oil, a light-coloured yellow oil, has excellent properties as an emollient along with *Aloe vera* and *Curcuma caesia* extracts. The said oil is equivalent to olive oil in its benefits. In addition, Moringa oil contains 6-10% behenic acid, which provides a unique skin feel and is attributed to the oxidative stability of the oil. Similarly, Aloe Vera is an excellent moisturizer used commercially in the cosmetic industry (Nandagopal *et al.,* 2011) [37]. Pharmacological investigations have proved that the *Moring oleifera* seed oil (MOSO) has several biological activities, including antioxidant, anticancer, antibacterial, antifertility, antifungal (Ramachandran *et al.,* 1980; Chuang *et al.,* 2007) [50, 12], skin protecting effect, anti-rheumatic and anti-hypertensive properties (Mahmood *et al.,* 2010) [34]. These findings were in line with the present study as the colour of the moringa oil isolated was light-yellow and manifested several properties in due course of the experiment.

c. MOPPAE

M. oleifera pods powder (MOPP) has an array of antimicrobial activities (Sayeed *et al.,* 2012) [56], are a good source of saponins, the plant glycosides with triterpene or steroid aglycone (Paliwal *et al.,* 2011) [40]. The mean values of inhibitory zones ranged between 15 to 25 mm against *Streptococcus agalactiae*, 15 to 26 mm against different strains of *E. coli*, 9 to 27 mm against different strains of *Staphylococcus aureus*, 17 to 25 mm against *Klebsiella pneumonia* for the different concentrations of 1:5 dilution in the present study. Similarly, the mean values of inhibitory zones ranged between 14 to 20 mm against *Streptococcus agalactiae*, 18 to 27 mm against different strains of *E. coli*, 9 to 28 mm against different strains of *Staphylococcus aureus*, 16 to 24 mm against *Klebsiella pneumonia* for the different concentrations of 1:4 dilution. For 1:5 dilution, the NCDC cultures depicted the highest zone of inhibition values compared to the NRCMQS cultures at different concentrations of MOPPAE, except for the NRCMQS *S. aureus* at 250 and 300µl of MOPPAE. For 1:4 dilution, the NRCMQS *S. aureus* depicted the highest zone of inhibition values compared to the NCDC 133 at different concentrations of MOPPAE, and a reverse pattern is observed with NRCMQS *E. coli* and NCDC 133. Kheir *et al.* (2014) [30] reported MOPP alcoholic extract showed antibacterial activity against all the tested gram-positive bacteria. MOPP alcoholic extract showed a maximum inhibition zone of 28 mm at 500 mg/ml concentration for *S. aureus.* The aqueous extracts also showed antibacterial activity against gram-positive bacteria. Jabeen *et al*. (2008) [27] observed that the crude extracts of MOPP exhibited zone inhibition of 21, 31, 36, and 20 mm against *Pasturella multocida*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, which was in line with the present study. Several studies reported the antimicrobial activity of moringa pods (Suganandam *et al.,* 2022 [60]; Premi and Sharma, 2017 [46]; Govardhan *et al.,* 2013 [21]; Karmakar *et al.,* 2010) [29] that are in agreement with the present study.

d. MOGPAE

Panda (2014) reported that Moringa gum is hydrophilic, and the emulsion created is stable compared to acacia gum. The emulsion of moringa gum is least affected by centrifugation, pH, temperature changes, and electrolytes. Moringa gum is a non-adhesive mucopolymer (Singh and Kumar, 2018) [58] (Rimpy *et al.,* 2017) [51]. Thus, it can be used as an agent of thickening, stabilizing, and gelling (Kumar and Renuka, 2019) [33]. The MIC of MOGPAE was carried out at 1:5 and 1:4 dilutions for different concentrations against the selected microorganism in the present study. The mean values of inhibitory zones ranged between 5 to 10 mm against *Streptococcus agalactiae*, 11 to 21 mm against different strains of *E. coli*, 4 to 15 mm against different strains of *Staphylococcus aureus*, 12 to 16 mm against *Klebsiella pneumonia* for the different concentrations of 1:5 dilution. Similarly, the mean values of inhibitory zones ranged between 7 to 12 mm against *Streptococcus agalactiae*, 12 to

24 mm against different strains of *E. coli*, 8 to 17 mm against different strains of *Staphylococcus aureus*, 13 to 20 mm against *Klebsiella pneumonia* for the different concentrations of 1:4 dilution. The mean values of the zone of inhibition observed for the antibiotic solution used against the selected microorganisms ranged between 30 to 32 mm for the present study. For both dilutions, the NRCMQS *S. aureus* depicted the highest zone of inhibition values compared to the NCDC 133 at different concentrations of MOGPAE. For 1:4 dilution, the NRCMQS *E. coli* showed the highest zone of inhibition values compared to the NCDC 135 to varying concentrations of MOGPAE. Still, for 1: 5 dilutions, a reverse trend was observed except for the concentration of 300µl MOGPAE. Irfan *et al.* (2021) [26] conducted an experiment and successfully synthesized zinc oxide and silver nitrate nanoparticles (NPs), where Moringa gum was the reducing and capping agent. They further analyzed their antimicrobial activity against MRSA, *E. coli*, and *S. aureus*. The concentration of gum was 50 per cent in NPs. The zone of exhibition exhibited by NPs of AgNPs and ZnO against *E. coli*, *S. aureus*, and MRSA were 21 and 22, 20 and 21, and 16 and 17 mm, respectively. The validation of the present test needs additional citations as similar studies about the mean values obtained for MOGPAE against the selected microorganisms are lacking.

The nature and amount of active ingredients present in the various extracts of Moringa depend on the method and the extraction solvent (Vongsak *et al.*, 2013) [63]. Not only the technique of extraction but also the geographical location, climatic variations, soil, and intensity of the solar radiation received (Wink, 2003) [65] affect the quality of active compounds present in Moringa. Several studies reported various bioactive compounds and secondary metabolites such as quercetin, gallic acid, chlorogenic acid, glucosinolates, ferulic acid, phenolic acids, vanillin, kaempferol, ellagic acid, and flavonoids (Coppina *et al.*, 2013) [13] in moringa flowers, seeds, leaves, pods, stem and bark (Mbikay, 2012) [35]. The major antibacterial compounds isolated from *M. oleifera* (Thilza *et al.*, 2010) [61] include isothiocyanates (4-(4'-O-acetyl-a-L rhamnopyranosyloxy) benzyl isothiocyanate, 4-(a-L-rhamnopyranosyloxy) benzyl isothiocyanate, benzyl isothiocyanate, pterygospermin, niazimicin,4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate), pterygospermin, glucosinolates and rhamanose (Fuglie, 2000) [18]. Thus, the antibacterial activity manifested by the various extracts of Moringa in the present study is due to the bioactive compounds and secondary metabolites. The degree of antimicrobial activity exhibited by the various dilutions used in the current study is due to the variations in the amount of bioactive compounds in the respective extracts.

Conclusion

The different bacterial cultures of NCDC and NRCMQS showed a significant increase in the mean values of the zone of inhibition of different concentrations of MOLAE, MOSO, MOPPAE, and MOGPAE to the control group ($p<0.05$), and the different cultures differ significantly ($p<0.01$) within the MIC each extract. Mastitic cultures showed the highest zone of inhibition with the different Moringa extracts, indicating the aqueous extract's effectiveness over NCDC cultures. Thus, Moringa could be utilized in developing prophylactic solutions against mastitis.

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