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To Study Antimicrobial Activity of Aloe Vera Extract on Staphylococcus Aureus and Escherichia Coli

[1]Snehal Wagh *Dr. Samir Pachpute [2]

1. Tutor, Department of Microbiology, Dr. D Y Patil Medical College, Hospital & Research Centre and Dr. D Y Patil Vidyapeeth
2. Professor, Department of microbiology, MGM Medical College, Navi Mumbai-410218

***Corresponding Author**

*Dr. Samir Pachpute, Professor, Department of microbiology, MGM Medical College, Navi Mumbai-410218

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KEYWORDS

aloe vera, antibacterial, extract, skin infection, zone inhibition

ABSTRACT:

Aloe vera is reputed to have medicinal properties. For centuries, it has been used for an array of ailments such as mild fever, wounds and burns, gastrointestinal disorders, diabetes, sexual vitality and fertility problems to cancer, immune modulation, AIDS and various skin infections. In present study, antibacterial activity of aloe vera gel extracts was tested against some common skin infection pathogens, that is, Escherichia coli, Shigella, Salmonella spp. and Staphylococcus aureus all were recorded positive. Antibiotic resistance and susceptibility pattern of above isolates were also studied against 10 clinically significant antibiotics (ampicillin [AMC], amoxicillin, augmentin, cefotaxime, ceftazidime [CAZ], cefuroxime [CXM], ciprofloxaci, tetracycline, cefpodoxime and imipenem). AMC and CXM were found to be most effective antibiotic followed by CXM with highest efficacy against Gram-negative bacteria. In case of CAZ showed highest efficacy was showed against Gram-positive bacteria. Aloe vera leave gel was extracted with four different solvent-like aloe vera leaf extract, root extract, leaf ethanol extract and root ethanol extract; however, Gram-negative as well Gram-positive isolates was found highest susceptibility with aloe leaf and aloe root ethanol extract. Moderate sensitivity observed with aloe leaf extract and aloe root extract against both Gram-positive as well as Gram-negative bacterial isolates. This result showed that ethanol extracts of aloe vera both leaf and root can be used alongside conventional antibiotics to fight agents of infections that are so prevalent in the skin infection.

Introduction

The widespread use of antibiotics, selection pressure on bacterial strains, and a lack of new vaccines, drugs and diagnostic tools have all contributed to multi-drug resistance becoming a global concern. These flaws have promoted a worldwide request for new antimicrobial medications, especially those derived from natural resources. [(1)] In recent years, medicinal plant research has gotten a lot of attention all over the world. A large body of evidence has developed to show the promising potential of medicinal plants employed in many traditional, complementary, and alternative methods of disease treatment. [(2)] The majority of the medicinal plants studied showed antibacterial activities after being analysed for physiologically active components known to have pharmacological characteristics. [(3)] As a result of pathogenic organisms developing resistance to antibiotics, consumer demand, side effects, and scientists are focusing their efforts on extracting, analysing, and developing potent active ingredients for use in drugs, topical products, herbal supplements, and various surfactants for internal use. [(3)] Aloe vera (Aloe barbadensis miller) is a succulent plant that belongs to the Liliaceae family and has a whorl of elongated, pointed leaves. The term comes from the Arabic word 'alloeh,' which meaning 'bitter,' and refers to the bitterness of the liquid contained in the leaves. It features stiff green lance-shaped leaves with a centre

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mucilaginous pulp that contains transparent gel. The leaves can retain a lot of water in extremely hot and dry areas. When the green epidermis of a leaf is cut, a clear mucilaginous fluid appears, including water, chemicals and a fibre that helps the leaf keep its moisture. [4] Native Americans have traditionally used aloe to treat stomach and intestinal aliments such as haemorrhoid, constipation, colitis and colon difficulties .It is said to be natural cleaner, effective in penetrating tissues, releasing pain associated with joints and muscles ,a powerful antibiotic, virucidal, bactericidal when in contact with bacteria for long periods of time ,fungicidal ,antiinflammatory and instrumental in increasing circulation to the area, breaking down and reabsorbing dead tissue, and moisturising tissues. Aloe vera appears to assist pores open and accept moisture and nutrients from the plants by absorbing up to three times faster than water. Also, aloe vera's ingredients have been shown to improve the body's immune system performance. [5] It is rich in nutritive and antipathogenic chemicals. The lead pulp and exudates of the aloe vera plant have been shown to have over 200 chemical components. [3] The gel has 99.3 percent water and 0.7 percent solids, with mannose and glucose accounting for the majority of the solids. Aloesin, aloemannan, anthraquinones, aloeride, acemannan, verectin, carbohydrates, enzymes, flavonoids, saponins, glycoprotein, vitamins, amino acid are some of the active components in aloe vera. [1] The antimicrobial efficiency of Aloe gel has been found to be effective against both Gram-positive and Gram-negative microorganisms. Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi have all been found to be killed, considerably reduced, or eliminated by the antimicrobial compounds in aloe vera gel [5] . So, in a view of this, we have conducted a study to show the efficacy of Aloe vera plant extract on Gram positive and Gram-negative bacteria isolated from the clinical samples in a tertiary care hospital Navi Mumbai.

