ISSN: 2456-2912
VET 2024; 9(1): 216-223

www.veterinarypaper.com
Received: 08-01-2024
Accepted: 17-02-2024

Gavali Sandeep
MVSc Scholar,
Department of Veterinary Pharmacology & Toxicology,
Mumbai Veterinary College, Parel,
Mumbai, Maharashtra, India

Karande Vikas
Assistant Professor,
Department of Veterinary Pharmacology & Toxicology,
Mumbai Veterinary College, Parel,
Mumbai, Maharashtra, India

Ghadigaonkar Sushma
Assistant Professor,
Department of Veterinary Pharmacology & Toxicology,
Mumbai Veterinary College, Parel,
Mumbai, Maharashtra, India

Joshi Purva
MVSc Scholar,
Department of Veterinary Pharmacology & Toxicology,
Mumbai Veterinary College, Parel,
Mumbai, Maharashtra, India

Corresponding Author:
Gavali Sandeep
MVSc Scholar,
Department of Veterinary Pharmacology & Toxicology,
Mumbai Veterinary College, Parel,
Mumbai, Maharashtra, India

Plant-derived drug against drug-resistant *Staphylococcus aureus*

Gavali Sandeep, Karande Vikas, Ghadigaonkar Sushma and Joshi Purva

DOI: https://doi.org/10.22271/veterinary.2024.v9.i2d.1203

Abstract
The study was planted to assess the *in vitro* antibacterial properties of ethanolic and aqueous extracts of Turmeric (*Curcuma longa*), Pudina (*Mentha arvensis)*, Garlic (*Allium sativum*), Tulsi (*Ocimum sanctum*), and Curry leaves (*Murraya koenigii*), against Multi Drug Resistant strains of *S. aureus* sourced from bubaline mastitis. Concentrations of 2.5, 5.0, and 10mg/100µL were tested using agar well diffusion, while Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined via broth dilution and MSA plates respectively. Ciprofloxacin served as the standard antibiotic, showing an 18.2 ± 0.66mm zone of inhibition. Ethanolic extracts from all plants, except Garlic, exhibited superior activity compared to Ciprofloxacin and aqueous extracts. Ethanolic extract of Turmeric showed the highest activity with a 26.4±2.42mm zone of inhibition. Pudina, Curry leaves, and Tulsi had moderate activity with 22±2.09, 22.2±1.82, and 22.8±2.26mm zones of inhibition at a 10mg/100ml concentration. Ethanolic garlic extract had lower activity, with a 12±0.83mm zone of inhibition at the same concentration. Aqueous extracts were less effective than ethanolic ones at all concentrations with statistically significant differences ($p<0.01$).
Pudina, Curry leaves, Tulsi, and Turmeric had similar MIC and MBC values (1.562 mg/ml) against all resistant strains, lower than other extracts. Both ethanolic and aqueous Garlic extracts shared similar MIC and MBC values (6.250 mg/ml). This study suggests the potential use of these plants in treating diseases caused by MDR *S. aureus*. Further research is needed to identify the active phytochemicals responsible for their antibacterial activity.

Keywords: Garlic, Pudina, curry leaves, Tulsi, turmeric, ciprofloxacin, *in-vitro*, zone of inhibition

Introduction
Antibiotics have long been considered the "magic bullets" to end infectious diseases Though the use of antibiotics has improved the countless health of human and animals, many antibiotics have also been losing their effectiveness since the beginning of the antibiotic era. Bacteria have adapted defenses against these antibiotics and continue to develop new resistance patterns. Inappropriate use of antibiotics in human and animal medicine, as more microbial species and strains become resistant, results in difficulty in treating many diseases. The use of antibiotics in raising food animals has also contributed significantly to the pool of antibiotic-resistant organisms globally.
Mastitis is an inflammation of the udder accompanied by physical, chemical, and bacteriological changes in milk. Bovine mastitis has become the costliest disease in India affecting nearly 50% of the herd population. It has been estimated that mastitis alone can cause 70% of all the unavoidable losses incurred during milk production (Sumathi *et al*., 2008) [32]. Bubaline mastitis is one of the devastating diseases causing huge losses to the dairy industry worldwide. The costs associated with mastitis are numerous and include antibiotic treatment, reduced milk quality, reduced milk yield, increased culling rate, and hazardous to public health (Kurijogi and Kaliwal, 2011) [20]. The large population of low-yielding dairy animals and mastitis are the major constraints of the Indian dairy industry. Mastitis is found to be widespread among dairy animals in the country. The disease also results in partial or complete damage to udder tissues and decreases the productive lifespan of the animal. Hundreds of microorganisms are isolated from bovine mammary infections but the majority of infections are caused by Staphylococcus, Streptococcus, and Gram-negative bacteria like E. *coli*, Klebsiella, etc. (Tenhagen *et al*., 2006) [33].

The versatile nature of Staphylococcus produces resistance and spreads it to other susceptible organisms which results in the ineffectiveness of antimicrobials. Therefore, the selection of a more effective drug for mastitis therapy by the evaluation of antimicrobial resistance may become a reality for clinical veterinarians (Li *et al*., 2010) [23]. S. aureus is a prevalent bacterium carried by animals that can cause several problems, like wound infections, pneumonia, and mastitis. Several virulence factors determine the pathogenicity of S. aureus. These include several toxins that can cause such effects as toxic shock syndrome and food poisoning. Antibiotic resistance in Staphylococci is very common and was observed very early in the antibiotic era. When general use of Penicillin began, nearly all Staphylococcal isolates were susceptible when tested in the laboratory, but later on, resistant strains of S. aureus developed in hospitals fastly & grew from less than 1% incidence to 14% in 1946, to 38% in 1947 and more than 80 to 90% today. Worldwide, Ampicillin and Penicillin resistance can be found together in more than 80% of S. aureus strains (Gangle, 2005) [13]. Resistance to other antibiotics similarly appeared shortly after their introduction. In 1953, Staphylococcal resistance to Streptomycin, Tetracycline, Chloramphenicol, and Novobiocin had been reported. The use of antibiotic therapy to treat and prevent udder infections in dairy animals is a key component of mastitis control. Due to the widespread use of antibiotics in mastitis in dairy livestock, much effort and concern have been directed towards the proper management and monitoring of antibiotics usage in treatments to prevent contamination of raw milk.

