

Antimicrobial Activity of Some Medicinal Plants against Pathogenic Microorganisms.

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Abstract

The present research work carries out the screening of some medicinal plants and evaluates the antimicrobial activity of plant extract against the pathogenic microorganisms by using the agar well diffusion assay method. Working concentrations (20mg/mL) of the Microbial extracts. Standardized broth cultures of test bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella spp*, and *Enterococci spp*) were spread aseptically onto the surface of Mueller-Hinton Agar (MHA) plates, respectively, using sterile cotton swabs. All plant crude extracts showed significant activity against organisms. Zone of inhibition of the extract compared with the standard antibiotics Gentamycin.

Keywords:- Medicinal plants, Screening, Bacterial isolates, Antimicrobial activity, Gentamycin, and Zone of inhibition, etc.

Introduction

Bactericidal drugs kill bacteria, and bacteriostatic drugs slow or stop bacterial growth. These definitions are not absolute; bacteriostatic drugs may kill some bacteria, and bactericidal and fungicidal drugs may not kill all of the bacteria *in vitro*. More precise quantitative methods identify the minimum *in vitro* concentration at which an antibiotic can inhibit growth (minimum inhibitory concentration (MIC)). *Staphylococcus aureus* is a bacterial species known as "golden staph" and Oro staphira. It is a facultative anaerobic Gram-positive coccil bacterium, which was first discovered in the pus of surgical abscesses by Sir Alexander Ogston in 1883. The golden appearance is the etymological root of the bacteria's name, aureus means "golden" in Latin. *Staphylococcus aureus* is a "Golden Cluster Seed" and is also known as golden Staph. *S. aureus* frequently colonizes the skin and has a niche preference for the anterior nares of the nose, with persistent nasal carriage occurring in 25–30% of the population. *Staphylococcus aureus* is one of the most prevalent organisms responsible for nosocomial infections, and cases of community-acquired *S. aureus* infection have continued to increase despite widespread preventative measures. *Enterococci* are associated with a variety of different clinical syndromes, including bacteremias, endocarditis, and skin or soft tissue and urinary tract infections. The emergence of resistance has made clinicians keenly aware of these opportunistic pathogens. Molecular methods have delineated the epidemiology of VRE and have conclusively demonstrated healthcare-associated acquisition and transmission.

Colonization with VRE occurs approximately 10 times more frequently than actual infection, and occurs in patients with severe underlying illness or who are receiving antibiotics with broad-spectrum anti-anaerobic activity. Infection control efforts have been established to limit the spread of this pathogen. Treatment of serious enterococcal disease requires a synergistic combination of a cell-wall active agent and an aminoglycoside. The relatively few antimicrobial agents available to treat serious VRE infections make therapeutic decision-making for these cases quite challenging. Although enterococci are generally considered safe for use in food production, their role as probiotics is not established, and alternatives should be sought, due to their involvement in therapeutically challenging diseases.

According to Hoge, Adams, Buchanan, & Sears stated that the *Enterococci* can cause a variety of infections. For some of these, other microorganisms are also frequently isolated from the same site. In those situations, it is often not clear whether the manifestations of infection are the result of enterococci, or whether these relatively virulent and opportunistic organisms are merely bystanders or are playing a minor role in the infection. However, for other types of infections, most notably endocarditis and bacteremia, enterococci can cause serious and often life-threatening disease. *Escherichia coli*, commonly abbreviated *E. coli*, is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Vogt & Dippold, reported that the most of *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls then further Reid *et al*²⁵¹ reported *E. coli* strains harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K₂ and by preventing the establishment of pathogenic bacteria within the intestine. Pathogenic strains of *E. coli* are responsible for three types of infections in humans: urinary tract infections (UTI), neonatal meningitis, and intestinal diseases (gastroenteritis).

According to Ryan *et al*, *Pseudomonas aeruginosa* is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility. An opportunistic human pathogen, *P. aeruginosa* is also an opportunistic pathogen of plants. *P. aeruginosa* is the type species of the genus *Pseudomonas*. The word *Pseudomonas* means "false unit", from the Greek pseudo (Greek: 'false') and Monas (Latin: Monas, from Greek: 'a single unit'). The stem word mon was used early in the history of microbiology to refer to germs, e.g., Kingdom Monera. The species name *aeruginosa* is a Latin word meaning "copper rust," as seen with the oxidized copper patina on the Statue of Liberty. *Pseudomonas aeruginosa* causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, *bacteremia*, bone and joint infections, gastrointestinal infections, and a variety of systemic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed. So, all these bacteria are taken for screening of antimicrobial activity with crude extracts of plants *Vitex negundo*. *Vitex negundo* is generally known as Negundo in India. It is also known as the five-leaved chaste tree, is a large aromatic shrub with quadrangular, densely whitish, tomentose branchlets. It is widely used in folk medicine, particularly in South and Southeast Asia. It belongs to the family *Verbanaceae* and is found throughout India. *Vitex negundo* has been used for various medicinal purposes in Ayurveda and Unani systems of medicine. Various medicinal properties

