Full Length

ANTIBACTERIAL ACTIVITY OF *ELAEOCARPUS GANITRUS* AND *ACACIA NILOTICA* EXTRACTS AGAINST MULTIDRUG RESISTANT *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

L. Kalaiselvi[1]*, S. Ramesh[2] and M. Jayaraman[3],

Department of Veterinary Pharmacology and Toxicology
Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University
Chennai-600 007

ABSTRACT

Bovine mastitis is a significant inflammatory disease in dairy cattle, complicated by the rising threat of antimicrobial resistance (AMR) among its causative pathogens. This study investigated antimicrobial resistance in pathogens causing bovine mastitis and evaluated the antibacterial activity of Elaeocarpus ganitrus and Acacia nilotica ethanolic extracts against multidrug-resistant Escherichia coli and Staphylococcus aureus. S. aureus (64%) and E. coli (36%) were isolated from mastitis milk samples which exhibited multidrug resistance with MAR indices >0.2. E. coli showed complete resistance to cefepime and high resistance to streptomycin (88.9%) and tetracycline (77.8%), while S. aureus exhibited complete cefepime resistance and significant resistance to gentamicin (75%), amoxicillin-clavulanic acid (62.5%), and streptomycin (62.5%). The extract yields were 9.56% and 13.66% for E. ganitrus and A. nilotica, respectively, both containing alkaloids, tannins, phenols, flavonoids, terpenoids, phytosterols, and steroids. Both the extracts and their combination extracts demonstrated significant antibacterial activity against multidrug-resistant isolates. These findings suggest their potential as alternative therapeutics to combat multidrug-resistant mastitis pathogens.

Key words: Mastitis, *Escherichia coli, Staphylococcus aureus, Elaeocarpus ganitrus*, *Acacia nilotica*, multidrug resistance

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[1]Associate Professor and Head, Veterinary College and Research Institute, Theni - 625 534
*Corresponding author Email id: kalaiselvivet@gmail.com
[2]Professor and Head
[3]Project Student, VCRI, Theni - 625 534

INTRODUCTION

Mastitis is an inflammatory condition of the udder that causes significant economic losses for farmers due to decreased milk production, treatment costs, and potential culling of affected animals. It is primarily

due to bacterial infections, with common causative pathogens including *Escherichia coli, Staphylococcus aureus, Streptococcus agalactiae*, *Klebsiella pneumoniae* and others. Treatment typically involves the use of antimicrobial agents; however, the emergence and rapid spread of antimicrobial resistance (AMR) among mastitis pathogens pose a serious challenge to effective disease management. Development of AMR leads to reduced treatment efficacy, prolonged infections, and increased economic burden, underscoring the need for alternative therapeutic strategies against resistant pathogens. Plant-derived natural products, due to their structural complexity and diverse mechanisms, offer a promising avenue for developing novel drugs against multidrug-resistant pathogens.

Elaeocarpus ganitrus, commonly known as rudraksha, is an evergreen plant belonging to the family Elaeocarpaceae and is predominantly found in the Himalayas. The seeds, bark, and leaves of this tree have been widely used in traditional medicine to treat various ailments, including arthritis, fever, wound healing, liver diseases and skin disorders (Gaurav *et al*., 2014). Previous studies have identified numerous phytochemicals in the plant such as alkaloids, glycosides, flavonoids, tannins, and terpenoid, highlighting its potential for antibacterial activity against multidrug-resistant bacteria (Singh and Singh, 2024).

Acacia nilotica, commonly known as Indian gum arabic or babul, is a thorny tree belonging to the family Fabaceae and is widely distributed in tropical and subtropical regions, including India, Africa, and the Middle East. Traditionally, various parts of the plant including bark, pods, leaves, and gum have been used in folk medicine to treat conditions like diarrhea, dysentery, skin infections, and respiratory ailments. Phytochemical analyses have revealed the presence of bioactive compounds including tannins, flavonoids, saponins and phenolics, which may be contributing to its broad spectrum of antimicrobial properties (Kumari and Swer, 2025). Although the antibacterial properties of *E. ganitru*s and *A. nilotica* have been documented, their efficacy and possible synergistic effects against multidrug-resistant pathogens remain unexplored.This study highlights plant-derived antimicrobials as promising tools against MDR pathogens, with potential to reduce antibiotic use and develop novel, natural antimicrobial strategies.