Phytotherapy is a traditional remedy for different diseases where herbs and their products are used. It is a very well-applied method of therapy for both humans and animals. India is one of the nations blessed with a rich heritage of traditional medicinal systems and rich biodiversity to complement the herbal needs of the treatment administrated by these traditional medical systems (Pei, 2001) [30]. Medicinal plants are important resources to traditional society's health care system and it is estimated that 70% to 80% of rural population in developing Asian nations depends on home care and traditional medicines for therapies.

Medicinal plants constitute major sources of several primary and secondary metabolites which are bioactive compounds of great therapeutic value (Evans *et al*., 2002) [12]. The various phytochemicals found in the plant extracts are tannins, flavonoids, saponins, and alkaloids. They have antidiabetic, antioxidant, antibacterial, anti-inflammatory, antipyretic, gastro-protective effects, and many more such important medicinal properties.

The emergence of resistance to conventional antimicrobials is a serious problem. Hence more studies about the use of plants as therapeutic agents for the control of antibiotic-resistant microbes should be emphasized (Mishra and Behal, 2010) [28]. Medicinal plants or Spices are important due to their medicinal, antimicrobial, and antioxidant properties (Joe *et al*., 2009; Anjeza *et al*., 2012) [18, 5]. The spices have unique aroma and flavor which are derived from phytochemicals or secondary metabolites (Avato *et al*., 2006) [7]. Keeping this view in mind, the present study was carried out to assess the antimicrobial effect of aqueous and ethanolic extracts of selected plants or spices available in India namely Turmeric (Curcuma longa), Pudina (Mentha arvensis), Garlic (Allium sativum), Tulsi (Ocimum sanctum), Curry leaves (Murraya koenigii) against Multi Drug Resistant Staphylococcus aureus bacteria sourced from bubaline mastitis cases. The present work was planned to determine the efficacy of selected plant extracts against MDR Staphylococcus aureus organisms and also to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of selected plant extracts.

Materials and Methods

Sampling was done to detect the presence of clinical and subclinical mastitis by using CMT from the buffalo herds in and around the Mumbai region. Positive samples were transported on ice to the laboratory of the Department of Veterinary Pharmacology and Toxicology for isolation and identification of bacteria.

The positive samples were tested for the presence of S. aureus by using enrichment broth, Mannitol Salt Agar (MSA), Gram staining, and various biochemical identification tests like Catalase test, Coagulase test, Sugar fermentation tests, etc.

Selection of plant material

For ethanolic and aqueous extracts, were selected following parts of plants:

Ocimum sanctum (Tulsi) - Leaves
Murraya koenigii (Curry leaves) - Leaves
Allium sativum (Garlic) - Fresh bulbs
Curcuma longa (Turmeric) - Dried rhizomes
Mentha arvensis (Pudina) - Leaves and inter nodal callus

These parts of the plants were selected for the evaluation of their antimicrobial properties against the isolated S. aureus strains (Ismail *et al*., 2012; Argal *et al*., 2011) [16, 6].

Preparation of plant extracts

Aqueous and Ethanolic crude extracts of all the selected plants were prepared with slight modifications in the methods of extraction (Khusro *et al*., 2013; Mukhtar and Ghori, 2012) [22, 29]. A total of 100 g of air-dried powder of the selected plant was filled in the thimble and extracted successively with 1000 ml of ethanol using a Soxhlet extractor (which used continuous heat and cold-water flow for 48-72 hrs). The extract was concentrated using a rotary flash evaporator and preserved at 50 C in an airtight bottle and the extract was subjected to an antibacterial activity assay (Bansode and Chavan, 2014) [9]. Various concentrations (100mg/100µl, 50mg/100µl and 25mg/100µl) were prepared from the fixed amount of paste, obtained from the above-mentioned procedures, by mixing with an appropriate volume of 5% DMSO (Dimethyl Sulfoxide) for antibacterial testing by agar well diffusion method.

Antimicrobial susceptibility testing

Bacterial isolates i.e. S. aureus from the representative milk samples were tested for antimicrobial susceptibility using the disc agar diffusion method according to procedures recommended by the CLSI, 2015. Bacterial isolates from the above microbiological analysis were grown on selective media. Standard antibiotic discs procured from HI-Media were tested on these plates having isolates. These plates were incubated at 37 ºC for 24 hrs and thereafter zones of inhibition were measured with a metric ruler provided by HI-Media and interpreted as resistant or sensitive strains according to the CLSI guidelines.

Identification of MDR organisms

Every isolate of S. aureus was tested with a maximum of 12 different antibiotics which are used commonly by field veterinarians for mastitis as well as other infectious diseases

of animals i.e. (Tetracycline, Streptomycin, Oxacillin, Methicillin, Ampicillin, Ceftazidime, Cefepime, Ciprofloxacin, Gentamicin, Penicillin G, Ceftriaxone, Cefixime) and zone of inhibition produced on Mueller Hinton agar was measured. The strain of S. aureus which showed resistance to a minimum of five or more antibiotics was interpreted as resistant (according to CLSI, 2015 guidelines) and then this isolate was considered as an MDR organism for further study. The inherent resistance was not accounted for by MDR.

The antimicrobial activity of the crude extracts of plants was studied using agar well diffusion method.