are attributed to it, particularly in the treatment of anti-inflammatory, fungal diseases, antioxidant, and hepatoprotective disorders, and as an anti-microbial. The leaves and whole plant are used as an anti-inflammatory, antiseptic, antipyretic, diuretic, and also as an antibiotic (Loganthan *et al.*, 2004; Ragasa, 1999). Second plant is *Holarrhena antidysenterica* (Family *Apocynaceae*), also known as Kutaj, seeds and bark of this tree have been used in Ayurveda since ancient time and possesses significant antimicrobial properties attributed to the presence of various bioactive compounds, especially steroidal alkaloids and third plant is *Adhatoda vasica* used in India's indigenous medical system and is a well-known expectorant in both the Ayurvedic and Unani systems of medicine (Kapoor LD, 2018). It has a large number of uses, including antibacterial, antipyretic, and so on (Dhuley, J. N. 1999). Vasicine, deoxyvasicine, vasicinone, vasicol, vasicinol, and adhatodinine are among the alkaloids found in the plant (Claeso *et al.*, 2000). The fourth plant is *Catharanthus roseus*, is one plant recognized well in Ayurveda. It is known for its anti-tumour, anti-diabetic, anti-microbial, anti-oxidant oxidant and antimutagenic effects. It is an evergreen plant first originated from the island madagascar. The flowers may vary in colour from pink to purple, and leaves are arranged in opposite pairs. It produces nearly 130 alkaloids, mainly ajmalicine, vincine, reserpine, vincristine, vinblastine, and raubasin. Vincristine and vinblastine are used for the treatment of various types of cancer such as Hodgkin's disease, Breast cancer, skin cancer, and lymphoblastic leukemia.

Gloriosa superba L. is an important medicinal plant belonging to the *Colchicaceae* family. It is a semi-woody herbaceous branched climber reaching plant approximately 5 meters height, with brilliant wavy-edged yellow and red flowers. It is one of the endangered species among the medicinal plants, and Different parts of the plant have a wide variety of uses, especially within traditional medicine practised in tropical Africa and Asia. The tuber is used traditionally for the treatment of bruises and sprains, colic, chronic ulcers, haemorrhoids, cancer, impotence, nocturnal seminal emissions, and leprosy, also for inducing labour pains and abortion. Some researchers reported the pharmacological properties of *Gloriosa superba* L. Also shows Antibacterial, antifungal, and mutagenic activities, and Kumarappan, *et al.*, 2011 reported that tuber extract in Alcohol shows Antihaemolytic activities.

MATERIAL AND METHODS

Collection of Plant Parts

Collections of plant parts were collected from the local Gadchiroli forest area, Maharashtra state, India. Plant part tuber and leaves cleaned of soil dust with tap water, then dried in the shade and prepared fine powder, and kept in an airtight bottle. The plant materials were identified by using standard floras like Naik 1979 and Yadav and Sardesai 2002.

Preparation of Plant Part Extract

Methanol, Chloroform, and n-butanol extracts were prepared by using a Soxhlet extractor. 30g of each plant part powder was placed in a thimble, which was placed in the chamber of the Soxhlet apparatus. 300ml solvent in the flask and the temperature was maintained at 55 °C. for 72 hours. Then the extracts were filtered through Whatman filter paper No. 1. Solvent was evaporated at 40-50 °C by using a Rotary evaporator. The collected powder

was weighed and dissolved in Dimethyl sulfoxide (DMSO) with 10% concentration. The extracts were used for antimicrobial activity. (Handa *et al.*, 2008., Subramanian *et al.*, 2011).

Antimicrobial Activity

Preliminary antimicrobial screening of the crude extracts was carried out using the agar well diffusion assay method. Working concentrations (20mg/mL) of the Microbial extracts. Standardized broth cultures of test bacterial isolates (*Staphylococcus aureus*, *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella*, *Enterococci*) were spread aseptically onto the surface of Mueller-Hinton Agar (MHA) plates, respectively, using sterile cotton swabs. All culture plates were allowed to dry for about 5 min, and agar wells were made by using a sterile cork-borer (8 mm in diameter). These wells were respectively filled with 200 µL of the crude extracts and controls. The plates were then kept at room temperature for 1 h to allow the agents to diffuse into the agar medium and incubated accordingly. Gentamycin (50 µg/mL) was used as a positive control in the antibacterial assay, while Nutrient broth and PDB were used as the negative control. The MHA plates were then incubated at 37°C for 24 hrs. The inhibition zones diameters (IZDs) were measured.

Observation and Results

Table No 1. Antimicrobial activity of *Vitex negundo* :

S N o	Culture	+ve	- v e 1	- v e 2	A	B	H	I	F 1	F 2	F 3	F4	F5	F 1 7	F 1 8	F1 9	F20	F2 1
Zone of inhibition (mm)																		
1.	<i>Staphylo coccus</i>	20 mm	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2.	<i>E.Coli</i>	21 mm	—	—	—	—	—	—	—	—	—	9 m m	6 m m	—	—	—	—	6 m m
3.	<i>Pseudo monas</i>	17 mm	—	—	6 m m	—	—	—	—	—	—	7 m m	—	—	—	—	—	6 m m
4.	<i>Klebsiell a</i>	18 mm	—	—	—	—	—	—	—	—	—	7 m m	7 m m	—	—	8 m m	12 mm	6 m m
5.	<i>Enteroc occi</i>	19 mm	—	—	6 m m	—	—	—	—	—	—	—	7 m m	—	—	—	—	—

