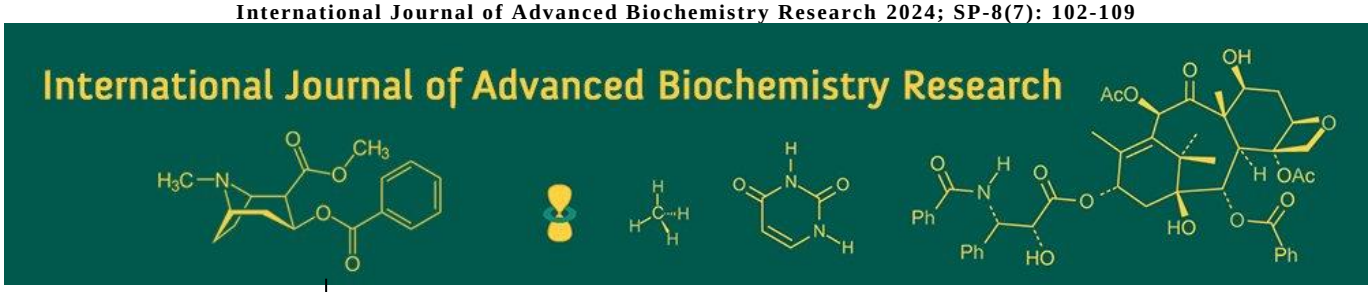

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(7): 102-109
www.biochemjournal.com
Received: 23-04-2024
Accepted: 30-05-2024

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Antimicrobial activity of six different plant extracts against clinical isolates of *Staphylococcus* spp. from milk origin

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DOI: https://doi.org/10.33545/26174693.2024.v8.i7Sb.1456

Abstract
Two aqueous and four methanolic extracts from six Indian medicinal plants, which are known for its antibacterial activity, were investigated for their ability to inhibit clinical isolates of *Staphylococcus* spp. from milk origin. The antibacterial activity of the extracts against *Staphylococcus* spp. was evaluated by Disk diffusion method and Minimum Inhibitory Concentration (MIC). The plant extracts from *Piper nigrum (seed)*, *Kigali pinnata* DC. (fruit), *Azadirchata indica* (leaves), *Cymbopogon citrates* (leaves), *Tagetes erecta* (leaves) and *Emblica officinalis Gaertn*. (fruit) showed antibacterial activity with an inhibition zone size of 8.10±0.40 mm, 10.02±0.19 mm, 9.98±0.21 mm, 10.60±0.15 mm, 12.02±0.14 mm and 7.71±0.12 mm, respectively against all the isolates. The antibacterial potency of plant extracts was determined in terms of MIC by the micro broth dilution method. The extracts from the leaves of *Tagetes erecta* showed better activity against all the isolates followed by *Cymbopogon citratus*, *Kigellia pinnata* DC., *Azadirachta indica*, *Piper nigrum* and *Emblica officinalis Gaertn*., respectively. MIC and Minimum Bactericidal Concentration (MBC) values, of the plant extracts, ranged from 16.96±0.00 to 200.00±0.00 mg/ml and 22.32±0.00 to 257.14±0.00 mg/ml respectively, against all the isolates. According to the results of the current investigation, each extract exhibited antibacterial activity and offers hope for the creation of novel drugs with significant biomedical applications and may be investigated further to identify its bioactive component.

Keywords: Soxhlet apparatus, antibacterial activity, plant extracts, antibiotics, disk diffusion assay, minimum inhibitory concentration, minimum bactericidal concentration

1. Introduction
Since the dawn of time, thousands of years ago, nature has been a source of medicinal agents (Tyler *et al*., 1988; Chopra and Doiphode, 2002) [28, 9]. Nearly all plants in India have therapeutic properties, and use of medicinal plants, particularly in traditional medicine, are now widely accepted (Clark, 1996) [6]. The presence of secondary metabolites such as alkaloids, tannins, saponins, flavonoids, anthraquinones, glycosides, volatile oils, terpenes, essential oils, and resins may account for the therapeutic benefit of the plants (Subramani *et al*., 2017) [25]. Around 70 to 80% populations in the developing countries use medicinal plants to treat diseases (Palhares *et al*., 2015) [21]. The World Health Organization states that one of the best sources for antibacterial drug is medicinal plants (Manandhar *et al*., 2019) [19]. In the last 20 years, research into various extracts made from conventionally used medicinal plants as possible sources of novel antimicrobial agents has gained more and more attention to combat the drug resistance (Charlandy *et al*., 1999) [7]. Antibacterial resistance to the medications has started to emerge as a result of the abuse and overuse of antibiotics in the treatment of diseases, and it has gotten worse due to selective pressure (Bhalodia and Shukla, 2011) [4]. *Staphylococcus* spp. one of the most common genus of bacteria frequently implicated in cases of bovine mastitis, has been documented multiple times as an antibiotic-resistant organism (Costa *et al*., 1992; Taponen *et al*., 2006) [5, 30]. Because of the emergence of resistant bacterial strains and limited discovery of novel antimicrobial compound (Yadav and Agarwal, 2011) [31], for the global management of highly contagious and recurrent infectious diseases necessitates the development of novel medicines, especially derived from naturally occurring plant metabolites. This study aimed to evaluate antibacterial activities of aqueous and methanolic extracts from six different plants *viz*., *Piper nigrum* (seed), *Kigellia pinnata* DC. (fruit), *Azadirachta indica* (leaves), *Cymbopogon citrate* (leaves),