Determination of MIC and MBC

The crude extracts of plants were found effective as an antimicrobial agent. The MIC and MBC value for each isolate was determined by using the method described by Jahan *et al*. (2011) [17] with slight modifications. MIC was determined using the broth dilution method and the extracts were serially diluted to obtain different concentrations per ml. 100 µl of 10 5 CFU/ml of the S. aureus isolates were added in 96 well microtitre plates with equal volumes of nutrient broth and plant extracts. The plates were incubated aerobically at 37°C for 24-48 hrs. Three controls were maintained for each isolate (media control, organism control, and extract control). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24 hrs when compared with the controls was considered as initial MIC. The dilution that showed no turbidity was incubated further for 24 hrs at 37 °C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 hrs was regarded as the final MIC. MBC value was determined by sub-culturing the test dilution (which showed no visible turbidity) onto freshly prepared nutrient agar media. The plates were incubated further for 18-42 hrs at 37 °C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was considered as MBC (Jahan *et al*., 2011) [17].

Results and Discussion

The use of antibiotic therapy to treat and prevent udder infections in dairy animals is a key component of mastitis control. The emergence of resistance to conventional antimicrobials is a serious problem. Many organisms causing mastitis have become resistant nowadays and several antibiotics have become ineffective. Hence to investigate alternatives to chemical antibiotics needs to search for new, safe, and effective bioactive agents of herbal origin. Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. Hence, in the present study, some plants were screened for their antimicrobial activity against resistant strains of S. aureus found in mastitic milk samples.

Sampling was done to detect the presence of clinical and SCM by using CMT from the buffalo herds in and around the Mumbai region. A total of 544 milk samples were collected from Maharaj dairy (Goregaon)/(90), Gaiwadi (Santa Cruz)/(116), Palghar district dairy farms/(150), Marol - Maroshi dairy farms/(74), Kandivali dairy farms/(114). Among all the screened samples, 224 (41.18%) were positive for mastitis, out of which 28.68% (156/544) and 12.50% (68/544) cases suffered from SCM and clinical mastitis respectively. Abera *et al.* (2010) [1] reported prevalence from Adama town, Ethiopia for clinical mastitis and SCM were 10.00% and 36.7% respectively. Prabhu *et al.* (2015) [31] also reported the total prevalence of mastitis as 45.33%, of which 13.33% and 32% were clinical and SCM, respectively. from Karnataka, India, Similar findings were reported by Meh *et al*. of Bangladesh (2011) [24] from Sodo town, Ethiopia and prevalence of SCM as 28.50% and 32.92% respectively.

A total of 224 (68 from clinical and 156 from SCM cases) milk samples were used for screening or detection of S. aureus presence by following routine microbiological procedures: From all positive mastitic milk samples, only 102 (45.53%) samples produced bacterial growth (yellow colonies) on MSA (24 from clinical and 78 from SCM cases). Abera *et al.* (2010) [1] observed 42.14% growth of S. aureus in their study.

MSA was used for the identification of S. aureus, it is both a differential and selective media used for isolation of pathogenic Staphylococci. It contains protease peptone as a nitrogen source, beef extract as a source of growth factors, nutrients, and trace elements, and mannitol as a source of carbon. The differential nature of the media is due to the phenol red indicator, which detects a change in pH due to the fermentation of mannitol, by some strains of Staphylococci. S. aureus ferments mannitol to produce yellow colonies surrounded by a yellow zone. Coagulase-negative strains of S. aureus and Staphylococcus epidermidis, which are usually non-fermenters, produce pink-red colonies. The selectivity of the media towards Staphylococci is due to the high concentration of sodium chloride (Addis *et al*., 2011) [3]. Plates with only yellow colonies were initially presumed as S. aureus and colonies from those plates were recruited for identification of S. aureus by biochemical tests for confirmation (Plate No. 2).

Concentrations used for testing

Ethanolic extract - Different (100mg, 50mg, 25mg) concentrations were prepared based on results obtained in the pilot study by using obtained ethanolic extract by mixing with appropriate (100µl) volume of 5% DMSO (Dimethyl Sulfoxide) for antibacterial testing by agar well diffusion method.

Aqueous extract - Different (100mg, 50mg, 25mg) concentrations were prepared based on results obtained in the pilot study by using obtained aqueous extract by mixing with appropriate (100µl) volume of sterile distilled water for antibacterial testing by agar well diffusion method.

Antimicrobial susceptibility testing and evaluation of MDR organisms

Bacterial isolates that were identified as S. aureus were tested for antimicrobial susceptibility using the disc agar diffusion method for the evaluation of MDR organisms according to procedures recommended by the CLSI in the year 2015.

Discs used for AST: Tetracycline, Streptomycin, Oxacillin, Methicillin, Ampicillin, Ceftazidime, Cefepime, Ciprofloxacin, Gentamicin, Penicillin G, Ceftriaxone and Cefixime discs were used for testing antimicrobial activity and to find out MDR S. aureus organisms.

Resistance pattern produced by S. aureus strains to antibiotics

All confirmed strains of S. aureus (102) were screened for identification of MDR by AST and obtained resistance to all used antibiotics as Methicillin (67%) followed by Oxacillin (60%), Cefixime (32.6%), Penicillin G (27.7%), Streptomycin (27.5%), Ampicillin (24. 3%), Cefepime (24%), Ceftriaxone (23.8%), Ceftazidime (22.9%), Gentamicin (22.3%), Tetracycline (18.2%), Ciprofloxacin (15.2%). The highest resistance was observed to Methicillin and the lowest to

Ciprofloxacin among the tested S. aureus organisms. Chile reported a resistance pattern produced by S. aureus to some antibiotics that as Lincomycin (38.9%) followed by Amoxicillin (38.1%), Penicillin G (28.8%), Ampicillin (26.0%), and Cefquinome (24.7%). West Central region of Iran also reported resistance pattern produced by S. aureus to Penicillin and Streptomycin as 14.28% and 28% respectively. Prabhu *et al*. (2015) [31] from Hassan district, Karnataka, India reported resistance pattern produced by S. aureus against some antibiotics were Tetracycline (74.28%) followed by Penicillin (71.42%) and Ampicillin (45.71%). Similarly, Adesola (2012) [2] from Nigeria reported a resistance pattern of S. aureus against some antibiotics like Tetracycline (100%) followed by Ampicillin (68%) and streptomycin (32%) which were higher than the results obtained in the present study.