The graphical representation showing the zone of inhibition of *Vitex negundo* plant extract against *S. aureus*, *E. Coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*

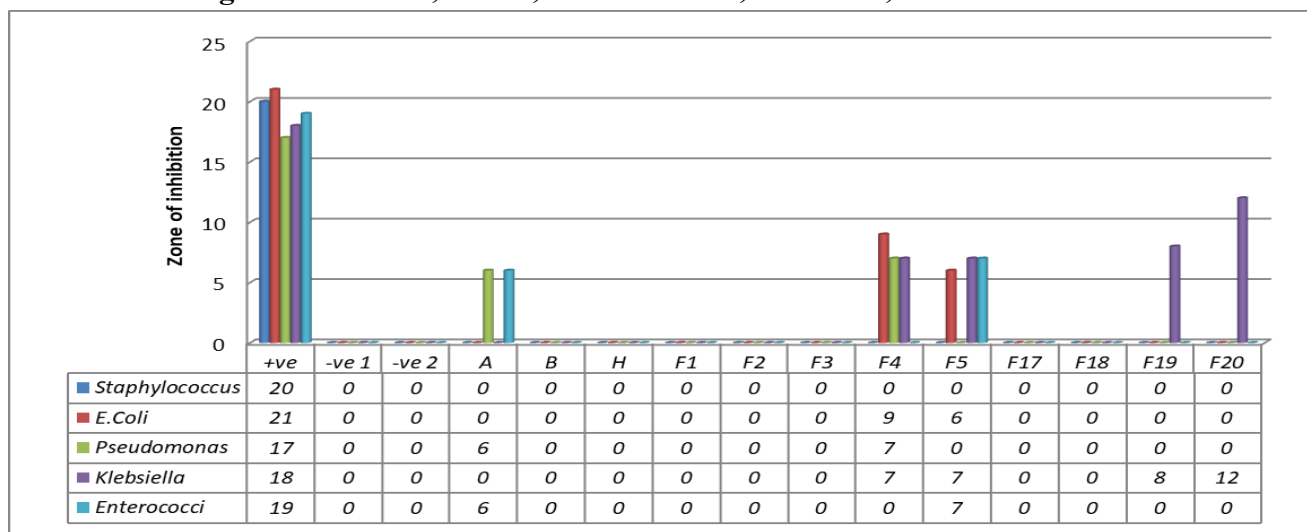
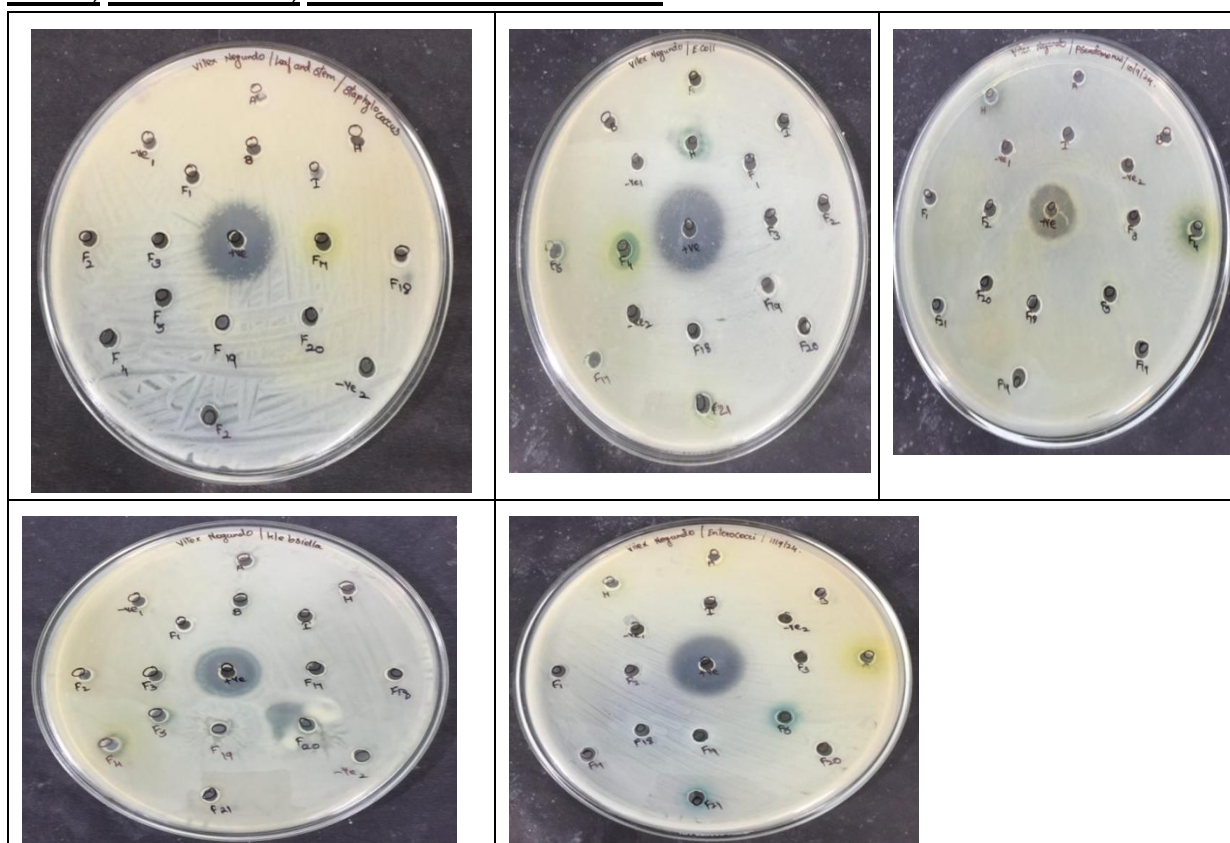