Tagetes erecta (leaves) and *Emblica officinalis Gaertn.* (fruit) against clinical isolates of *Staphylococcus* spp. from milk origin.

2. Materials and Methods

2.1 Plants extraction preparation

2.1.1 Aqueous plant extracts preparation

The aqueous extracts of *Piper nigrum* (seed) and *Kigellia pinnata* DC. (fruit) are prepared using distilled water. Seeds and fruits were collected from in and around Indian Veterinary Research Institute, Bareilly, Uttar Pradesh and subsequently dried and crushed them into a powdered form. For preparation of the aqueous extracts, 50 g of dried plant materials were weighed and extracted with 300 ml of distilled water in a Soxhlet apparatus. The extracted material was then concentrated under reduced pressure and stored at 4 °C for later use (Muhammad *et al*., 2019) [20].

2.1.2 Preparation of methanolic plant extracts

Methanol (40%) was used to make methanolic extracts from dried leaves of *Azadirachta indica*, *Cymbopogon citratus*, *Tagetes erecta*, and the fruit of *Emblica officinalis Gaertn*. Fresh and disease-free leaves and fruit were collected from in and around Indian Veterinary Research Institute, Bareilly, Uttar Pradesh and then dried and crushed them into a powdered form in a grinder. 50 g of dried plant materials were extracted with 300 ml of methanol (40%) in a Soxhlet apparatus and subsequently concentrated under reduced pressure and stored in 4 °C for further use (Imran *et al*., 2021) [14].

2.2 Antibacterial activity of the plant extracts and commercially available antibiotics

2.2.1 Bacterial cultures

A total of 14 clinical *Staphylococcus* spp. isolates of mastitis milk origins were collected from Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly. All the isolates were subcultured and stored at −80 °C and maintained on Muller-Hinton agar (MHA) plates at 4 °C, and grown at 37 °C when required.

2.2.2 Preparation of the inoculums

The cultures were grown on Mueller Hinton broth (MHB) (Himedia, India) at 37 °C for 18 hours. The turbidity was adjusted to 0.5 McFarland standards.

2.2.3 Antibacterial activity of plants extract

2.2.3.1 Disk diffusion method

MHB was inoculated with isolates of *Staphylococcus* spp. and incubated at 37 °C overnight. Bacterial suspension was adjusted to 0.5 McFarland standards before use. As per Imran *et al*., (2021) [14] the inoculum was spread uniformly over the entire surface of the MHA plates. These plates were allowed to dry for few minutes and then a sterile filter paper disc of 6 mm diameter, to which different extracts at a concentration of 100 mg/ml were absorbed, and were placed, with the help of forceps. Commercially available antibiotic discs were used as positive control while methanol was used as vehicle control. The plates were incubated at 37 °C for 18- 24 hrs. Inhibitory zone diameters (mm) were measured with the help of scale. Each assay was repeated thrice, and the mean diameters were recorded.

2.2.3.2 Determination of Minimum Inhibitory Concentrations (MIC) of plants extract by

2.2.3.2.1 Micro Broth dilution method

The antimicrobial activity of extracts against all isolates was investigated at various concentrations in a 96-well microtiter plate. As per Sultana *et al*. (2016) [24], each extract was serially diluted (two-fold) upto 9th well using a sterile MHB medium, and the 10th, 11th and 12th wells serving as vehicle, media and culture controls, respectively. Each plant extract dilution was inoculated with 100 µl of log phase microorganism (0.5 McFarland). The 96-well microtiter plate was then kept at 37 °Celsius for 18 hours. The MIC value of the extract was recorded after 18 hours. Each extract was tested for antibacterial activity in triplicate Zihadi *et al*., 2019 [32].

2.2.3.2.2 Minimum Bactericidal Concentration (MBC)

The MBC was determined by collecting a loop full of broth from those wells which did not show any growth in MIC assay, two wells above and two wells below the MIC value are inoculated on sterile MHA plate by streaking and incubated at37 °C for 18- 24 hours. The highest dilution that did not yield a colony on a solid medium was considered as MBC Zihadi *et al*., 2019 [32].