Staphylococcus aureus

Staphylococcus aureus is a common bacterial human infection that can cause a range of clinical manifestation. S. aureus can be found in the environment as well as in the normal flora of humans [6]. Skin and the nasopharynx are the natural habitats of S. aureus in humans. [7] Direct touch is the most common method of transmission. Other infection transmission techniques, on the other hand, are used in some cases. [6] Infections are widespread in both community and hospital settings, and treatment is difficult to manage because of the growth of multi-drug resistant forms like MRSA (Methicillin-Resistant Staphylococcus aureus). S. aureus does not generally cause infection on healthy skin, but if it enters the bloodstream or internal tissues, these bacteria can cause a variety of potentially fatal illnesses. [7].

Escherichia coli

E. coli are gram negative and facultative anaerobic bacillus normally present as a commensal in the lower intestine of many warm-blooded organisms. [8] E. coli constitute up to 1% of the population of gut flora of mammals and is one of the most prominent commensals. It also acts as an important human pathogen causing not only intestinal but also extra-intestinal infections. It has fecal-oral route of transmission and enters in soil and water making them their secondary habitats. The bacterium can persist only for a short span of time in these habitats and hence considered as an ideal indicator organism in environment especially water for detection of fecal contamination. [9]

Material and Methods

Place of study: Central Laboratory,Department of Microbiology MGM Medical College and Hospital, Kamothe, Navi Mumbai.

Period of study: February 2021 to February 2022.

Type of study: Experimental study and observational prospective

study Inclusion criteria: - Adult patient admitted in the hospital with history of fever, cough, wound or UTI or any source of bacterial infection.

Exclusion Criteria: Patient not willing to participate in the study.

Sample size: All non- repetitive pathogenic E. coli (50) and S. aureus (50). aureus samples were collected during the given study period. (Sample size: 100) All the samples were processed in biosafety cabinet taking strict personal protective measures.

Study group: OPD and IPD patient with various types of infections.

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Specimens

Urine culture and sensitivity 2. Sputum and throat swab culture and sensitivity 3. Blood culture and sensitivity 4. Frank pus/ pus swab culture and sensitivity 5. Body Fluids (C.S.F, Peritoneal Fluid, Pleural Fluid, Synovial Fluid) 6. Catheter tip/ Endotracheal tube secretions culture and sensitivity.

Maintenance of clinical isolates

Stock cultures were maintained in vials by growing the skin isolates in 3-ml nutrient broth and next day overlaying with 3 ml 40% glycerol. Vials were than frozen at −70°C. [10]

Determination of antibiotic resistance profile

Skin wound isolates were subjected to antibiotic resistance screening by disk diffusion method. For this purpose, inoculates were prepared by diluting overnight cultures in sterile sodium chloride (0.9%) suspension and then match with the 0.5 standard Mac Farland index. Bacterial suspensions were then plated onto Mueller-Hinton agar (Oxoid) and the commercially available antibiotic discs were placed on lawn of culture and the plates were incubated over night at 37°C. Sensitivity, intermediate sensitivity and resistances were determined by the zone of complete growth inhibition around each disk according to reference standards. The following antibiotic discs were used ampicillin (AMC) (10 µg), amoxicillin (AMX) (25 µg), augmentin (AUG) (30 µg), cefotaxime (CTX) (30 µg), ceftazidime (CAZ) (30 µg), cefuroxime (CXM) (30 µg), ciprofloxacin (CPX) (10 µg), tetracycline (30 µg), cefpodoxime (CP) (10 µg) and imipenem (10 µg).[10]

Collection of plant material: Plant of Aloe vera was collected from different places of New Mumbai and grown in Microbiology department, MGM Medical college and hospital and MGM Central Research Laboratory.

Preparation of plant extract: -The fully expanded leaves with gel of aloe vera were selected from the plant, washed with distilled water and were surface sterilized with 70% ethyl alcohol, air dried and crushed to small pieces and powdered in an electric grinder. -Extraction was done with a methanol using hot continuous extraction (Soxhlet) method. - 40g of powdered material was weighted and packed in muslin cloth and mixed with 100ml of methanol. -The packed material was placed inside the extractor. -The tip of the extractor was fixed to round bottom flask placed on a mantel. Condenser was fixed at the top of the extractor. -The aloe vera plant was extracted with methanol for about 40 - 50 complete cycles maintaining a constant temperature of about 45 - 50°C. -After complete solvent evaporation 20mg (0.02g/ml) and 40mg (0.04g/ml) leaf extract were dissolved in 1% DMSO.[11]

Disc Preparation: The 6mm (diameter) discs were prepared from whatmann No. 1 filter Paper the discs were sterilized by autoclave at 12°C. After the sterilization the moisture discs were dried on hot air oven at 50°C.Then various solvent extract discs and control discs were prepared.[11]

Source of tested organism: Microorganisms selected for this study were- E. coli, S. aureus, Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922. Tested sample were inoculated with 5mm nichrome wire loop, which carries 0.01ml of sample on Blood agar (BA) and MacConkey agar (MAC). The plates were incubated for overnight at 37 degree C.