Antimicrobial assay by agar well diffusion method

The antimicrobial activity of the crude extracts (in triplicate) of plants was evaluated using the agar well diffusion method. Consideration of zone of inhibition for extract: Due to the various colors of plant extracts, the zone of inhibition was considered based on the exact circle formed around the agar well which filled with a specific concentration of plant extract

Antibacterial activity of Turmeric/*Curcuma longa*

The antibacterial activity of aqueous extract of Turmeric, Tulsi, Curry leaves, Pudina and garlic were tested at concentrations of 100, 50, and 25mg per 100µl of distilled water against all resistant isolates of S. aureus.

The mean zones of inhibition (in mm) observed were 6.2 ± 1.85, 4.6 ± 1.88, and 3.4 ± 1.46 for the concentrations of 100, 50, and 25mg of Turmeric extract respectively. The concentration of 100mg showed a greater zone of inhibition than the remaining two concentrations of 50 and 25mg. The results were analyzed statistically, where a non-significant difference was observed between mean zones obtained at different concentrations of aqueous extract of Turmeric. It was observed that a higher concentration of ethanolic extract (100mg) was more effective than all other concentrations of ethanolic and aqueous extract of Turmeric (Table No 1 and 2). Chhattisgarh reported that the methanolic extract of Turmeric had high antimicrobial activity against *S. aureus* (range of zone of inhibition, 6- 16 mm) due to the content of a high number of secondary metabolites. Mukhtar and Ghori (2012) [29] from Rawalpindi, Pakistan reported that the ethanolic extract of Turmeric produced greater activity as 14mm and 11mm zones of inhibition against *B. subtilis* and *E. coli* respectively which was greater than the aqueous extract.

Antibacterial activity of Tulsi

The antibacterial activity of aqueous extract of Tulsi was tested at concentrations of 100, 50, and 25mg per 100µl of distilled water against all resistant isolates of S. aureus. The mean zones of inhibition (in mm) observed were 11.6 ± 1.12, 10.2 ± 1.39, and 8.4 ± 1.43 for the concentrations of 100mg, 50mg, and 25mg, respectively. The concentration of 100mg showed a greater zone of inhibition than the remaining two concentrations of 50 and 25mg. The results were analyzed statistically, where a non-significant difference was observed between mean zones obtained at different concentrations of aqueous extract of Tulsi. It was observed that a higher concentration of ethanolic extract (100mg) was more effective than all other concentrations of ethanolic and aqueous extract of Tulsi (Table No.1 and 2). The antibacterial effect of Tulsi against S. aureus was reported from Sindh, Pakistan by Tasneem *et al*. (2015) [4]. They reported that the ethanolic extract of Tulsi produced a 19.7 ± 0.6mm zone of inhibition. Many scientists have also determined antibacterial activity and phytochemical evaluation of extracts of Tulsi. Jahan *et al.* (2011) [17] from Allahabad studied the antimicrobial activity of Tulsi against sensitive and resistant S. aureus strains, where, Tulsi had moderate antibacterial activity. Chennai, Tamilnadu evaluated the antibacterial activity of aqueous and ethanolic extracts of Tulsi against S. aureus and reported that Tulsi had good antibacterial activity against S. aureus as the extract contains phytochemicals like triterpenoid, phenol and tannin.

Antibacterial activity of curry leaves

The antibacterial activity of aqueous extract of Curry leaves was tested at concentrations 100, 50, and 25mg per 100µl of distilled water against all resistant isolates of S. aureus. The mean zones of inhibition (in mm) observed were 11.8 ± 1.15, 10.8 ± 0.80, and 10.2 ± 0.80 for the concentrations of 100, 50 and 25mg, respectively. The concentration of 100mg showed a greater zone of inhibition than the remaining two concentrations of 50 and 25mg. The results were analyzed statistically, where a non-significant difference was observed between mean zones obtained at different concentrations of aqueous extract of Curry leaves.

It was observed that a higher concentration of ethanolic extract (100mg) was more effective than all other concentrations of ethanolic and aqueous extract of Curry leaves (Table No.1 and 2). Many reports are stating the antibacterial activity of Curry leaves from India and other countries. From Nagaland, India Argal *et al*. (2011) [6] studied the antimicrobial activity of curry leaves against S. aureus, where extract of Curry leaves had intermediate antibacterial activity as it contains phytochemicals like glycosides, steroids, tannins, alkaloids, flavonoids, saponins, quinone, protein and sugar. Similarly, Baskaran *et al*. (2011) [8] from Chennai, Tamilnadu reported the antimicrobial activity of hot water extract of Curry leaves against S. aureus. Where the zone of inhibition was 28.17± 0.29 mm. Reported that methanolic and aqueous extracts of Curry leaves Showed 10.64 and 8.09 zones of inhibition (in mm) against S. aureus respectively.

The antibacterial activity of aqueous extract of Pudina was tested at concentrations 100, 50, and 25 mg per 100 µl of distilled water against all resistant isolates of S. aureus. The mean zones of inhibition (in mm) observed were 9.80 ± 0.48, 6.4 ± 1.69, and 4 ± 1.70 for the concentrations of 100, 50, and 25mg, respectively. The concentration of 100mg showed a greater zone of inhibition than the remaining two concentrations of 50 and 25mg. The results were analyzed statistically, significant difference was observed between mean zones obtained at different concentrations of aqueous extract of Pudina ($p \leq 0.05$). It was observed that a higher concentration of ethanolic extract (100mg) was more effective than all other concentrations of ethanolic and aqueous extract of Pudina (Table No. 1 and 2). Nascimento *et al*. (2009) [25] from Crato, Brazil reported that Pudina inhibited the growth of S. aureus by producing a 21 mm mean zone of inhibition. S. aureus was the most susceptible to Pudina in a similar way to that of antibacterial activity produced by Tetracycline. This result indicates that Pudina extract has an antibacterial potential similar to that of a commercial drug. Britto *et al*. (2012) [10] from Chandigarh reported the antibacterial efficacy of the extract of Pudina by disc diffusion method and they observed that Pudina had intermediate activity against MRSA with 7mm of zone of inhibition.