2.2.4 Antibacterial activity with commercially available antibiotics

2.2.4.1 Disk diffusion test

A disc diffusion test was conducted in accordance with National Committee for Clinical Laboratory Standards (CLSI) guideline 2023 to determine the antibiotic susceptibility against all clinical isolates. Commercially available 6mm diameter antibiotic discs procured from Himedia were used in this study *viz.*, Gentamicin (10 mcg), Enrofloxacin (5 mcg), Tetracycline (30 mcg), Erythromycin (15 mcg) and Chloramphenicol (30 mcg). 0.5 McFarland bacterial suspension was seeded over the MHA plate followed by placement of the disc with the help of forceps. The plates were incubated at 37 °C for 18- 24 hrs. Zone of Inhibition (ZOI) in mm were measured with the help of scale. Each assay was repeated thrice, and the mean diameters were recorded.

2.2.4.2 MIC of Antibiotics

As per CLSI (2023) guideline the MIC of antibiotics was determined by first adding 100 µl of MHB to a 96-well sterile microtiter plate. Twofold dilution of antibiotics, from the first to the tenth well was done. The 11th and 12th wells are used for media and culture control respectively. Next, the microorganism suspension was diluted in MHB broth by a ratio of 1:100. Subsequently, the adjusted bacterial suspension was introduced into each well that had varying antibiotic concentrations. The microtiter plate was then incubated at 37 °C for 18 hours. MIC was defined as the concentration at which the organism did not appear to be growing. The bactericidal activity of each drug was examined in triplicate.

3. Results and discussion

3.1 Disk diffusion assay of plants extract

Six plant extracts from *Piper nigrum* (P), *Kigellia pinnata* DC. (K), *Azadirachta indica* (A), *Cymbopogon citrates*

(Cy), *Tagetes erecta* (T) and *Emblica officinalis Gaertn*. (E) are investigated to evaluate their antibacterial activity against clinical isolates of *Staphylococcus* spp. using disk diffusion method. Evaluation of antibacterial activity of these plant extracts are presented in Table 1 and graphically represented in Fig 1. The results revealed that all plant extracts were potentially effective in suppressing microbial growth with 100 mg/ml concentration. The highest zone of inhibition was observed with the T (12.02±0.14 mm) followed by Cy (10.60±0.15 mm), K (10.02±0.19 mm), A (9.98±0.21 mm), P (8.10±0.40 mm) and E (7.71±0.12 mm), respectively. Very less growth inhibition zone was seen with the extracts of E (7.71±0.12 mm). T (12.02±0.14 mm) was the most effective extract retarding microbial growth at concentration of 100 mg/ml. Similar studies was reported by Aldaly, 2010 [2]; Ewansiha *et al*., 2012 [12]; Latifian *et al*., 2021 [17] with extracts of *Piper nigrum*; *Cymbopogon citrates*; *Tagetes erecta* showing almost similar ZOI. On the other hand, Agyare *et al*., 2013 [3] studied the susceptibility of methanolic extracts of *Kigellia Africana* (leaf and stem) and found 14.50±0.50 mm ZOI which is slightly higher than our findings. This may be due to different range of activity with different parts of the plants as per Latifian *et al.*, 2021 [17] or may be due to extraction media (Gupta *et al*., 2019) [13]. Similarly, for *Emblica officinalis* extracts our finds for antimicrobial susceptibility is poor when compared to findings of Gupta *et al*., 2019 [13]. In contrast to our findings, Kumar *et al*., 2018 [16] observed methanolic extracts of *Azadirachta indica* that had lower ZOI.