With this background, the present study was conducted to evaluate the antibacterial activity of *E. ganitrus* seed and *A. nilotica* bark extracts against antibiotic-resistant bacteria isolated from clinical cases of bovine mastitis.

MATERIALS AND METHODS

Drugs and Chemicals

Nutrient broth, brain heart infusion broth, eosin methylene blue agar, mannitol salt agar, Mueller Hinton agar and antibiotic discs utilized in this study were purchased from M/s HiMedia Laboratories, Mumbai. All other solvents and chemicals used were of analytical grade

Preparation of Plant Extract

The seeds of *E.ganitrus* and bark of *A. nilotica* were commercially procured and authenticated by the botanist, Department of Medical Botany, Government Siddha Medical College, Chennai. The plant materials were dried, powdered and stored. Approximately 50 g of the dried and powdered plant material was weighed, and extraction was performed using 200 mL of ethanol by hot continuous extraction in a Soxhlet apparatus. Following extraction, the solvent was concentrated under reduced pressure using a rotary evaporator, and the resulting extracts were stored for further analysis. Phytochemical screening of the extracts was performed as per the following methods described by Deyab *et al*. (2016).

- Alkaloids: 2 mL of extract was mixed with 2 mL of concentrated HCl, followed by a few drops of Mayer's reagent. Formation of a green color or white precipitate indicated alkaloids.

- Flavonoids: 2 mL of extract was mixed with 1 mL of 2N NaOH. Yellow color formation indicated flavonoids.

- Steroids, Terpenoids, and Phytosterols: 0.5 mL of extract was treated with 2 mL of chloroform and concentrated H_2SO_4 A reddish-brown color at the interface indicated terpenoids; greenish-yellow fluorescence indicated phytosterols, and red color formation confirmed steroids.

- Tannins: 1 mL of extract was mixed with 1 mL of 5% $FeCl_3$. Dark blue or greenish-black color indicated tannins.

- Phenols: 1 mL of extract was mixed with 2 mL of water and a few drops of 10% $FeCl_3$. Blue or green coloration confirmed phenols.

- Saponins: 2 mL of extract was mixed with 2 mL of water and shaken for 15 minutes. Formation of a stable foam layer confirmed saponins.

Collection of Samples and Isolation of bacteria

Twenty-five milk samples were collected from clinical cases of bovine mastitis at the Madras Veterinary College Teaching Hospital. Bacterial isolation was performed following aseptic techniques. Briefly, 100 µL of each milk sample was inoculated into 10 mL of sterile nutrient broth and brain heart infusion broth, then incubated overnight at 37°C. A loopful of the overnight culture was streaked onto eosin methylene blue agar and mannitol salt agar for the selective isolation of *E. coli* and *Staphylococcus* species, respectively. Isolates were identified based on colony morphology on selective media, Gram staining, and biochemical tests, following the protocols described in Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1993) Pure cultures were stored in glycerol stocks at −20°C for further analysis.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the isolates were performed using the Kirby Bauer Disc diffusion method following the Clinical and Laboratory

Standards Institute guidelines (CLSI, 2010). Standardized bacterial inocula were prepared by transferring isolated colonies from culture plates into sterile normal saline using a sterile loop. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard, corresponding to approximately 108 CFU/mL. Using a sterile swab, the inoculum was uniformly streaked over the entire surface of Mueller-Hinton agar plates, and excess moisture was allowed to dry. Antibiotic discs were then placed on the agar surface using sterile forceps. Plates were incubated inverted at 37°C for 16–18 hours. Zones of inhibition were measured with an antibiotic zone reader and interpreted according to CLSI standards. The antibacterial activity of the plant extracts was also evaluated by the Kirby-Bauer method, using sterile discs loaded with 20 µL of plant extract in DMSO at a concentration of 500 µg/mL. The concentration of 500 µg/mL was selected based on the preliminary studies and previous literature (Okoro *et al*., 2014). Sterile disc loaded with 10% DMSO was used as a negative control.

Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic Resistance (MAR) index of an isolate was determined using the formula (Mir *et al*., 2021)

$$\frac{\text{(No.of antibiotics to which an isolate was resistant)}}{\text{(Total number of antibiotics tested)}}$$

Statistical analysis

Statistical analysis was conducted using a one-way ANOVA followed by Duncan's post hoc test in SPSS to determine significant differences in the antibacterial activity of the plant extracts.

RESULTS AND DISCUSSION

Mastitis is one of the most prevalent inflammatory conditions affecting dairy cattle, manifesting in two forms, viz., clinical and subclinical mastitis. The condition is caused by a range of pathogens, broadly categorized as either contagious or environmental. *S. aureus* is a major contagious pathogen, predominantly colonizing the udders of infected cows and serving as a reservoir for ongoing infection. This bacterium primarily induces subclinical mastitis, with intermittent episodes of clinical flare-ups. In contrast, *E. coli* is a significant environmental pathogen responsible for clinical mastitis, typically characterized by a short duration of illness (Shah *et al*., 2025).

In the present study, nine (9) *E. coli* and sixteen (16) *S. aureus* were isolated from the milk samples collected. Both *E. coli* and *S. aureus* were present in three samples. *S. aureus* was the most prevalent pathogen, detected in 64% of samples, followed by *E. coli* at 36%. These findings align with previous reports by Bhat *et al*. (2017), Verma *et al.* (2018), Shakir *et al*. (2024) and Rana *et al.* (2022), all of whom documented a higher prevalence of *S. aureus* compared to *E. coli*

in cases of bovine mastitis. Specifically, Bhat *et al.* (2017) reported a similar prevalence of *S. aureus* (60.87%) in the Jammu and Kashmir region of India, whereas Verma *et al.* (2018) observed a lower prevalence of 42.55% in Meerut. Globally, the prevalence of *S. aureus* in bovine mastitis cases has been reported to vary widely, ranging from 39.13% to 66.67%. (Shah *et al.*, 2025; Shakir *et al*., 2024, Rana *et al*., 2022, Islam *et al*, 2025).

Compared to our findings, a lower prevalence of E. coli in bovine mastitis has been reported in India, ranging from 13.04% to 21.28% (Bhat *et al*., 2017; Verma *et al.*, 2018). Conversely, Manasa *et al*. (2019) documented a higher prevalence of 41% in India, exceeding the levels observed in our study. On a global scale, prevalence rates comparable to our results have been reported in Pakistan and Bangladesh (Shakir *et al*., 2024; Rana *et al*., 2022).

Antimicrobial susceptibility of isolates was tested by Kirby Bauer Disc diffusion method and the results have been given in Table 1 and Fig. 1. The prevalence of multidrug resistance among bacterial isolates have been given in Table 2 and 3.

In the present study, all *S. aureus* and *E. coli* isolates were found to be multidrug-resistant, exhibiting a MAR index greater than 0.2 and demonstrating resistance to two or more classes of antibiotics. The MAR index is a valuable method for assessing the source of contamination and a value greater than 0.2 indicates exposure to high-risk sources where antibiotics are frequently used, reflecting significant antibiotic pressure in the environment. This threshold is commonly used as a marker to identify bacteria originating from environments with heavy antibiotic use, such as hospitals or intensive farming settings (Mir *et al*., 2022). Among *E. coli,* the highest frequency observed was 33.33%, with resistance to seven antibiotics. Similarly, 25% of *S.aureus* isolates showed resistance to seven antibiotics with an MAR index of 1.0.