Inoculums: Plates were incubated within 15 minutes of preparation of the suspension (peptone broth) so that the density does not change. A sterile cotton swab was dipped into the suspension and surplus removed by rotation of the swab against the side of the tube above the fluid level. The medium was inoculated by even streaking of the swab over the entire surface of the Mueller-Hinton agar plate in three directions.

➢ Incubation: Plates were incubated for 24hrs at 37 degree Celcius aerobically.

➢ Reading of zone of inhibition: The zones of inhibition were then read as resistant and sensitive using calibrated ruler and compared with the standard chart, Disc diffusion method.

➢ Antimicrobial activity of Aloe vera extract: Antibacterial activity of whole leaf extracts of aloe vera was detected as zone of inhibition in mm using kirby-Bauer disk diffusion method. Sterile disc of 6 mm was impregnated by soaking with methanolic extract having different concentrations (20mg/ml and 40mg/ml of 1% DMSO).[12]

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Test Control: Sterile disc impregnated with Methanol compound were used as test control. The diameter of Zone of inhibition was measured and the mean was recorded.

Antibiotics sensitivity testing:

Disk diffusion method (Kirby-bauer test) Antibiotic susceptibility testing was done by Kirby- Bauer's disc diffusion method. The principle behind this being antibiotic impregnated discs absorb moisture from the agar and the antibiotic diffuses in the agar medium. Visible growth of bacteria occurs on the surface where the concentration of antibiotic has fallen below its inhibitory level for the test strain.

Testing of antibiotics: The antimicrobial impregnated antibiotic discs are placed on the agar surface with the help of a forceps. Gently tap the disc to ensure uniform contact with the agar surface. The minimum distance between the centre if 2 discs should be no less than 24 mm and incubate the plates at 37oC for 16-18 hours. Interpretation: After overnight incubation, the plates were examined and the zone diameters were measured. With the help of sliding callipers, the diameter of zone of inhibition around the antibiotic discs were measured. All the measurements are made with the unaided eye while viewing the back of the Petri dish using the reflected light to measure. An interpretative correlation (susceptible, intermediate, or resistant) is provided by reference to published guidelines [13].

Pic no. 1 Aloe vera leaf

Pic no. 2 Aloe vera powder

Pic no. 3 Soxhlet apparatus

Pic no.4 Extractor filter

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Result

Initially, the isolation of E. coli and S. aureus was done from the clinical samples, biochemical tests were performed to confirm the organism. Later, these organisms were tested using both commercially available antibiotics and aloe vera extract.

Table no. 1 Prevalence of *E. coli* in different clinical samples. (n=50)

Clinical sample	Total isolated organism	Percentage (%)
Blood	5	10%
pus	12	24%
Urine	19	38%
Sputum	10	20%
Body fluids	1	2%
Foleys tip	1	2%
ET tip	2	4%