3.2 MIC and MBC of the plant extracts

The MIC of six plant extracts was employed by micro broth dilution method**.** The mean MIC values of all plant extracts were reported in Table 2 and the graphical representation was shown in Fig 2. The lowest MIC was observed with the extract of T (16.96±0.00 mg/ml) followed by Cy (68.57±0.00 mg/ml), K (108.57±0.00 mg/ml), A (112.50±0.00 mg/ml), P (126.79±0.00 mg/ml), where as E, showed the highest MIC of 200.00±0.00 mg/ml, respectively. Out of all prepared plant extracts, *Tagetes erecta* shows a good antibacterial activity with least MIC value among all the prepared plant extracts against all the isolates followed by *Cymbopogon citratus*, *Kigellia pinnata* DC., *Azadirachta indica*, *Piper nigrum* and *Emblica officinalis Gaertn*., respectively. The MBC of the six plant extracts was determined by sub-culturing the well that exhibits no growth on a fresh media, two wells above and two wells below the MIC value. The MBC values of the plant extracts T, Cy, K, A, P, and E are 22.32±0.00 mg/ml, 90.00±0.00 mg/ml, 120.00±0.00 mg/ml, 143.75±0.00 mg/ml, 152.14±0.00 mg/ml, and 257.14±0.00 mg/ml, respectively. The MBC values are presented in Table 3 and graphically represented in Fig 3. The plant extracts of *Piper nigrum*, *Cymbopogon citrates* and *Emblica officinalis* exhibit lower antibacterial activity in terms of MIC and MBC value when compared to study of antibacterial activity of those of Aldaly 2010 [2], Ewansiha *et al*., (2013) [12] and Sharma and Pundir *et al*., 2018, respectively. This could be the result of the extraction medium (Gupta *et al*., 2019) [13] or the plant extracts themselves, which might still include impurities that would inhibit their function (Tereschuk *et al*., 2004) [29]. As a result, extraction in various media is necessary to determine which media performs best. Purification is also crucial since it concentrates the active component and increases its activity (Ewansiha *et al*., 2012) [12]. Extracts of *Kigellia pinnata* DC., *Azadirachta indica*, and *Tagetes erecta* were reported to have good antibacterial activity by Kimutai 2014 [15], Muhammad *et al*., 2019 [20], and Motamedi *et al*., 2015, respectively. However, our findings show that the plant extracts have very good antimicrobial activity with lower minimum inhibitory concentrations than their reporting. Several studies were done on P, K, A Cy, T and E plant extracts to determine their antimicrobial activity against *Staphylococcus aureus;* nevertheless, very few studies have been conducted on clinical isolates of *Staphylococcus* spp. from milk origin. In general, the prepared plant extracts exhibit a good antibacterial activity against the clinical isolates of *Staphylococcus* spp. However, studying a greater number of isolates as well isolation and purification are required in future to give a good antimicrobial picture by the plant extracts.

3.3 Susceptibility Test with antibiotics

To determination the susceptibility of microorganisms to antimicrobials, disk diffusion test and MIC test was performed. Disk diffusion test also commonly known as Kirby Bauer test was used to determine the susceptibility of the clinical isolates to different commercially available antibiotics. The results of disk diffusion test performed against 5 antibiotics *viz*., Gentamicin (Gen), Enrofloxacin (EX), Tetracycline (TE), Erythromycin (E) and Chloramphenicol (C) in freshly prepared MHA plates are given in Table 4. The mean growth inhibited zone is given in mm±standard error. Generally, all the tested isolates were found to be susceptible to extracts with variable zones of inhibition. Studies revealed ZOI for Gen, EX, TE, E and C were 14.69±0.27, 21.36±0.26, 20.64±0.33, 14.52±0.33 and 14.62±0.40, respectively against all the isolates of *Staphylococcus* spp. MIC is defined as the lowest concentration at which antimicrobial inhibits the visible growth of a microorganism after overnight incubation. MIC of 5 antibiotics was performed against all the isolates by micro broth dilution method in a 96 well microtiter plate. The mean MIC (µg/ml) value against all the isolates was presented in Table 5. Studies revealed that TE shows 78.57 % sensitivity, which is highest among all the antibiotics followed by Gen (57.14%), C (28.57%), whereas EX and E shows only 7.14% sensitivity. The intermediate sensitivity was observed against EX and C (28.57%), Gen and TE (7.14%). The isolates show highest resistance against E (92.86%), followed by EX (64.26%), C (42.86%), Gen (35.71%) and TE (14.29%), respectively. The percent susceptibility of the antibiotics against all isolates is graphically represented in Fig 4. Similar studies have been done by other workers like Sweeney *et al*., 2024; Pumipuntu *et al*., 2019; Ralbetli *et al*., 2016; Choi *et al*., 2012; Costa *et al*., 2000 [27, 22, 23, 10, 8] and Archer and Climo, 1994 [1]. They studied the antibiotic resistance pattern in *Staphylococcus* spp. of milk origin and found that most of the isolates were resistant to Erythromycin and sensitive to Tetracycline and Gentamicin.

Table 1: Zone diameter (mm) in Disk diffusion assay with six plant extracts against *Staphylococcus* spp.