The antibacterial activity of aqueous extract of Garlic was tested at concentrations 100, 50, and 25mg per 100µl of distilled water against all resistant isolates of S. aureus. The mean zones of inhibition (in mm) observed were 11.2 ± 0.48, 10.4 ± 0.6, and 9.4 ± 0.87 for the concentrations of 100, 50 and 25mg, respectively. The concentration of 100mg showed a greater zone of inhibition than the remaining two concentrations of 50 and 25mg. The results were analyzed statistically where a non-significant difference was observed between mean zones obtained at different concentrations of aqueous extract of Garlic. It was observed that all concentrations of ethanolic and aqueous extracts of Garlic had shown similar antibacterial activity (Table No.1 and 2).b Iwalokun (2004) [15] from Nigeria from Misurata, Libya reported antimicrobial effects of AGE against S. aureus. They observed that AGE-produced zones of inhibition (in mm) ranged from 20.2 to 22.7 and 48 at 1000µg/ml concentration respectively. Iraq reported a 23mm zone of inhibition produced by ethanolic extract of Garlic against S. aureus. Karuppiah and Rajaram (2012) [19] from Tamilnadu evaluated the antibacterial properties of ethanolic extract of Garlic cloves against MDR S. aureus whereas they observed that Garlic had intermediate antibacterial activity against S. aureus and Ismail *et al*. (2012) [16] screened the alcoholic extract of Garlic juice against the pathogenic strain of S. aureus and they observed that Garlic juice showed broad-spectrum antimicrobial activity.

In the present study, the mean zone of inhibition (in mm) was observed as 22 ± 2.096, 22.2 ± 1.825, 22.8 ± 2.261, 12 ± 0.836, and 26.4 ± 2.427 for Pudina, Curry leaves, Tulsi, Garlic and Turmeric respectively, at concentration of 100mg/100µl of ethanolic extract of all plants. Similarly, the mean zone of inhibition (in mm) was observed as 9.8 ± 0.488, 11.8 ± 1.155, 11.6 ± 1.124, 11.2 ± 0.488 and 6.2 ± 1.857 for Pudina, Curry leaves, Tulsi, Garlic and Turmeric respectively, that concentration of 100mg/100µl of aqueous extract of all plants (Table No.2).

All the resistant strains of S. aureus were found susceptible to Ciprofloxacin. Hence, Ciprofloxacin was kept as a standard antibiotic for the comparison of antimicrobial activity of all plants. The mean zone of inhibition was 18.2 ± 0.663mm observed for Ciprofloxacin. The activity of the aqueous extract, though found lesser, they were effective against these resistant isolates. Whereas, ethanolic extracts of all the plants except Garlic had shown either equivalent or sometimes greater antibacterial activity to that of the standard drug used.

Among all the tested plant extracts, the ethanolic extract of Turmeric showed greater antibacterial activity than all the other plant extracts at different concentrations as well as that of standard drug /Ciprofloxacin. Also, ethanolic extracts of Curry leaves, Tulsi, and Pudina had shown intermediate antibacterial activity and Garlic was showing poor antibacterial activity when compared with Turmeric (Table No. 2)

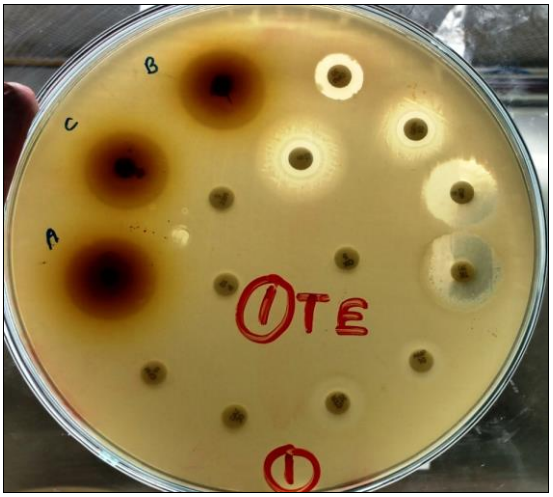

Fig 1: Antimicrobial activity of ethanolic extract of Tulsi against resistant strain of *S. aureus*

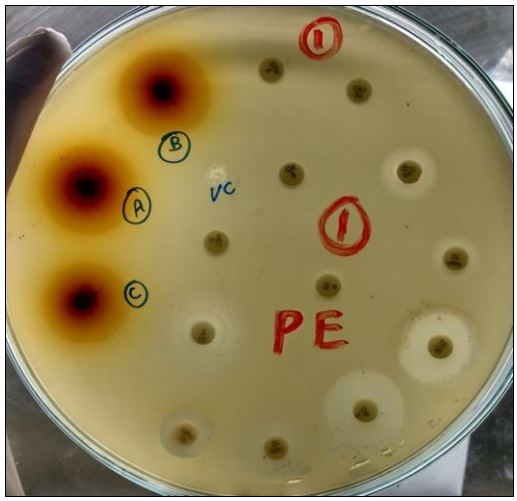

Fig 2: Antimicrobial activity of ethanolic extract of Pudina against resistant strain of *S. aureus*

Table 1: ANOVA of comparative antimicrobial activity between plant extracts against MDR strains of S. aureus.