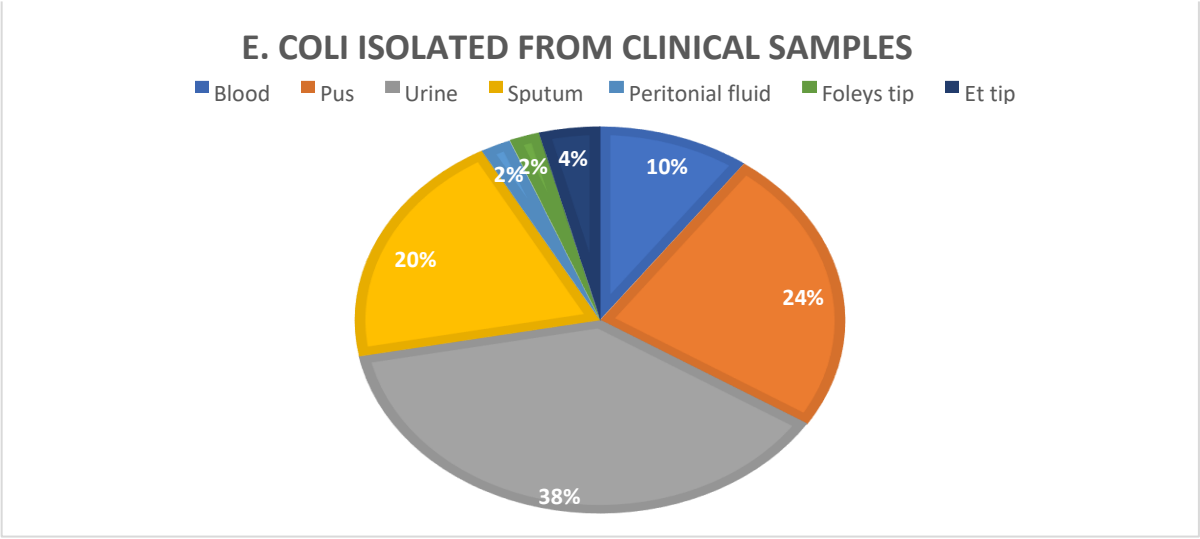

Fig No 1: *E. coli* isolated from Clinical samples

Table no. 2 Prevalence of *S. aureus* in different clinical samples. (n=50)

Clinical sample	Total isolated organism	Percentage (%)
Blood	15	30%
pus	18	36%
Urine	7	14%

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Sputum	8	16%
Body fluids	1	2%
Suction tip	1	2%

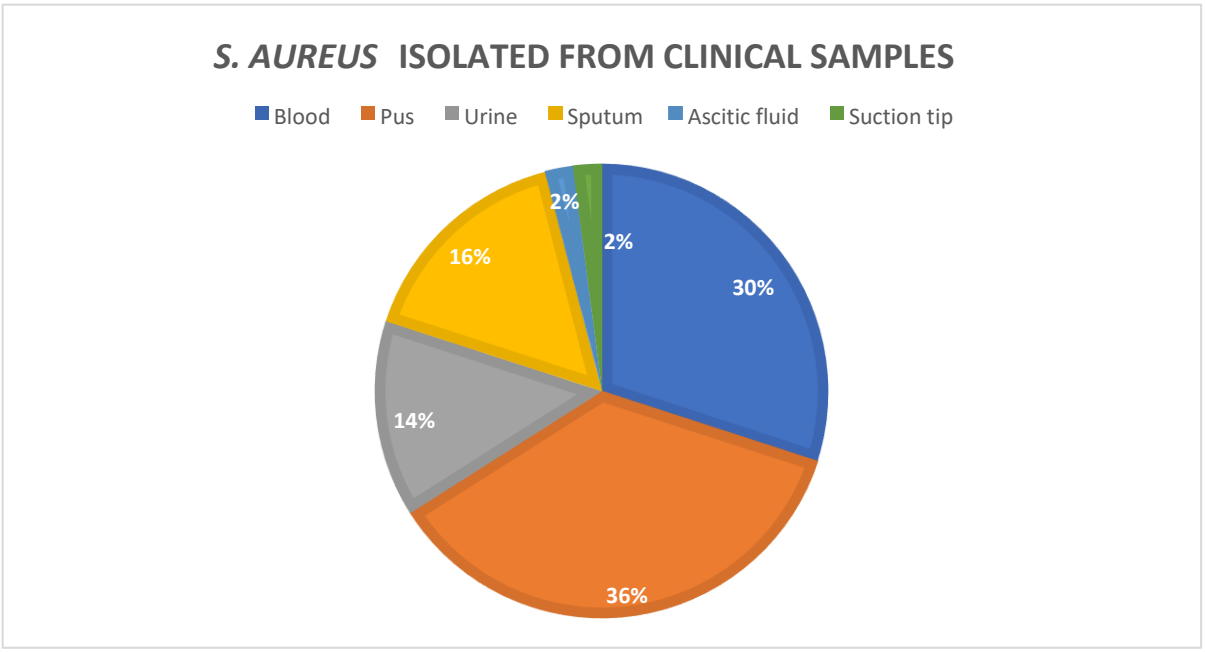

Fig No 2 :*S. aureus* isolated from Clinical samples

Antibiotic Sensitivity Testing

- Done by Kirby Bauer Method.

For *E. coli*:

Table 3 – Antibiotic (1st Line Sensitivity) Pattern

Antimicrobial	**Concentration**	**Sensitive**	**Percentage (%)**
AMC	30mcg	7	14%
TOB	10mcg	34	68%
CZ	30mcg	6	20%
GEN	10mcg	21	76%
CXM	30mcg	6	12%
AK	30mcg	34	68%
CTX	30mcg	8	16%

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CIP	5mcg	7	14%
CPZ	30mcg	7	14%
OF	5mcg	21	42%
CAZ	30mcg	11	22%
TE	30mcg	17	34%
CEP	30mcg	1	5%
CZX	30mcg	1	5%
NIT	30mcg	12	60%
TR	5mcg	3	15%
NX	10mcg	10	50%
Methanolic Aloe Vera Extract 1	20mg	40	80%
Methanolic Aloe Vera extract 2	40mg	41	82%