Photo plate showing zone of inhibition of *Vitex negundo* plant extract against *S. aureus*, *E. Coli*, *Pseudomonas*, *Klebsiella* and *Enterococci*



The minimum inhibitory concentration (MIC) of with 200 μ L of the crude extracts and controls against bacteria was compared with the MIC values of the standard drug Gentamycin (50 μ g/mL) was used as a positive control in the antibacterial. The MIC values were observed against *S. aureus*, *E. Coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci* respectively. The values exhibit similar activities as compared with the standard drug Gentamycin. The MIC values of

shows *Vitex negundo* plant extract against *Pseudomonas*, *Enterococci* showed 6mm and 6mm zones of inhibition, as compared to the standard drug Gentamycin drug 17mm and 19mm, respectively. Only positive control Gentamycin showed that the inhibit growth of *S. aureus*, *E. Coli*, and *Klebsiella*, and *Vitex negundo* plant extract also showed a positive impact on fungal growth shown in Table no 1. The antimicrobial activity was expressed at varying degrees, with the activity being all microbial strains and dose dependent. The various crude extracts of *V. negundo* showed significant activity against all the microbes tested. Similar results of biological activity of *V. negundo* against bacterial strains were reported by (Agrawal *et. al.*, 2012, Zaidan *et. al.*, 2005) and (Perumal Samy *et. al.*, 1998). Meena *et al* in 2016 reported that the leaf extract of *Vitex negundo* Linn. Indeed possessed significant antimicrobial activity against all the bacteria tested, but the effect was greater towards *S aureus*. The maximum zone of inhibition observed for *S. aureus* was 15 mm at the concentration of 80 mg/ml and 100 mg/ml, and for *E. coli* and *K. pneumoniae* maximum ZOI noted was 12 mm and 11 mm at 100 mg/ml concentration, respectively.

Table No. 2. Antimicrobial activity of *Holarrhena antidysenterica* :

S N o	Culture	+ve	-ve 1	-ve 2	E	G	O	P	Q	F1 1	F1 2	F1 3	F1 4	F1 5	F1 6	F3 2	F3 3
Zone of inhibition (mm)																	
1.	<i>Staphylococcus</i>	20 m m	—	—	—	—	—	—	—	—	—	—	—	9m m	—	—	—
2.	<i>E. Coli</i>	21 m m	—	—	—	—	—	—	—	8 m m	—	—	—	8m m	7 m m	—	—
3.	<i>Pseudomonas</i>	17 m m	—	—	—	7 m m	7 m m	6 m m	—	7 m m	—	10 m m	6 m m	6m m	—	6 m m	—
4.	<i>Klebsiella</i>	18 m m	—	—	—	—	—	—	—	6 m m	—	—	—	12 m m	—	—	6 m m
5.	<i>Enterococci</i>	19 m m	—	—	6 m m	—	6 m m	6 m m	6 m m	—	—	7m m	—	11 m m	6 m m	6 m m	—

The graphical representation showing the zone of inhibition of *Holarrhena antidysenterica* plant extract against *S. aureus*, *E. Coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*.