Plant extracts	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean
P	10.00±0.00	8.00±0.00	9.33±0.27	7.33±0.27	9.67±0.27	5.00±2.05	8.33±0.27	7.00±0.00	5.33±2.18	9.00±0.00	10.00±0.00	7.00±0.00	10.00±0.00	7.33±0.27	8.10±0.40
K	11.33±0.54	10.33±0.27	10.00±0.00	9.00±0.00	10.33±0.27	9.00±0.00	8.67±0.27	9.00±0.47	10.00±0.00	11.00±0.00	11.67±0.27	9.67±0.27	11.33±0.27	9.00±0.00	10.02±0.19
A	10.67±0.27	10.33±0.27	10.00±0.00	8.67±0.27	10.00±0.00	9.00±0.00	8.33±0.27	9.33±0.27	10.33±0.27	10.67±0.27	11.33±0.54	10.00±0.00	11.67±0.27	9.33±0.27	9.98±0.21
Cy	12.00±0.00	10.67±0.27	11.00±0.00	10.00±0.47	10.33±0.27	9.67±0.27	9.67±0.27	9.67±0.27	10.33±0.27	12.00±0.00	12.00±0.00	10.00±0.00	12.00±0.00	9.00±0.00	10.60±0.15
T	14.33±0.27	12.00±0.00	14.00±0.00	10.67±0.27	14.00±0.00	10.67±0.27	10.00±0.00	10.67±0.27	10.67±0.27	13.00±0.00	12.67±0.27	10.67±0.27	15.00±0.00	10.00±0.00	12.02±0.14
E	9.00±0.00	7.00±0.00	8.33±0.27	7.00±0.00	8.33±0.27	7.33±0.27	7.00±0.00	7.00±0.00	7.00±0.00	8.67±0.27	8.67±0.27	7.00±0.00	8.67±0.27	7.00±0.00	7.71±0.12

Values are expressed as Mean±Standard Error. Experiments were performed in triplicates.
P = *Piper nigrum*, K = *Kigellia pinnata* DC., A = *Azadirachta indica*, Cy = *Cymbopogon citrates*,T = *Tagetes erecta* and E = *Emblica officinalis Gaertn*.

Table 2: Determination of MIC (mg/ml) with six plant extracts against clinical isolates of *Staphylococcus* spp.

Plant Extracts	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean
P	88.75±0.00	88.75±0.00	88.75±0.00	177.50±0.00	88.75±0.00	177.50±0.00	88.75±0.00	177.50±0.00	177.50±0.00	88.75±0.00	88.75±0.00	177.50±0.00	88.75±0.00	177.50±0.00	126.79±0.00
K	40.00±0.00	160.00±0.00	40.00±0.00	160.00±0.00	80.00±0.00	160.00±0.00	160.00±0.00	80.00±0.00	80.00±0.00	80.00±0.00	80.00±0.00	160.00±0.00	80.00±0.00	160.00±0.00	108.57±0.00
A	87.50±0.00	87.50±0.00	87.50±0.00	87.50±0.00	87.50±0.00	87.50±0.00	175.00±0.00	87.50±0.00	87.50±0.00	175.00±0.00	87.50±0.00	175.00±0.00	87.50±0.00	175.00±0.00	112.50±0.00
Cy	30.00±0.00	60.00±0.00	30.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	120.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	60.00±0.00	120.00±0.00	60.00±0.00	120.00±0.00	68.57±0.00
T	6.25±0.00	12.50±0.00	6.25±0.00	12.50±0.00	12.50±0.00	12.50±0.00	12.50±0.00	12.50±0.00	12.50±0.00	12.50±0.00	12.50±0.00	50.00±0.00	12.50±0.00	50.00±0.00	16.96±0.00
E	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00

Values are expressed as Mean±Standard Error. Experiments were performed in triplicates.
P = *Piper nigrum*, K = *Kigellia pinnata* DC., A = *Azadirachta indica*, Cy = *Cymbopogon citrates*, T = *Tagetes erecta* and E = *Emblica officinalis Gaertn*.

Table 3: MBC value in mg/ml with six plant extracts against clinical isolates of *Staphylococcus* spp.

Plant Extracts	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean
P	177.50±0.00	177.50±0.00	88.75±0.00	177.50±0.00	88.75±0.00	177.50±0.00	177.50±0.00	177.50±0.00	177.50±0.00	88.75±0.00	177.50±0.00	177.50±0.00	88.75±0.00	177.50±0.00	152.14±0.00
K	40.00±0.00	160.00±0.00	40.00±0.00	160.00±0.00	80.00±0.00	160.00±0.00	160.00±0.00	160.00±0.00	160.00±0.00	80.00±0.00	80.00±0.00	160.00±0.00	80.00±0.00	160.00±0.00	120.00±0.00
A	87.50±0.00	175.00±0.00	87.50±0.00	175.00±0.00	87.50±0.00	175.00±0.00	175.00±0.00	175.00±0.00	175.00±0.00	175.00±0.00	87.50±0.00	175.00±0.00	87.50±0.00	175.00±0.00	143.75±0.00
Cy	30.00±0.00	120.00±0.00	30.00±0.00	120.00±0.00	60.00±0.00	120.00±0.00	120.00±0.00	120.00±0.00	120.00±0.00	60.00±0.00	60.00±0.00	120.00±0.00	60.00±0.00	120.00±0.00	90.00±0.00
T	6.25±0.00	25.00±0.00	6.25±0.00	25.00±0.00	12.50±0.00	25.00±0.00	25.00±0.00	25.00±0.00	25.00±0.00	12.50±0.00	12.50±0.00	50.00±0.00	12.50±0.00	50.00±0.00	22.32±0.00
E	200.00±0.00	200.00±0.00	200.00±0.00	400.00±0.00	200.00±0.00	200.00±0.00	400.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	200.00±0.00	400.00±0.00	200.00±0.00	400.00±0.00	257.14±0.00