Fig no.3 Sensitivity Pattern of Antibiotics and Methanolic extract of aloe vera on *E. coli*

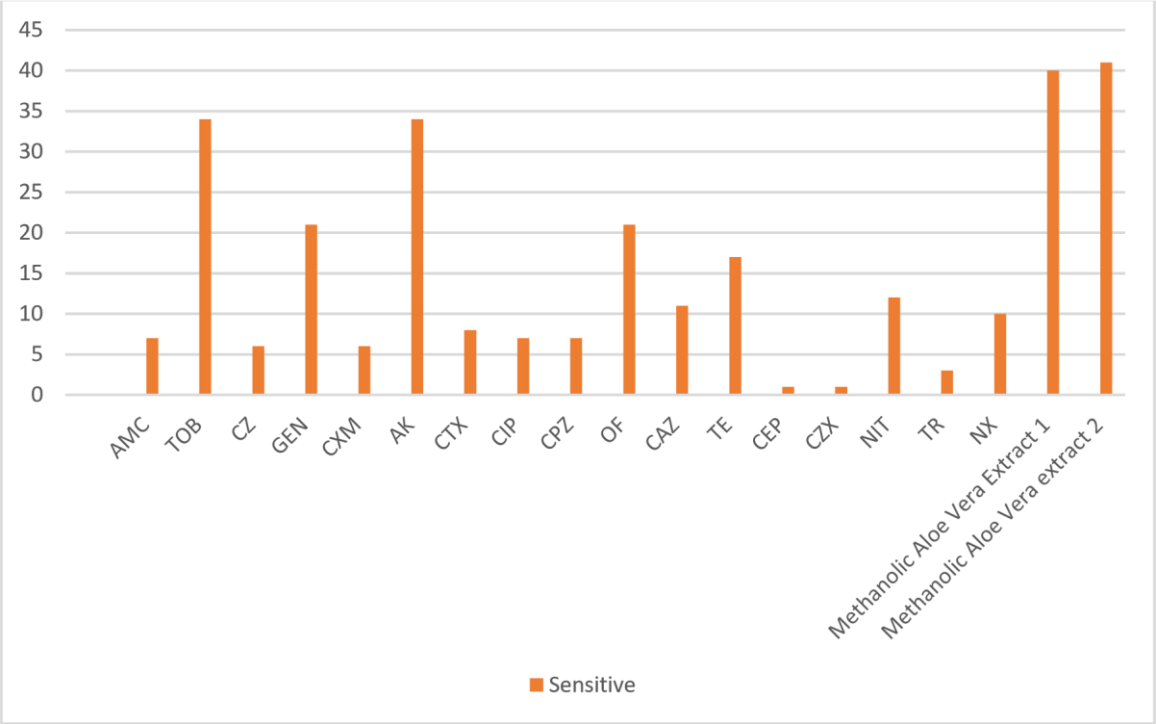

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Table no. 4 Antibiotics (2nd line antibiotics) in *E. coli*

Antibiotics	**Concentration(mcg)**	**Sensitive**	**Percentage**
CAT	30mcg	11	22%
IPM	10mcg	15	30%
CIS	30mcg	13	26%
LE	5mcg	31	62%
CPM	30mcg	14	28%
PRU	5mcg	12	24%
CEC	10mcg	11	22%
TCC	10mcg	9	18%
CMC	5mcg	9	18%
AT	30mcg	15	30%
ETP	10mcg	12	24%
TGC	300mcg	8	16%
PB	15mcg	20	40%
MRP	10mcg	14	28%
CFS	30mcg	9	18%
Methanolic Aloe Vera Extract 1	20mg	40	80%
Methanolic Aloe Vera extract 2	40mg	41	82%

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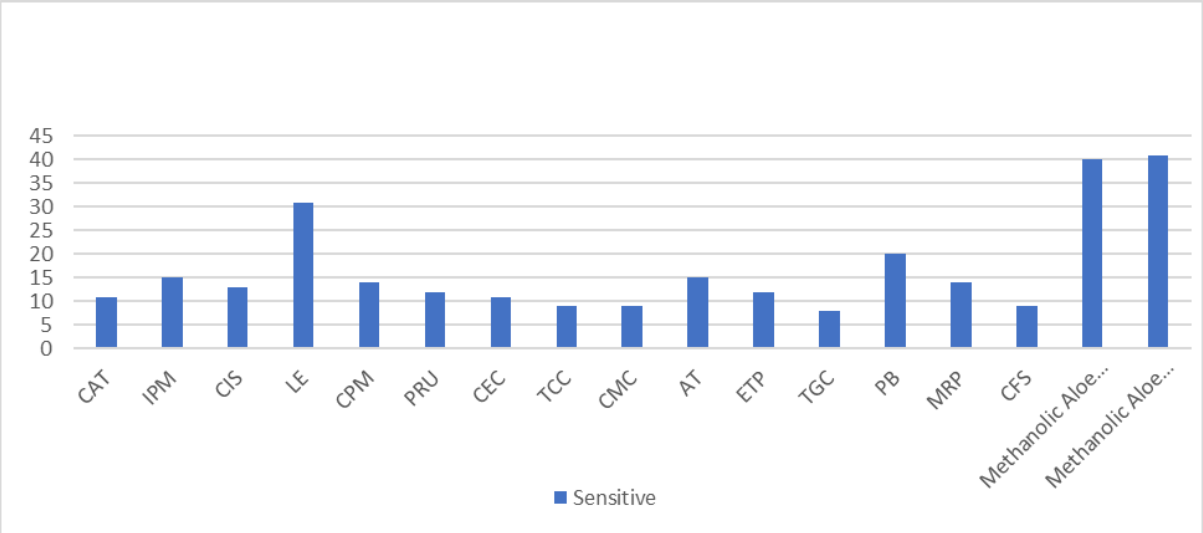
Fig no. 4 Sensitivity Pattern of Antibiotics and Methanolic extract of aloe vera on *E. coli*