In the present study, both *E. coli* and *S. aureus* isolates exhibited complete resistance to cefepime, a fourth-generation cephalosporin. This result contrasts with the findings of Fazal *et al.* (2023), who reported a cefepime resistance rate of 53.6% among *S. aureus* isolates. The complete resistance observed in this study highlights a potentially escalating concern regarding the effectiveness of advancedgeneration cephalosporins in treating infections caused by these pathogens.

In the present study, we observed high level of resistance to streptomycin (88.9%) and tetracycline (77.8%) among *E. coli* isolates. These findings are consistent with those reported by Verma *et al*. (2018) and Shakir *et al*. (2024).

S. aureus isolates demonstrated a high level of resistance to gentamicin (75%), followed by amoxicillin-clavulanic acid (62.5%) and streptomycin (62.5%). The observed resistance to amoxicillin-clavulanic acid aligns with the findings reported by Rana *et al*. (2022). However, Shah *et al*. (2025), Verma *et al.* (2018), and

Shakir *et al.* (2024) documented even higher resistance rates, reporting near-complete resistance to this antibiotic, which contrasts with the levels observed in our study. Notably, the gentamicin resistance detected in our study exceeded the rates reported by Shah *et al*. (2025), Verma *et al*. (2018), Shakir *et al*. (2024), Rana *et al.* (2022), and Bhat *et al.* (2017). In contrast, the level of streptomycin resistance observed in our study aligns closely with the findings of Verma *et al*. (2018).

The emergence of such high-level resistance in these clinically significant pathogens underscores the declining efficacy of critical antibiotics, thereby complicating treatment protocols and severely limiting available therapeutic options for managing infections caused by these organisms. This trend highlights the urgent need for enhanced antimicrobial stewardship, routine surveillance, and the development of novel therapeutic agents to effectively combat multidrug-resistant bacterial infections.

The soxhlet ethanolic extraction of *E. ganitrus* seeds and *A. nilotica* bark resulted in semi-solid extracts with yields of 9.56% and 13.66%, respectively. The *E. ganitrus* extract was greenish-brown, while the *A. nilotica* extract exhibited a reddish-brown color. Preliminary phytochemical screening revealed the presence of alkaloids, tannins, phenols, flavonoids, terpenoids, quinones, phytosterols, and steroids in the *E. ganitrus* extract. Similarly, the *A. nilotica* extract also contained alkaloids, tannins, phenols, flavonoids, terpenoids, saponins, phytosterols, and steroids.

The antibacterial activity of plant extracts and their combinations against *E. coli* and *S. aureus* isolates is presented in Tables 4 and 5 and Fig. 1. A zone of inhibition greater than 8 mm was considered indicative of significant antibacterial activity (Saquib *et al*., 2021). Extracts of *E. ganitrus*, *A. nilotica*, and their combination demonstrated significant activity against multidrug-resistant *E. coli* isolates, with *E. ganitrus* showing a slightly larger zone of inhibition compared to *A. nilotica*. However, no statistically significant difference was observed among the inhibition zones produced by *E. ganitrus*, *A. nilotica*, and their combination against MDR *E. coli.*

Similar to *E. coli,* both extracts and their combination exhibited significant antibacterial activity against multidrug-resistant *S. aureus.* The zone of inhibition produced by *A. nilotica* was slightly larger than that produced by *E. ganitrus*. However, no statistically significant difference was observed among the inhibition zones produced by *E. ganitrus*, *A. nilotica*, and their combination against MDR *S. aureus*.

Our findings are consistent with those of Dalei and Sahoo (2016), who reported significant antibacterial activity of methanolic and acetone extracts of the epicarp and endocarp of *E. ganitru*s against MTCC (Microbial Type Culture Co llection of India) strains of *E. coli* and *S. aureus.* Similarly, Kiromah *et al*. (2023) reported the antibacterial activity of ethanolic, methanolic, and aqueous extracts of *E. ganitrus* leaves against *E. coli.* In agreement with our findings, Sadiq *et al.* (2017)

observed antibacterial activity of *A. nilotica* against *E. coli* isolated from beef and chicken meat samples. Khan *et al*. (2009) also documented the antibacterial potential of ethanolic extracts of *A. nilotica* against ATCC strains of *E. coli* and *S. aureus*. Furthermore, Okoro *et al.* (2014) reported antibacterial activity of ethanolic extracts of *A. nilotica* against human clinical isolates of *E. coli* and *S. aureus*. The antibacterial activity of the extracts against multidrug-resistant isolates of *E. coli* and *S. aureus* observed in our study indicates its potential as an effective agent against resistant bacterial strains.