Values are expressed as Mean±Standard Error. Experiments were performed in triplicates.
P = *Piper nigrum*, K = *Kigellia pinnata* DC., A = *Azadirachta indica*, Cy = *Cymbopogon citrates*,T = *Tagetes erecta* and E = *Emblica officinalis Gaertn*.

Table 4: ZOI diameter (mm) in Disk diffusion test with antibiotics against clinical isolates of *Staphylococcus* spp.

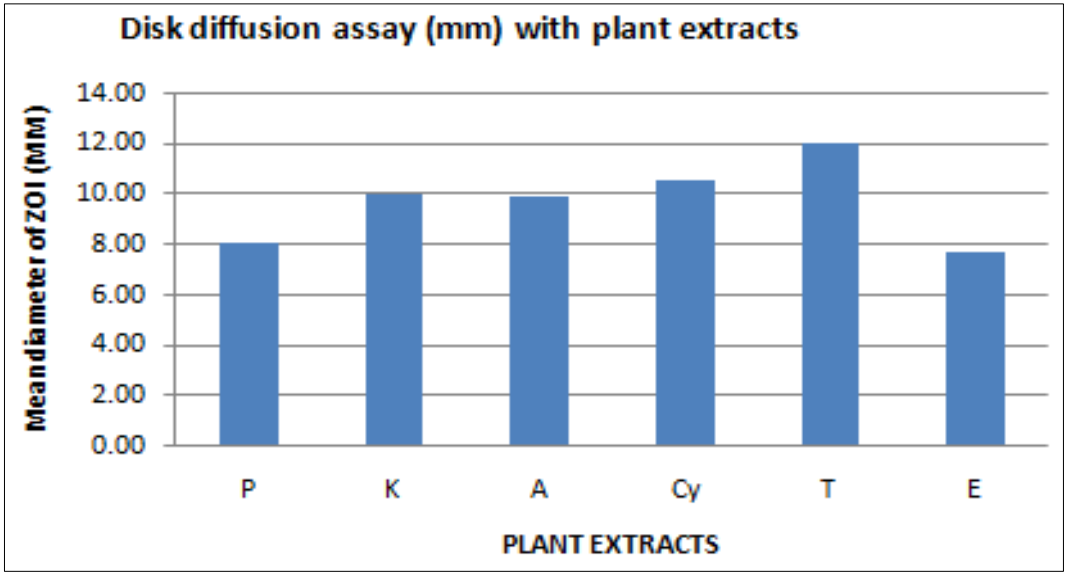
Antibiotics	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gentamicin (GEN) 10 mcg	14.67±0.33	20.33±0.33	14.33±0.33	15.00±0.00	12.67±0.67	24.00±0.58	11.67±0.33	15.67±0.33	15.33±0.33	12.00±0.00	12.00±0.00	11.00±0.58	15.00±0.00	12.00±0.00
Enrofloxacin (EX) 5 mcg	25.33±0.33	25.33±0.33	24.67±0.33	20.33±0.33	14.33±0.33	24.67±0.33	26.67±0.33	24.33±0.33	26.33±0.33	17.00±0.00	15.67±0.33	15.00±0.00	24.33±0.33	15.00±0.00
Tetracycline (TE) 30 mcg	19.33±0.33	18.67±0.33	20.33±0.33	18.67±0.33	27.00±1.00	14.67±0.33	19.00±0.00	22.00±0.00	18.67±0.33	18.00±0.00	23.33±0.33	26.00±1.00	25.00±0.00	18.33±0.33
Erythromycin (E) 15 mcg	12.67±0.33	13.33±0.33	10.33±0.33	15.33±0.33	13.67±0.33	17.00±1.00	21.67±0.33	10.67±0.33	12.33±0.33	13.33±0.33	12.67±0.33	13.00±0.00	24.33±0.33	13.00±0.00
Chloramphenicol (C) 30 mcg	18.33±0.33	12.00±0.00	21.67±0.33	17.67±0.33	12.33±0.33	12.33±0.33	18.33±0.33	13.00±0.58	16.33±0.33	13.33±0.33	12.67±0.67	12.33±0.33	12.00±1.00	12.33±0.33

Values are expressed as Mean±Standard Error. Experiments were performed in triplicates.

Table 5: Determination of MIC (µg/ml) along with antibiotics against clinical isolates of *Staphylococcus* spp.