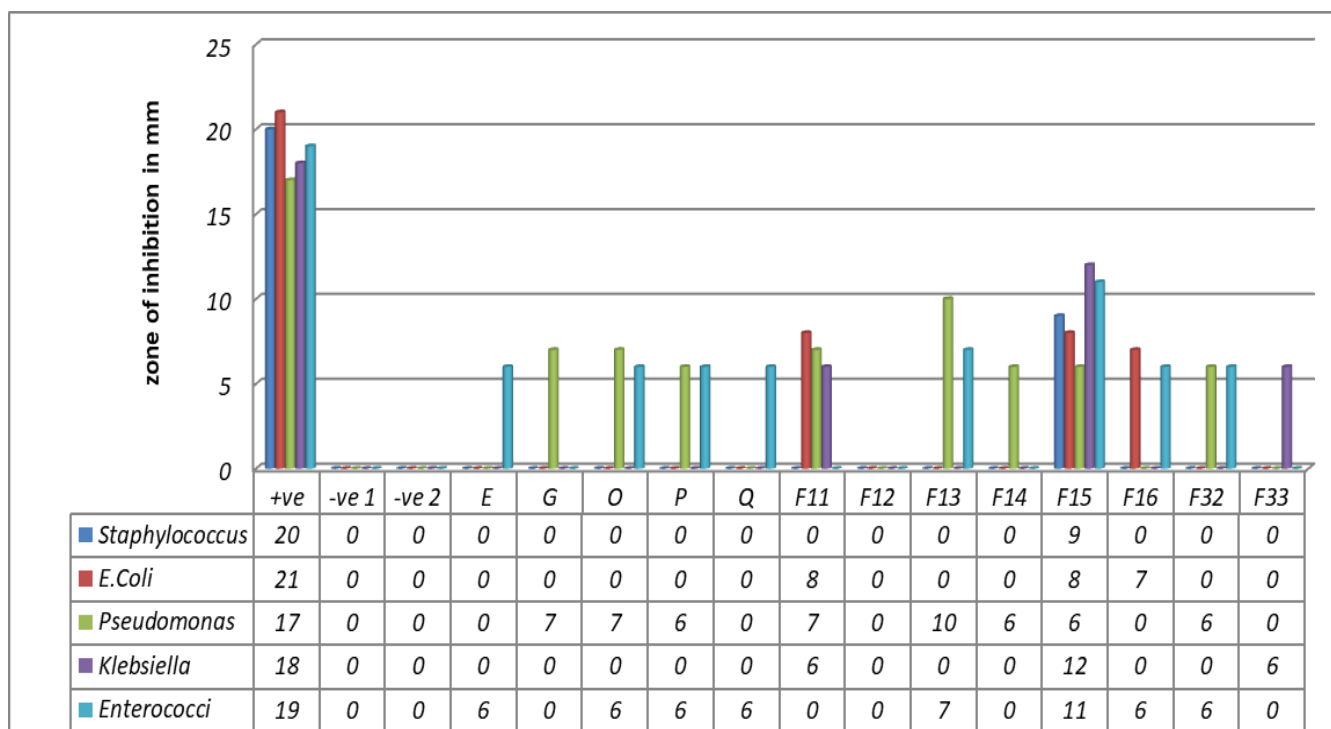
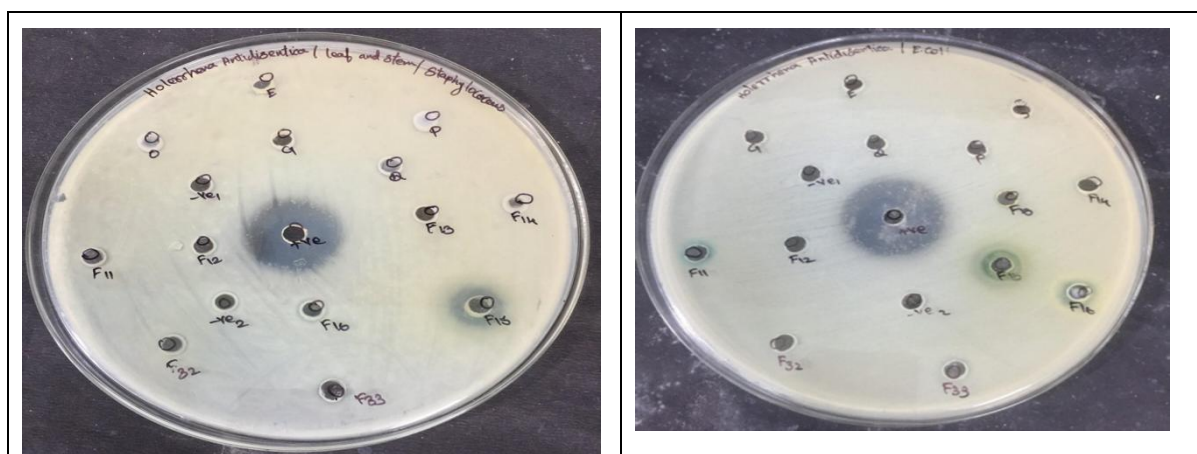
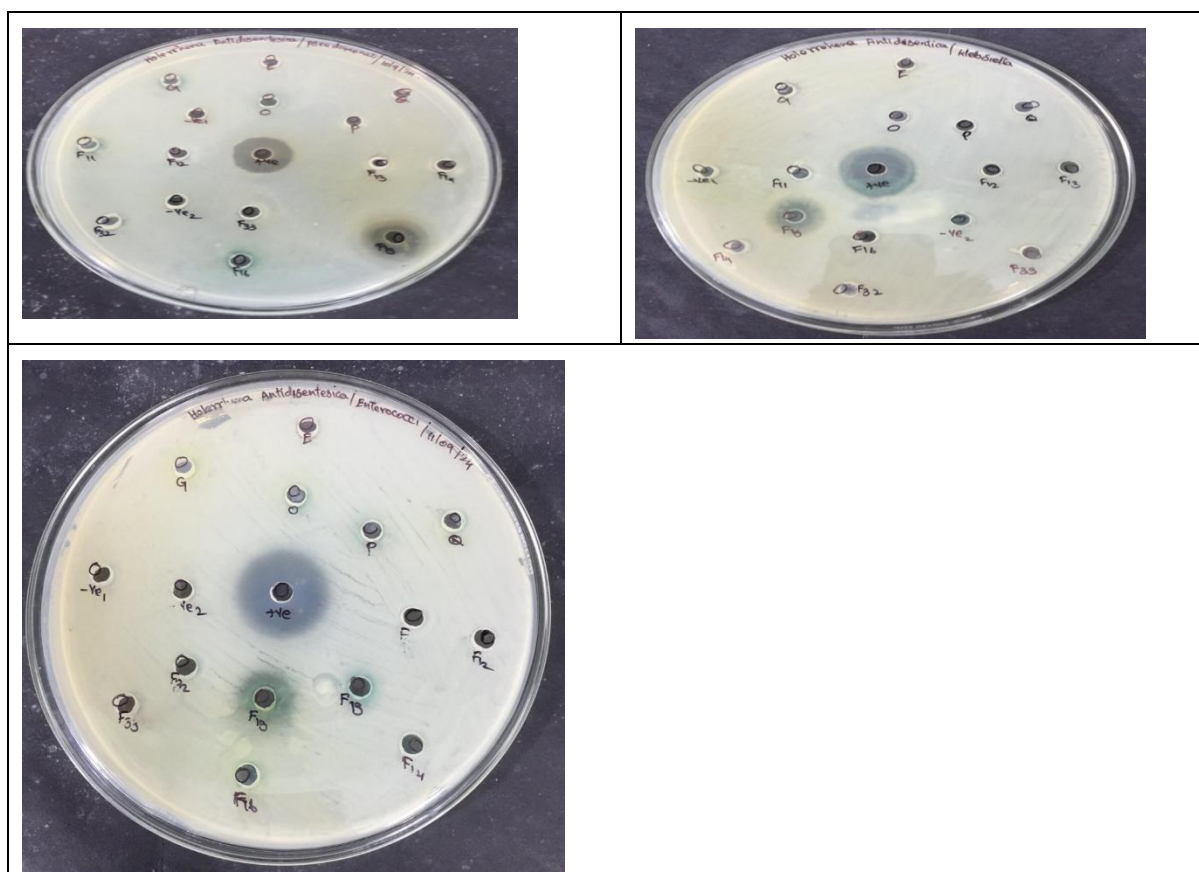