For *S. aureus*:

Table no 5- Antibiotic Sensitiviy pattern (1st line)

Antibiotics	Concentration	Sensitive	Percentage
COT	25mcg	24	48%
CXM	30mcg	21	42%
CX	30mcg	30	60%
CIP	5mcg	21	42%
CZ	30mcg	31	62%
GEN	10mcg	26	52%
AMC	30mcg	14	28%
TE	30mcg	28	56%
CD	2mcg	30	60%
AZM	15mcg	14	28%
P	10units	3	9%
RO	15mcg	6	12%
Methanolic Aloe Vera Extract 1	20mg	43	86%

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Methanolic Aloe Vera extract 2	40mg	43	86%

Fig no. 5 Sensitivity Pattern of Antibiotics and Methanolic extract of aloe vera on *S. aureus*

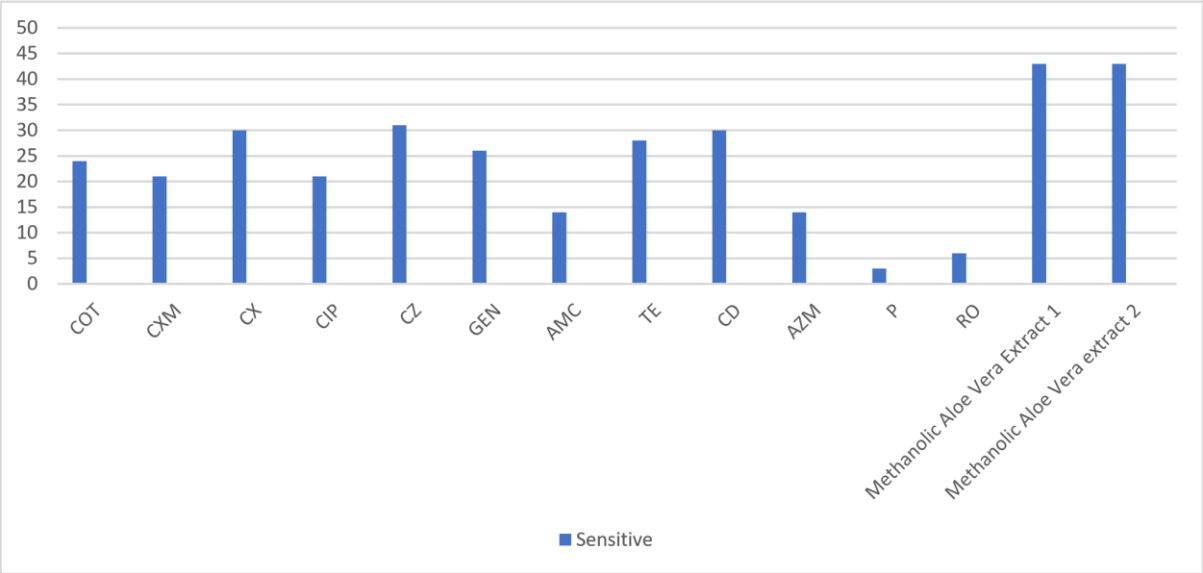

Table no. 6 Antibiotics (2nd line antibiotics) in *S. aureus*

Antibiotics	**Concentration**	**Sensitive**	**Percentage**
VA	30mcg	28	56%
LZ	30mcg	35	70%
RIF	30mcg	9	18%
IMP	10mcg	12	24%
CTX	30mcg	22	44%
NET	30mcg	9	18%
MRP	10mcg	24	48%
CLR	15mcg	18	36%
Methanolic Aloe Vera Extract 1	20mg	43	86%
Methanolic Aloe Vera extract 2	40mg	43	86%