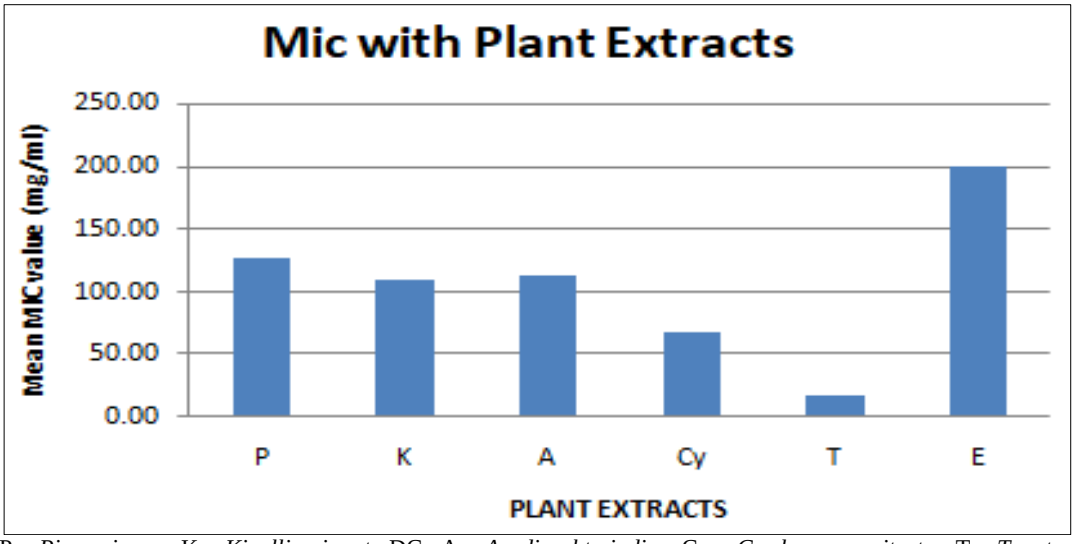
Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gentamicin (GEN)	0.50±0.00	1.00±0.00	0.50±0.00	0.50±0.00	64.00±0.00	2.00±0.00	64.00±0.00	0.25±0.00	0.25±0.00	64.00±0.00	64.00±0.00	8.00±0.00	1.00±0.00	64.00±0.00
Enrofloxacin (EX)	0.50±0.00	32.00±0.00	2.00±0.00	2.00±0.00	64.00±0.00	4.00±0.00	16.00±0.00	2.00±0.00	2.00±0.00	64.00±0.00	64.00±0.00	64.00±0.00	8.00±0.00	64.00±0.00
Tetracycline (TE)	2.00±0.00	4.00±0.00	4.00±0.00	2.00±0.00	4.00±0.00	16.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	8.00±0.00	2.00±0.00	1.00±0.00	4.00±0.00	64.00±0.00
Erythromycin (E)	32.00±0.00	64.00±0.00	64.00±0.00	64.00±0.00	64.00±0.00	16.00±0.00	16.00±0.00	16.00±0.00	16.00±0.00	32.00±0.00	32.00±0.00	64.00±0.00	0.50±0.00	64.00±0.00
Chloramphenicol (C)	1.00±0.00	32.00±0.00	16.00±0.00	16.00±0.00	64.00±0.00	32.00±0.00	4.00±0.00	16.00±0.00	16.00±0.00	8.00±0.00	32.00±0.00	32.00±0.00	4.00±0.00	32.00±0.00

Values are expressed as Mean±Standard Error. Experiments were performed in triplicates.