Photo plate showing zone of inhibition of *Holarrhena antidysenterica* plant extract against *S. aureus*, *E. Coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*





The result showed the antimicrobial activities against all the above bacterial pathogens studied. The maximum zone of inhibition observed for *E. coli* was 21 mm at the concentration of Gentamycin (50 µg/mL) as a positive control for *S. aureus*, *Pseudomonas*, *Klebsiella*, and *Enterococci* maximum ZOI noted was 20 mm and 17 mm, 18mm, and 19mm at Gentamycin (50 µg/mL) concentration, respectively. Some researchers carried out the same antimicrobial activities of *H. antidysenterica*. Mahato *et al.* (2013) found that bark, seed, and callus extracts of *H. antidysenterica* possess potential antibacterial activity against *S. aureus*, *Salmonella typhimurium*, and *E. coli*. Farrukh *et al.* (2006) found that ethanol bark extracts of *H. antidysenterica* showed an inhibition zone against the anti-methicillin-resistant *Staphylococcus aureus* (MRSA). Mule *et al.* (2013) found that *H. antidysenterica* stem bark extracts have antibacterial activity against *E. coli*, *Salmonella typhi*, and *S. aureus*. Preeti Kaundal and Anand Sagar in 2016 reported that the growth of almost all the tested bacteria was inhibited by both methanol and acetone extracts of leaves and bark of *H. antidysenterica* but the methanolic extract of leaves as well as bark showed a higher range of inhibition diameter compared to acetone extracts. So, it is hoped that this study will lead to the establishment of some compounds that could be used to develop effective and more potent antibacterial drugs of natural origin against human pathogenic bacterial strains.

Table No 3. Antimicrobial activity of *Adhatoda vasica*:

S N o	Culture	+ve	- ve 1	- ve 2	C	D	R	S	T	F6	F7	F8	F9	F10	F29	F30	F31
Zone of Inhibition																	
1.	<i>Staphylo coccus</i>	20 m m	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2.	<i>E.Coli</i>	21 m m	—	—	—	12 m m	—	7 m m	7 m m	7m m	—	—	—	—	—	—	—
3.	<i>Pseudom onas</i>	17 m m	—	—	6 m m	9m m	—	—	—	12 m m	—	—	—	—	—	—	—
4.	<i>Klebsiell a</i>	18 m m	—	—	—	—	6 m m	—	—	—	—	6 m m	—	—	—	—	—
5.	<i>Enteroco cci</i>	19 m m	—	—	7 m m	8m m	6 m m	6 m m	6 m m	8m m	8 m m	—	—	—	—	—	—

The graphically representation showing zone of inhibition of *Adhatoda vasica* plant extract against *S. aureus*, *E.Coli*, *Pseudomonas*, *Klebsiella* and *Enterococci*.