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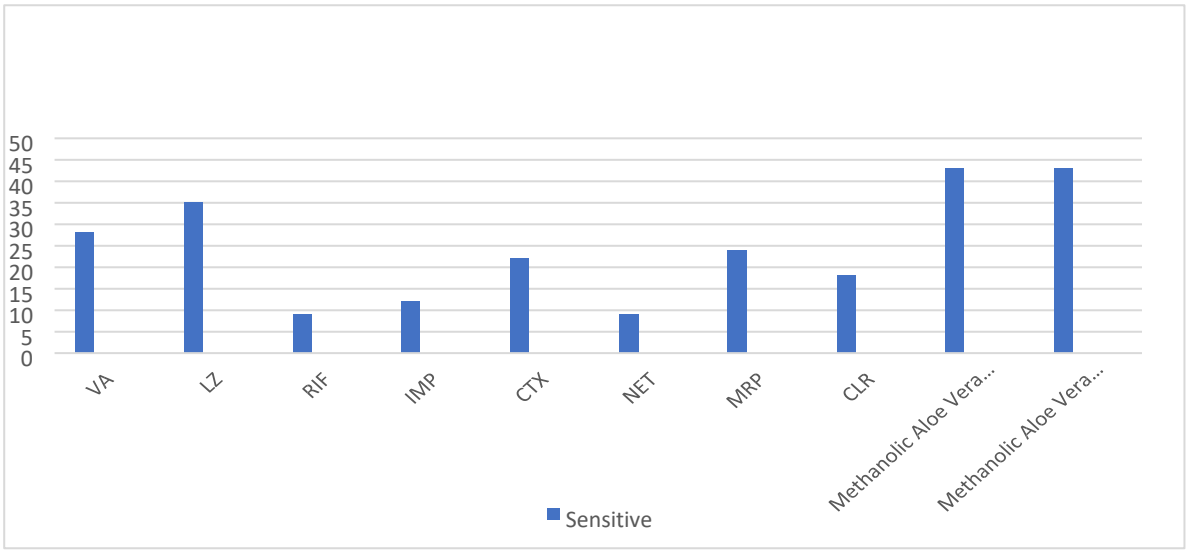

Fig no. 6 Sensitivity Pattern of Antibiotics and Methanolic extract of aloe vera on *S. aureus*

Table no-7: **Antimicrobial activity of methanolic extract of aloe vera on Standard ATCC *E. coli* and *S. aureus*:**

Aloe vera extract	**Zone of inhibition(mm)**	
	ATCC *E. coli*	ATCC *S. aureus*
20mg	15mm	19mm
40mg	26mm	28mm

Table no. 8 Susceptibility pattern of *E. coli* with Aloe vera extract

Concentration (mg)	**Total no of samples with *E. coli***	**Sensitive samples**	**% Sensitivity**
20 mg	50	40	80%
40 mg	50	41	82%

Table no. 9 Susceptibility pattern of *S. aureus* with Aloe vera extract

Concentration (mg)	**Total no of samples with *S. aureus***	**Sensitive samples**	**% Sensitivity**
20 mg	50	43	86%
40 mg	50	43	86%

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Discussion

The present study looks at the frequency of isolation of E. coli strains and S. aureus strains in clinical sample collected and received between February 2021 to February 2022 in Microbiology Laboratory, MGM Medical College and Hospital, Navi Mumbai. From all the samples received in the Laboratory, 100 samples were taken in consideration from which S. aureus and E. coli were isolated. Out of those 100 samples, 20 were Blood cultures, 30 were Pus samples, 26 were Urine samples, 18 sputum samples, 2 were Body fluids and 4 were for Tip culture sensitivity including Foley's tip, ET Tip, and Suction Tip. From Blood cultures 15 S. aureus were isolated while 5 E. coli were isolated. Similarly, from Pus samples 18 S. aureus and 12 E. coli, from Urine samples 7 S. aureus and 19 E. coli, from Sputum samples 8 S. aureus and 10 E. coli, from Body Fluid 1 S. aureus and 1 E. coli and from Tip Culture sensitivity 1 S. aureus and 3 E. coli were isolated. The methanolic extract of Aloe Vera was prepared using Soxhlet apparatus. Different concentration of extracts i.e., 20 mg and 40 mg were then prepared and tested against the ATCC Strains of S. aureus and E. coli as well as against the strains isolated from clinical samples. ATCC strains were also tested against the commercially available 1st line and 2nd line antibiotics. The results showed that the ATCC strains of S. aureus and E. coli were sensitive against all the commercially available antibiotic. In contrast to that, the ATCC strains of S. aureus and E. coli were also sensitive against the Aloe vera extract. The zone of inhibition of S. aureus against 20 mg and 40 mg Aloe vera extract was 19 mm and 28 mm respectively while the zone of inhibition of E. coli against 20 mg and 40 mg was 15 mm and 26 mm respectively.