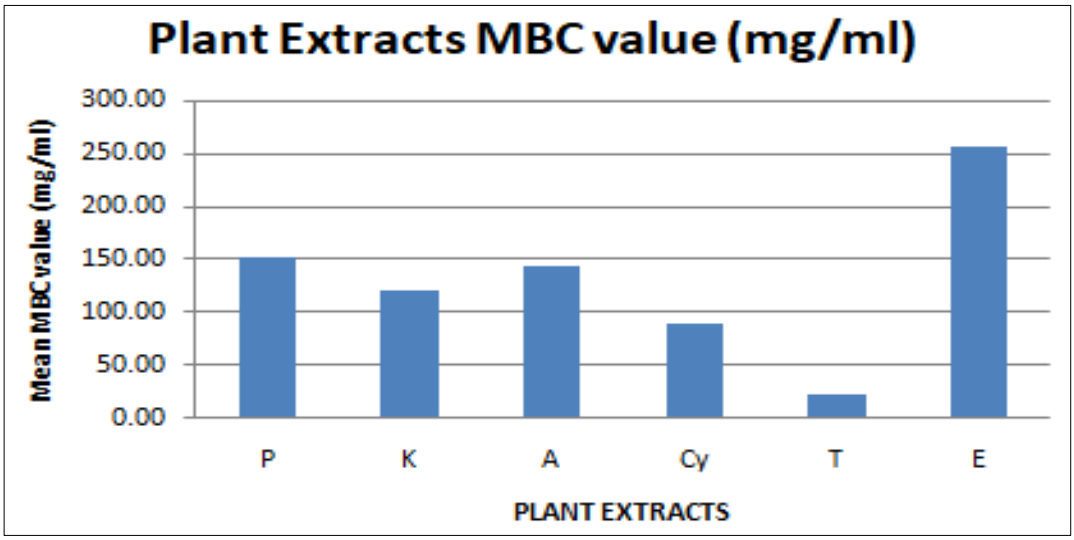
P = *Piper nigrum*, K = *Kigellia pinnata* DC., A = *Azadirachta indica*, Cy = *Cymbopogon citrates*, T = *Tagetes erecta* and E = *Emblica officinalis Gaertn*.
The highest zone of inhibition was observed with the T (12.02±0.14 mm) followed by Cy (10.60±0.15 mm), K (10.02±0.19 mm), A (9.98±0.21 mm), P (8.10±0.40 mm) and E (7.71±0.12 mm), respectively

Fig 1: Graphical representation of Disk diffusion assay with six plant extracts



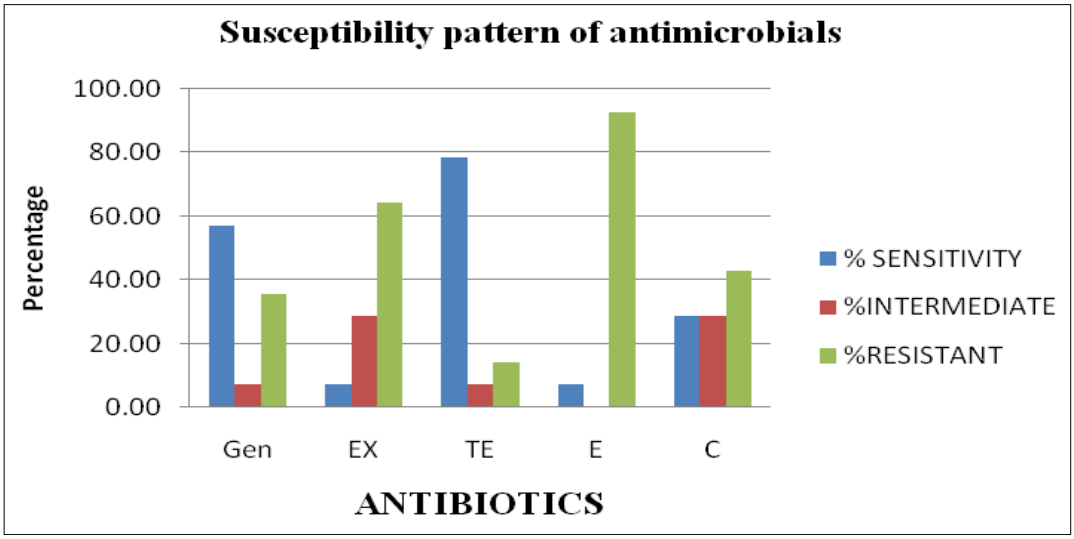
P = *Piper nigrum*, K = *Kigellia pinnata* DC., A = *Azadirachta indica*, Cy = *Cymbopogon citrates*, T = *Tagetes erecta* and E = *Emblica officinalis Gaertn.*
T shows a good antibacterial activity with least MIC value followed by Cy, K, A,P and E, respectively.

Fig 2: Graphical representation of MIC (mg/ml) with six plant extracts



P = *Piper nigrum*, K = *Kigellia pinnata* DC., A = *Azadirachta indica*, Cy = *Cymbopogon citrates*, T = *Tagetes erecta* and E = *Emblica officinalis Gaertn*

Fig 3: Graphical representation of MBC (mg/ml) with six plant extracts against clinical isolates of *Staphylococcus* spp.



Sensitive = TE (78.57%); Gen (57.14%); C (28.57%): EX and E (7.14%). Intermediate = EX and C (28.57%), Gen and TE (7.14%). Resistant = E (92.86%); EX (64.26%), C (42.86%), Gen (35.71%) and TE (14.29%).

Fig 4: Percent susceptibility of antimicrobials against all the clinical isolates of *Staphylococcus* spp.

4. Conclusion

The study's findings show that the prepared plant extracts exhibit strong antibacterial activity against *Staphylococcus* spp. clinical isolates from milk origin. They can therefore be used to exploit for novel drugs in near future. More isolates, as well as the isolation and purification of the phytochemical compound using different extraction media, may be studied in future research.

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