The therapeutic potential of *E. ganitrus* is likely attributable to its diverse phytochemical profile, which includes bioactive compounds such as quercetin, gallic acid, ellagic acid, elaecocarpine, and rudrakine (Dalei and Sahoo, 2016). Similarly, *A. nilotica* has been identified as a reservoir of pharmacologically active constituents, including rutin, ellagic acid, terpenoids, epicatechin, and various polyphenols. These phytochemicals have been independently reported to exhibit antimicrobial, anti-inflammatory, and antioxidant properties, which may collectively contribute to the observed efficacy of these plant (Kumari and Swer, 2025).

Beyond the antibacterial activity both *E. ganitru*s and *A. nilotica* extracts has been documented to exhibit a broad spectrum of biological activities, including anti-inflammatory and antioxidant properties (Kumari and Swer, 2025 and Dalei and Sahoo, 2016). These multifaceted properties are particularly advantageous in the management of mastitis, where they may contribute to combating multidrug-resistant pathogens, mitigating inflammation, and slowing disease progression.

The combination of crude plant extracts represents a promising alternative strategy for managing infections caused by antibiotic-resistant pathogens. Interactions among the diverse biomolecules present in crude extracts may result in synergistic, additive, or indifferent effects on antibacterial activity. In the present study, however, the combined extracts of *E. ganitrus* and *A. nilotica* did not demonstrate significantly enhanced antibacterial activity compared with the individual extracts. This outcome may be attributed to the possible masking or interference of active constituents by other components within the crude extracts.

This finding suggests that, while both plants contain bioactive compounds effective against resistant bacterial strains, their combined use may not enhance antimicrobial potency beyond their individual effects. Similar findings have been reported in previous studies, where combinations of plant extracts failed to produce synergistic outcomes, potentially due to antagonistic interactions among phytochemicals or concentration-dependent inhibitory effects (Patel *et al.*, 2019; Zhang *et al.*, 2021). These results highlight the complexity of phytochemical interactions and underscore the necessity of systematically evaluating plant extract combinations. A rigorous

assessment of potential synergistic, additive, or antagonistic effects is essential before such formulations can be recommended as viable alternative treatments for multidrug-resistant infections.

To conclude, the plant extracts were found to exhibit significant antibacterial activity against multidrug resistant *E. coli* and *S.aureus* isolates. The observed antibacterial activity in the present study suggests that the bioactive compounds in the plant may interfere with bacterial growth and resistance pathways, offering a promising avenue for developing novel therapeutics to address the escalating challenge of multidrug resistance.

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Table.1. Antibiogramof *E. coli* and *S. aureus* isolates

Organism	Susceptibility	AMC	CPM	CIP	GEN	STN	TET	VAN
E. coli (n=9)	% Sensitivity (No.)	33.3 (3)	0	55.6 (5)	55.6 (5)	11.1 (1)	22.2 (2)	55.6 (5)
	% Resistant (No.)	33.7 (6)	100 (9)	44.4 (4)	44.4 (4)	88.9 (8)	77.8 (7)	44.4 (4)
S. aureus (n=16)	% Sensitivity (No.)	37.5 (6)	0	43.7 (7)	25.0 (4)	37.5 (6)	50.0 (8)	43.7 (7)
	% Resistant (No.)	62.5 (10)	100 (16)	56.3 (9)	75.0 (12)	62.5 (10)	50.0 (8)	56.3 (9)