A similar study was done by **EL-mahmood MA (2009),** in which crude extract of garlic was tested against the clinically isolated *S. aureus, E. coli*, *Streptococcus* and *Pseudomonas* strains. The organisms were isolated from various samples like Blood, Pus, Urine, Stool, Sputum, Body fluids. Organisms isolated from all the specimens over 2 years of study period were tested against aqueous extracts of the crude garlic. *S. aureus* showed higher zone of inhibition against the extracts as to that of Gram-Negative Organisms. Hence, it was concluded that aqueous extracts can be used as a potent antimicrobial agent against Gram Positive organisms along with conventional antibiotics [(14)].

A similar study was conducted by FIŢ NI et.al., (2013) in which aqueous extract of Aloe vera and Goji Berry was tested against ATCC Strains of E. coli, S. aureus, Pseudomonas and Candida albicans. The results revealed that the Aloe vera extract was effective only against ATCC strains of S. aureus and no other organism showed any zone of inhibition against it. But our study yielded better results and our extract was effective against both E. coli and S. aureus [(15)] . In this study, the organisms isolated from clinical samples were also tested against the commercially available 1st and 2nd line antibiotics. Through AST pattern of S. aureus, it was found that the highest isolates were sensitive against CZ (n=3, 62%) and least were sensitive against P (n=9, %) in 1st Line antibiotics and in 2nd Line antibiotics highest isolates were sensitive against LZ (n=35, 70%) and least were sensitive against both RIF and NET (n=9, 18%). In AST pattern of E. coli, the highest isolates were sensitive against GEN (n=21, 76%) and lowest were sensitive against CEP and CZX (n=1, 5%) in 1st Line antibiotics and highest isolates were sensitive against LE (n=31, 62%) and lowest isolates were sensitive against TGC (n=8, 16%). Bashir A et.al. (2011), studied the antimicrobial activity of aloe vera extracts against the organisms isolated from skin infections. The aloe vera gel extract was prepared and tested against the isolated organisms. A comparison of 5 commercially available antibiotics was done with leaf and gel extracts of aloe vera. The results showed that, Gram-positive bacteria were highly sensitive against Vancomycin (80.5%) followed by Methicillin (68%), Erythromycin (55.6 %), Novobiocin (54.1 %) and Bacitracin (25%). Similar antibiotics were tested against Gram-Negative organism which revealed that 72 % of the isolates were sensitive against Vancomycin which was the highest and 42.4 % isolates were sensitive against Erythromycin which was the lowest. On comparing this with the aloe vera gel extract, 75.3 % of Gram-positive organisms were susceptible and 100 % Gram-Negative organisms were susceptible to the extract. Hence, in their study, the aloe vera extract showed similar findings to that of our study (41) . In our study, on testing E. coli isolates with Aloe Vera extract at a concentration of 20 mg, 40 isolates (80%) were sensitive with an average zone of 18 mm and at concentration of 40 mg, 41 isolates (82 %) were sensitive with an average zone of 28 mm. In S. aureus, at concentration of 20 mg, 43 isolates (86%) were sensitive with an average zone of 19 mm and at a concentration of

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40 mg, 43 isolates (86%) were sensitive with an average zone of 29 mm. Hence, in our study, the Gram-Positive organisms i.e., S. aureus showed an increased zone of inhibition in both the concentrations of Aloe Vera extract as compared to that of Gram-Negative organisms i.e., E. coli from both clinical isolates as well as against ATCC strains.

Therefore, it can be concluded that aloe vera extracts can be commercially used to treat certain infection parallelly with commercially available antibiotics.

Conclusion

Isolation and purification of methanolic extract of Aloe vera and evaluation of its antibacterial activity on Standard strains of Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 was done and found that these strains were sensitive to the aloe vera extract. • E. coli strains were isolated from various clinical samples such as Pus, urine, sputum, blood, body fluids and ET tip. E. coli was isolated more from urine sample (38%). • Staphylococcus strains were isolated from various clinical samples such as Pus, urine, sputum, blood, body fluids and ET tip. Staphylococcus was isolated more from Pus sample (36%). • In our study, on testing E. coli isolates with Aloe Vera extract at a concentration of 20 mg, 40 isolates (80%) were sensitive with an average zone of 18 mm and at concentration of 40 mg, 41 isolates (82 %) were sensitive with an average zone of 28 mm. • In S. aureus, at concentration of 20 mg, 43 isolates (86%) were sensitive with an average zone of 19 mm and at a concentration of 40 mg, 43 isolates (86%) were sensitive with an average zone of 29 mm. Therefore, our study results concludes that the methanolic extract of Aloe vera has desired effect of antibacterial activity against both gram positive and gram-negative bacteria. This study suggests that the Aloe vera plant extracts can be used to treat infections caused by organisms like E. coli and S. aureus and can also help to overcome MDR against commercially available antibiotics.

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