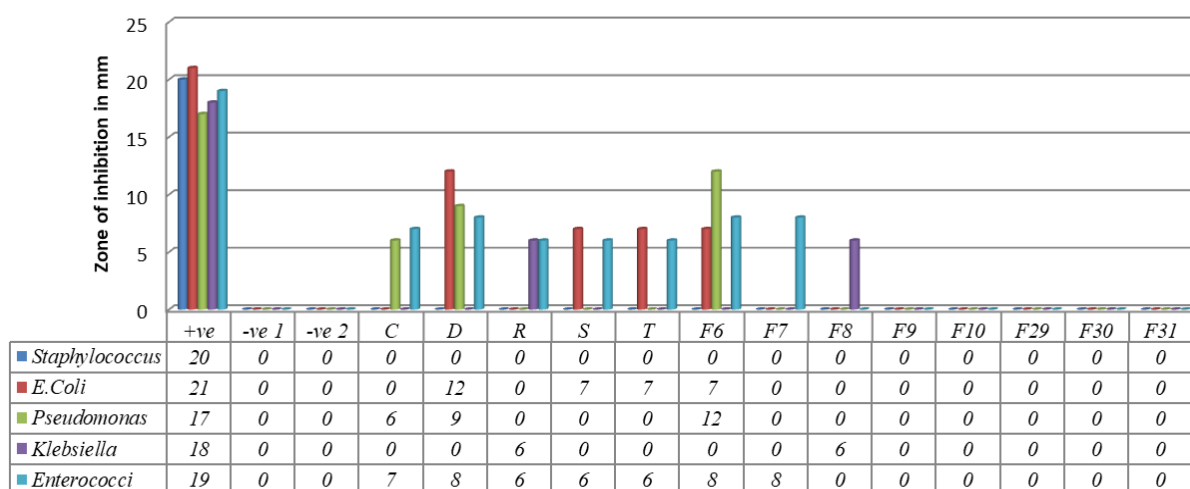
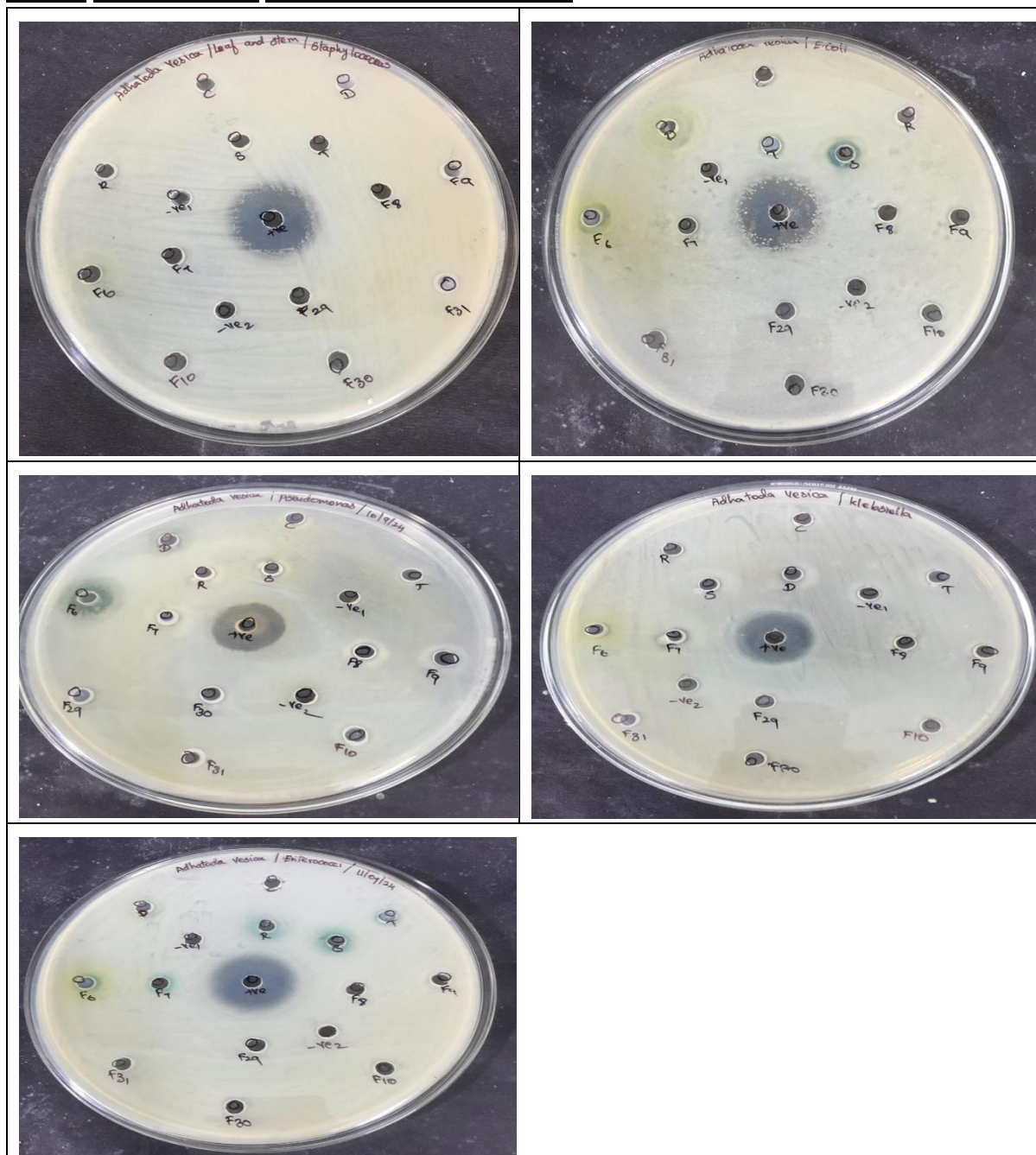


Photo plate showing zone of inhibition of *Adhatoda vasica* plant extract against *S. aureus*, *E. Coli*, *Pseudomonas*, *Klebsiella* and *Enterococci*



In the present studies antimicrobial activities of *Adhatoda vasica* plant extract showed antimicrobial potent activity against bacterial strains. The maximum zone of inhibition found in *E. coli* 21mm with positive control followed by 20mm, 19mm, 18mm and 17 mm in *S. aureus*, *Enterococci*, *Klebsiella* and *Pseudomonas* with Gentamycin (50 µg/mL) concentration respective and also zone of inhibition found in plant extract resist the growth of bacteria *E. coli* 12 mm with 200 µL and followed by *Pseudomonas* and *Enterococci* respectively. Sheeba JB, Mohan ST, in 2012 reported that the *Adhatoda vasica* has potent antimicrobial activity and in

future can be used as an antibacterial and antifungal agent for a number of pathogens, proper research is needed to find out the active principle for antimicrobial potential of this plant.