(S- Susceptible; R- Resistant; AMC - Amoxicillin / Clavulanic acid; CPM – Cefepime; CIP – Ciprofloxacin; GEN – Gentamicin; STN– Streptomycin; TET – Tetracycline; VAN– Vancomycin)

Table.2. Antibiotic Resistance pattern and MAR index of *E. coli* isolates

Antibiotic Resistance Pattern	No. of antibiotics (antibiotic Classes) resistant	Frequency of isolates	MAR Index (% of isolates)
CPM-STN	2(2)	1	0.29 (11.11%)
AMC-CPM-VAN; CPM-STN-TET; CPM-CIP-STN	3 (3)	1,1,1	0.43 (33.33%)
AMC-CPM-STN-TET	4 (4)	1	0.57 (11.11%)
AMC-CPM-GEN-STN-TET-VAN	6 (5)	1	0.86 (11.11%)
AMC-CPM-CIP-GEN-STN-TET-VAN	7 (6)	3	1.00 (33.33%)

AMC - Amoxicillin / Clavulanic acid; CPM – Cefepime; CIP – Ciprofloxacin; GEN – Gentamicin; STN – Streptomycin; TET – Tetracycline; VAN – Vancomycin)

Table.3. Antibiotic Resistance pattern and MAR index of *S. aureus* isolates

Antibiotic Resistance Pattern	No. of antibiotics (antibiotic Classes) resistant	Frequency of isolates	MAR Index (% of isolates)
CPM-GEN; CPM-VAN	2(2)	1, 1	0.29 (12.5%)
CPM-GEN-STN; CPM-GEN-TET; AMC-CPM-GEN	3 (3)	1, 1, 1	0.43 (18.75%)
AMC-CPM-CIP-STN; AMC-CPM-CIP-GEN; AMC-CPM-GEN-VAN	4 (4)	1, 1, 1	0.57 (18.75%)
CPM-CIP-STN-TET-VAN	5 (5)	1	0.71 (6.25%)
CPM-CIP-GEN-STN-TET-VAN; AMC-CPM-CIP-GEN-STN-VAN; AMC-CPM-CIP-GEN-STN-TET	6 (5)	1, 1, 1	0.86 (18.75%)
AMC-CPM-CIP-GEN-STN-TET-VAN	7 (6)	4	1.00 (25%)

Table.4. Antibacterial activity of extracts of *E.ganitrus, A.nilotica* and their combination against *E.coli* isolates

Isolate No.	Zone of inhibition in mm		
	E.ganitrus	A.nilotica	E.ganitrus + A.nilotica
1	12	14	15
2	10	10	10
5	10	10	10
6	18	12	12
9	13	10	12
18	12	10	10
22	11	10	11
23	10	10	10
24	12	12	15
Mean ±SEM	12^{NS} ±0.79	10.89^{NS} ± 0.46	11.67^{NS} ± 0.65

NS-Non-significant

Table.5. Antibacterial activity of extracts *E.ganitrus, A.nilotica* and their combination against *S. aureus* isolates

Isolate No.	Zone of inhibition in mm		
	E.ganitrus	A.nilotica	E.ganitrus + A.nilotica
3	15	12	11
4	10	11	13
7	10	10	10
8	13	12	12
10	16	18	11
11	13	14	12
12	10	13	11
13	11	12	10
14	13	13	11
15	11	12	12
16	11	14	11
17	10	12	10
19	11	10	11
20	14	14	14
21	10	10	10
25	13	13	12
Mean ±SEM	11.94^{NS} ±0.46	12.50^{NS} ± 0.47	11.31^{NS} ± 0.27