Sr. No.	Types of extract	Conc. of extract (mg/well)	Names of plants					Ciprofloxacin/Standard Antibiotic
			Pudina	Curry leaves	Tulsi	Garlic	Turmeric	
1.	Ethanolic	100mg/100µl	$22^{ab} \pm 2.096$	$22.2^{ab} \pm 1.825$	$22.8^{ab} \pm 2.261$	$12^{c} \pm 0.836$	$26.4^{a} \pm 2.427$	$18.2^{b} \pm 0.663$
		50mg/100µl	$19.8^{ab} \pm 2.261$	$19.8^{ab} \pm 1.800$	$20^{ab} \pm 1.878$	$10.4^{c} \pm 0.977$	$24^{a} \pm 2.028$	$18.2^{b} \pm 0.663$
		25mg/100µl	$18.2^{a} \pm 2.578$	$17^{a} \pm 2.584$	$18^{a} \pm 1.541$	$9^{b} \pm 0.836$	$20.4^{a} \pm 2.719$	$18.2^{a} \pm 0.663$
2.	Aqueous	100mg/100µl	$9.8^{b} \pm 0.488$	$11.8^{b} \pm 1.155$	$11.6^{b} \pm 1.124$	$11.2^{b} \pm 0.488$	$6.2^{c} \pm 1.857$	$18.2^{a} \pm 0.663$
		50mg/100µl	$6.4^{c} \pm 1.691$	$10.8^{b} \pm 0.800$	$10.2^{b} \pm 1.398$	$10.4^{b} \pm 0.600$	$4.6^{c} \pm 1.887$	$18.2^{a} \pm 0.663$
		25mg/100µl	$4^{c} \pm 1.709$	$10.2^{b} \pm 0.800$	$8.4^{b} \pm 1.432$	$9.4^{b} \pm 0.877$	$3.4^{c} \pm 1.466$	$18.2^{a} \pm 0.663$

MIC was determined using the broth dilution method and the extracts of each plant were serially diluted to obtain different concentrations per ml. 100 µl of 10 5 CFU/ml of the S. aureus isolates were inoculated in 96 well microtitre plates with equal volumes of nutrient broth and plant extracts. The plates were incubated aerobically at 37 °C for 24-48 hrs. Three controls were maintained for each isolate (media control, organism control, and extract control).

MBC value was determined by sub-culturing the test dilution (which showed slight and no visible turbidity during the determination of MIC) onto freshly prepared mannitol salt media. The plates were incubated further for 18-42 hrs at 37 °C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was considered MBC.

The Final mean MIC and MBC values obtained in the present study for aqueous and ethanolic extracts of Pudina were 125

and 15.62mg/ml respectively against all MDR strains of S. aureus. However, lower MIC values were reported by Britto *et al*. (2012) [10] and Dwivedi *et al*. (2012) [11] which were 6.25mg/ml and 0.090mg/ml respectively against S. aureus.

The final mean MIC and MBC values obtained in the present study for aqueous and ethanolic extracts of Turmeric were 125 and 15.62mg/ml respectively against all MDR strains of S. aureus. Lower MIC values were reported by Afrose *et al*. (2015) [4] and Mun *et al*. (2013) [27] which were 125 to 250µg/ml and 800µg/ml respectively.

The Final mean MIC and MBC values obtained in the present study for aqueous and ethanolic extracts of Tulsi were 62.50 and 15.62mg/ml respectively against all MDR strains of S. aureus. Almost similar MIC and MBC values of ethanolic extract were observed by Tasneem *et al*. (2015) [4] against S. aureus which were 26.3 ± 17.2 and 52.5 ± 34.4 mg/ml respectively.

The Final mean MIC and MBC values obtained in the present study for aqueous and ethanolic extracts of Curry leaves were 62.50mg/ml and 15.62mg/ml respectively against all MDR strains of S. aureus. Almost similar MIC values of ethanolic extract of Curry leaves were observed by Mathur *et al*. (2011) [26] against S. aureus which were 8.25 mg/ml to 30mg/ml.

The Final mean MIC and MBC values obtained in the present study for aqueous and ethanolic extract of Garlic were observed similar (62.50mg/ml). Lower MIC values were reported by Iwalokun (2004) [15] against MDR S. aureus, which was 15.6-48.3 mg/ml.

Among all the tested plant extracts, ethanolic extracts of Pudina, Curry leaves, Tulsi, and Turmeric showed similar mean MIC and MBC values i.e. 15.62mg/ml against all resistant strains which was lesser than all other aqueous and ethanolic plant extracts. However, ethanolic and aqueous extracts of Garlic showed similar i.e. 125mg/ml mean MIC and MBC value. The variations in the result may have occurred due to the solvent used, incubation temperature and duration, media used for making dilutions, amount of inoculum added, plant species and parts used, and methods used for determining MIC and MBC values (Jahan *et al*., 2011) [17].

Table 2: MIC of aqueous and ethanolic extracts of selected plants against resistant *S. aureus* strains

Name of plant extract ↓	MIC of Plants extracts against resistant *S. aureus* strains in (mg/ml)															Extract Control	Vehicle Control
	1			$1P_A$			22 P_{2s}			28P			$4P_{2(2)}$				
	Initial MIC	Final MIC	BC	Initial MIC	Final MIC	BC	Initial MIC	Final MIC	BC	Initial MIC	Final MIC	BC	Initial MIC	Final MIC	BC		
Aq. Pudina	125	125	TD	125	125	TD	125	125	TD	125	125	TD	125	125	TD	NTD	NTD
Aq. Curry leaves	62.50	62.50	TD	62.50	62.50	TD	62.50	62.50	TD	62.50	62.50	TD	62.50	62.50	TD	NTD	NTD
Aq. Tulsi	31.25	62.50	TD	31.25	62.50	TD	31.25	62.50	TD	31.25	62.50	TD	31.25	62.50	TD	NTD	NTD
Aq. Garlic	31.25	62.50	TD	31.25	62.50	TD	31.25	62.50	TD	31.25	62.50	TD	31.25	62.50	TD	NTD	NTD
Aq. Turmeric	125	125	TD	125	125	TD	125	125	TD	125	125	TD	125	125	TD	NTD	NTD
Eth. Pudina	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	NTD	NTD
Eth. Turmeric	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	NTD	NTD
Eth. Garlic	31.25	62.50	TD	31.25	62.50	TD	31.25	62.50	TD	31.25	62.50	TD	31.25	62.50	TD	NTD	NTD
Eth. Tulsi	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	NTD	NTD
Eth. Curry leaves	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	7.812	15.62	TD	NTD	NTD

Summary and Conclusion

Among all the tested plant extracts, ethanolic extracts of Pudina, Curry leaves, Tulsi, and Turmeric showed similar mean MIC and MBC values i.e. 15.62mg/ml against all resistant strains which was lesser than all other aqueous and ethanolic plant extracts. However, ethanolic and aqueous extracts of Garlic showed similar i.e. 125mg/ml mean MIC and MBC value. However detailed studies are needed to confirm and study the mechanism action of Turmeric, Garlic, Tulsi, Pudina, and Curry leaves. Based on the results of the present investigation the following conclusions were drawn.