NS-Non-significant

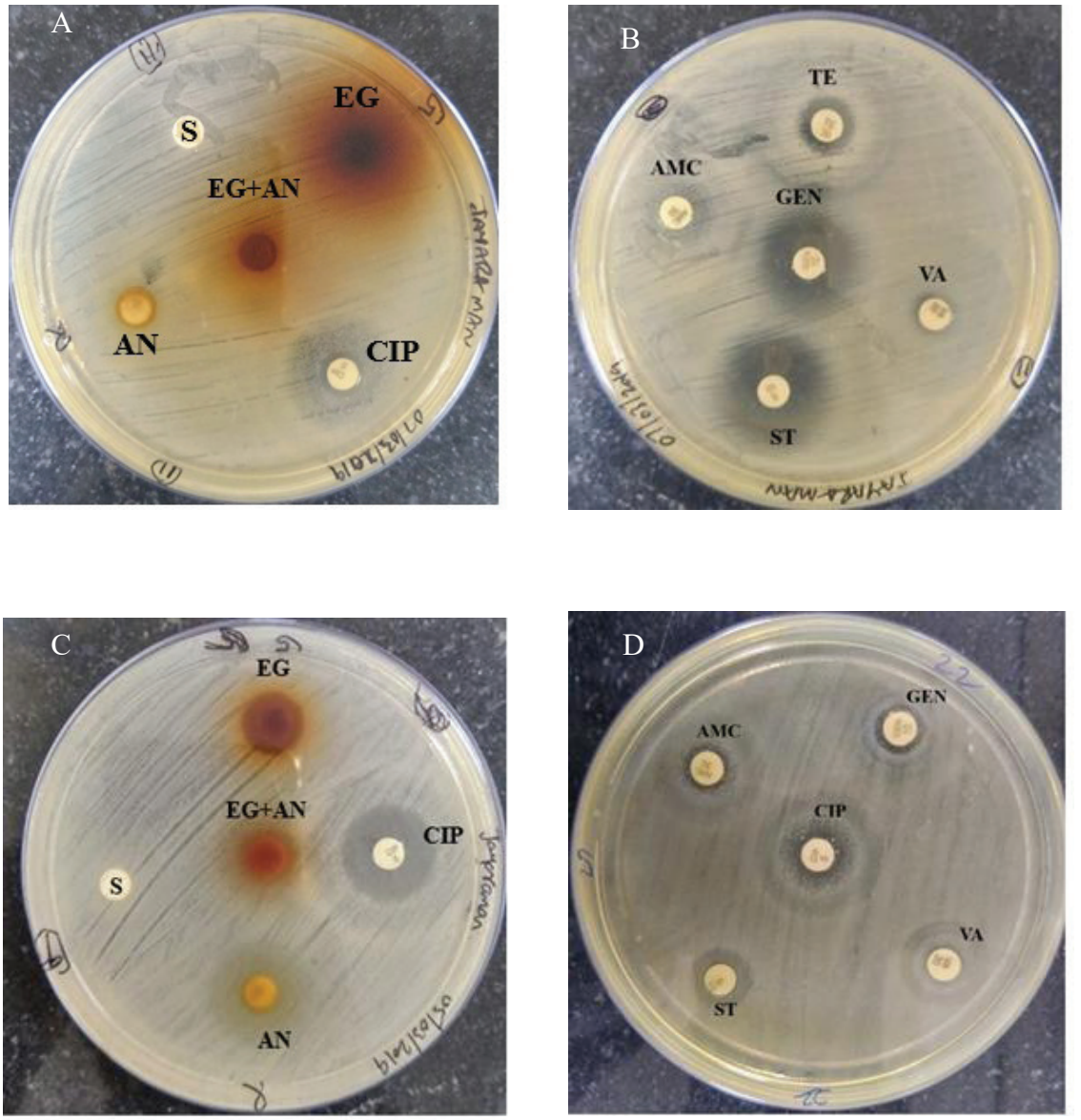

Fig.1. Inhibition zones of extracts of E. ganitrus (EG), A. nilotica (AN), their combination (EG + AN), and commonly used antibiotics against S. aureus (A & B) and E. coli (C & D). AMC – Amoxycillin-clavulanic acid; CIP-Ciprofloxacin; ST-Streptomycin; GEN-Gentamicin, VA-Vancomycin; TE-Tetracycline; S- Solvent Control (10% DMSO)

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