1. The observed prevalence of Bubaline mastitis in Mumbai and around the region was 41.18%, of which 28.68% and 12.50% of cases suffered from SCM and clinical mastitis respectively.
2. Out of all mastitis-positive milk samples, 45.53% samples were infected with the S. aureus organism and when strains of S. aureus were screened for identification of MDR, obtained resistance to all used antibiotics was Methicillin (67%) followed by Oxacillin (60%), Cefixime (32.6%), Penicillin G (27.7%), Streptomycin (27.5%), Ampicillin (24. 3%), Cefepime (24%), Ceftriaxone (23.8%), Ceftazidime (22.9%), Gentamicin (22.3%), Tetracycline (18.2%), Ciprofloxacin (15.2%).
3. Out of all 45.53% (102) strains, only 5 strains of S. aureus were confirmed as an MDR. Resistance obtained to a minimum of 5 antibiotics was considered as MDR.
4. In the present study Turmeric, Garlic, Tulsi, Pudina, and Curry leaves exhibited antimicrobial activity against MDR strains of S. aureus. Which ethanolic extract of Turmeric showed greater inhibitory activity than all other plants at all selected concentrations (100, 50, and 25mg per 100µl of 5% DMSO) of extract.
5. Ethanolic extracts of all selected plants except Garlic showed greater antimicrobial activity than aqueous extracts at all concentrations (100, 50, 25mg). Ethanolic and aqueous extracts of Garlic showed almost similar antimicrobial activity.
6. In the present study, it was observed that the MIC and MBC values of all the ethanolic extracts of plants except Garlic were lower than aqueous extracts and the final MIC values of all the extracts of plants were similar to that of the MBC values.
7. Based on promising *in-vitro* assay findings, Turmeric, Garlic, Tulsi, Pudina and Curry leaves were likely to be the best substances for antibacterial treatment of MDR S. aureus infection. However, further studies are needed for a better understanding of the antimicrobial effects of Turmeric, Garlic, Tulsi, Pudina, and Curry leaves.

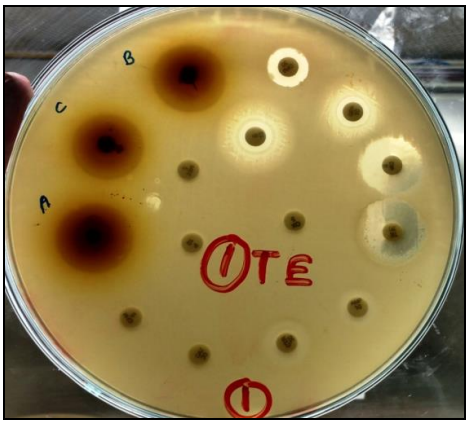

Fig 3: Antimicrobial activity of ethanolic extract of Tulsi against resistant strain of *S. aureus* (1)

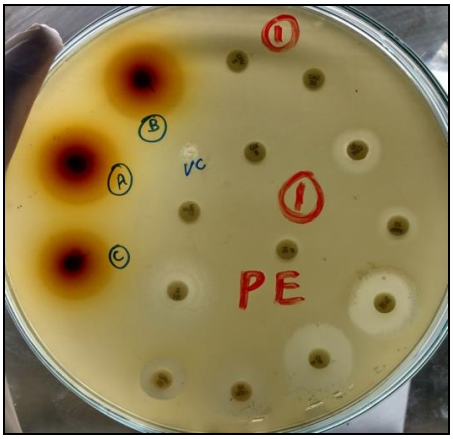

Fig 4: Antimicrobial activity of ethanolic extract of Pudina against resistant strain of *S. aureus*

Conclusion

From the present study, It was concluded that 41.18%, of which 28.68% and 12.50% cases were suffered from SCM and clinical mastitis respectively. The ethanolic extracts of all selected plants except Garlic showed greater antimicrobial activity than aqueous extracts at all concentrations (100, 50, 25mg). Ethanolic and aqueous extracts of Garlic showed almost similar antimicrobial activity and MIC and MBC values of all the ethanolic extracts of plants except Garlic were lower than aqueous extracts.

References

1. Abera M, Demie B, Aragaw K, Regassa F, Regassa A. Isolation and identification of Staphylococcus aureus from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. J Vet Med Anim Health. 2010;2(3):29-34.
2. Adesola AE. Antimicrobial resistance pattern of streptococci and staphylococci isolated from cases of bovine clinical mastitis in Nigeria. Nat Sci. 2012;10(11):96-101.
3. Addis M, Pal M, Kyule MN. Isolation and identification of Staphylococcus species from raw bovine milk in Debre Zeit, Ethiopia. Medwell Journals. 2011;4(2):45-49.
4. Afrose R, Saha SK, Banu LA, Ahmed AU, Shahidullah AS, Gani A, *et al*. Antibacterial effect of Curcuma longa (Turmeric) against Staphylococcus aureus and Escherichia coli. Mymensingh Med J. 2015;24(3):506-515.
5. Anjeza C, Mandal S. Synergistic or additive antimicrobial activities of Indian spice and herbal extracts against pathogenic, probiotic and food-sp. Int Food Res J. 2012;19(3):1185-1191.
6. Argal MS, Kumar S, Choudhary HS, Thakkar RM, Verma SK, Seniya C. The efficacy of *Murraya koenigii* leaf extract on some bacterial and a fungal strain by disc diffusion method. J Chem Pharm Res. 2011;3(5):697-704.
7. Avato P, Bucci R, Tava R, Vitali C, Rosato A, Bilay Z, *et al*. Antimicrobial activity of saponins from Medicago sp.: structure activity relationship. Phytotherapy. 2006;40(6):454-457.
8. Baskaran C, Ratha bai V, Kanimozhi D. Screening for antimicrobial activity and phytochemical analysis of various leaf extract of *Murraya koenigii*. IJRAP. 2011;2(6):1807-1810.
9. Bansode DS, Chavan MD. Phytochemical screening and antibacterial activity of curry (*Murraya koenigii*) leaf extracts against enteric pathogens. World J Pharm Sci. 2014;2(9):1075-1079.
10. Britto AJD, Sebastian SR, Sujin RM. Antibacterial activity of selected species of Lamiaceae against human pathogens. IJNPR. 2012;3(3):334-342.
11. Dwivedi D, Khandelwal G, Patidar RK, Singh V. Antimicrobial activity of *Mentha arvensis* against clinical isolates of human cariogenic pathogens - *in vitro* study. IJPSR. 2012;3(5):1355-1360.
12. Evans CE, Banso A, Samuel OA. Efficacy of some nupe medicinal plants against Salmonella typhi: an *in vitro* study. J Ethnopharmacol. 2002;80:21-24.
13. Gangle BJ. Sources and occurrence of antibiotic resistance in the environment. University of Maryland, College Park. 2005.
14. Islam MA, Islam MZ, Islam MA, Rahman MS, Islam MT. Prevalence of subclinical mastitis in dairy cows in selected areas of Bangladesh. Bangl J Vet Med. 2011;9(1):73-78.
15. Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J. *In vitro* antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and Candida species from Nigeria. J Med Food. 2004;7(3):327-333.
16. Ismail M, Essam TM, Mohamed AF, Mourad FE. Screening for the antimicrobial activities of alcoholic and aqueous extracts of some common spices in Egypt. Int J Microbiol Res. 2012;3(3):200-207.
17. Jahan F, Lawrence R, Kumar V, Junaid M. Evaluation of antimicrobial activity of plant extracts on antibiotic susceptible and resistant Staphylococcus aureus strains. J Chem Pharm Res. 2011;3(4):777-789.
18. Joe MM, Jayachitra J, Vijayapriya M. Antimicrobial activity of some common spices against some human pathogens. J Med Plant Res. 2009;3(11):1134-1137.
19. Karuppiah P, Rajaram S. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian Pac J Trop Biomed. 2012;2(8):597-601.
20. Kurjogi MM, Kaliwal BB. Prevalence and antimicrobial susceptibility of bacteria isolated from bovine mastitis. Adv Appl Sci Res. 2011;2(6):229-235.
21. Kurjogi MM, Kaliwal BB. Epidemiology of bovine mastitis in cows of Dharwad District. Int. Scholarly Res Notices; c2014. p. 1-9.
22. Khusro A, Aarti C, Preetamraj JP, Panicker SG. *In vitro* studies on antibacterial activity of aqueous extracts of spices and vegetables against *Bacillus licheniformis*

strain 018 and *Bacillus tequilensis* strain ARMATI. Int. J Curr Microbiol App Sci. 2013;2(9):79-88.
23. Li J, Zhou H, Yuan L, He T, Hu S. Prevalence, genetic diversity, and antimicrobial susceptibility profiles of *Staphylococcus aureus* isolated from bovine mastitis in Zhejiang Province, China. J Zhejiang Univ. Sci. B. 2010;10(10):753-760.
24. Meh K, Talukder M, Anower A. Prevalence of subclinical mastitis and its association with bacteria and risk factors in lactating cows of Barisal district in Bangladesh. Int. J Biol Res. 2014;2(2):35-38.
25. Nascimento EMM, Rodrigues FFG, Campos AR, Costa JGM. Phytochemical prospection, toxicity and antimicrobial activity of *Mentha arvensis* (Labiatae). J Young Pharm. 2009;1(3):210-213.
26. Mathur A, Dua VK, Prasad GBKS. Antimicrobial activity of leaf extracts of *Murraya koenigii* against aerobic bacteria associated with bovine mastitis. IJCEPr. 2010;1(1):12-16.
27. Mun SH, Joung DK, Kim YS, Kang OH, Kim SB, Seo YS, *et al*. Synergistic antibacterial effect of curcumin against methicillin-resistant *Staphylococcus aureus*. NCBI. 2013;20(8-9):714-718.
28. Mishra N, Behal KK. Antimicrobial activity of some spices against some microbes. Inter J Phar Pharmaceuti Sci. 2010;2(3):187-196.
29. Mukhtar S, Ghori I. Antibacterial activity of aqueous and ethanolic extracts of garlic, cinnamon and turmeric against *Escherichia coli* ATCC 25922 and bacillus subtilis DSM 3256. Int. J Appl. Biol. Pharm Technol. 2012;3(2):131-136.
30. Pei SJ. Ethnobotanical approaches of traditional medicine studies: Some experiences from Asia. Pharm Biol. 2001;39:74-79.
31. Prabhu KN, Ruban WS, Kumar GSN, Sharada R, Padalkar RD. Subclinical mastitis in buffaloes: Prevalence, isolation and antimicrobial resistance of *S. aureus*. Buffalo Bull. 2015;34(2):215-222.
32. Sumathi BR, Veeregowda BM, Gomes AR. Prevalence and antibiogram profile of bacterial isolates from clinical bovine mastitis. Vet World. 2008;1(8):237-238.
33. Tenhagen BA, Koster G, Wallmann J, Heuwieser W. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J Dairy Sci. 2006;89:2542-2551.