Table No. 4. Antimicrobial activity of *Catharanthus roseus*:

S N o	Culture	+ve	- ve 1	- ve 2	J	K	L	M	N	F 22	F2 3	F 24	F 25	F 26	F2 7	F2 8
		Zone of Inhibition														
1.	<i>Staphylococcus</i>	20 mm	—	—	—	—	—	—	9m m	—	—	—	—	—	—	—
2.	<i>E.Coli</i>	21 mm	—	—	—	7m m	10 mm	7m m	10 mm	—	6m m	—	—	—	—	—
3.	<i>Pseudomonas</i>	17 mm	—	—	—	9m m	10 mm	7m m	12 mm	—	—	—	—	—	7m m	—
4.	<i>Klebsiella</i>	18 mm	—	—	—	—	9m m	8m m	6m m	—	6m m	—	—	—	—	—
5.	<i>Enterococci</i>	19 mm	—	—	—	6m m	7m m	7m m	6m m	—	—	—	—	—	—	7m m

The graphical representation showing the zone of inhibition of *Catharanthus roseus* plant extract against *S. aureus*, *E.Coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*.

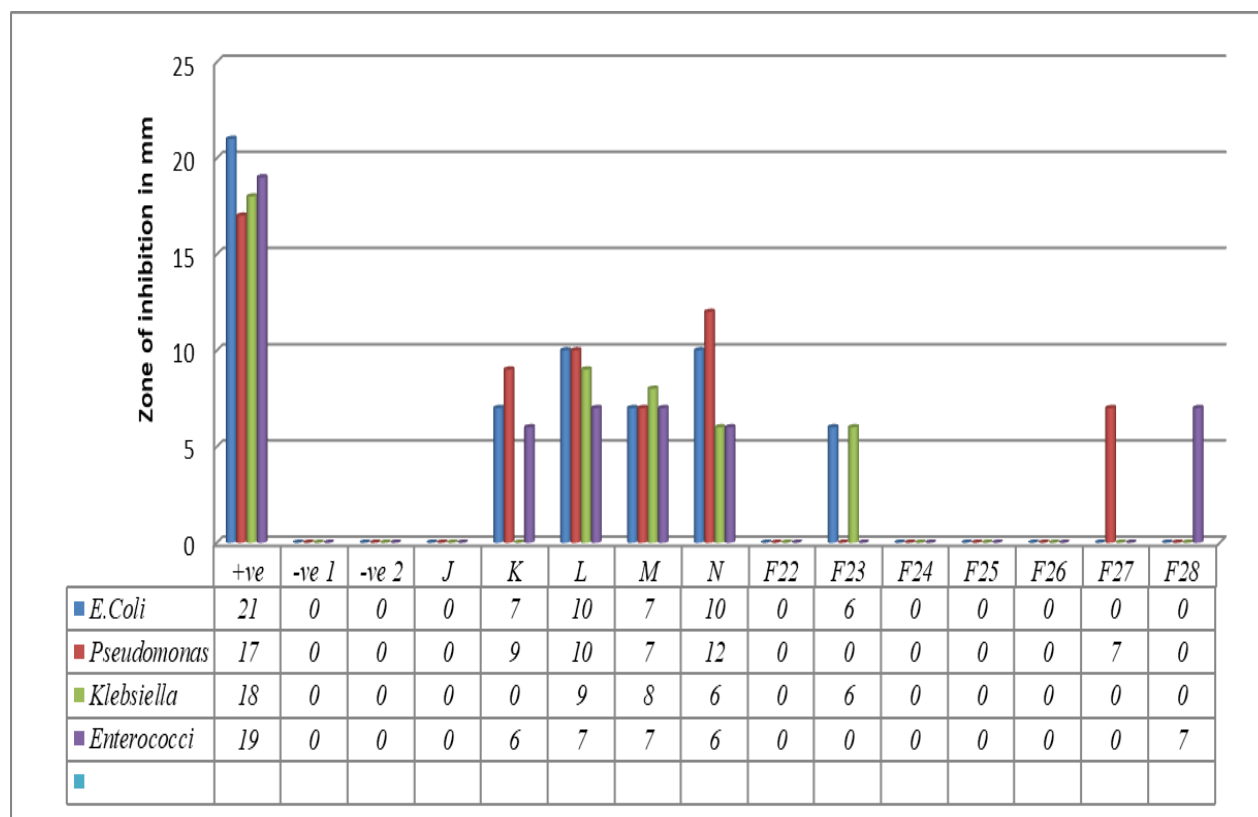
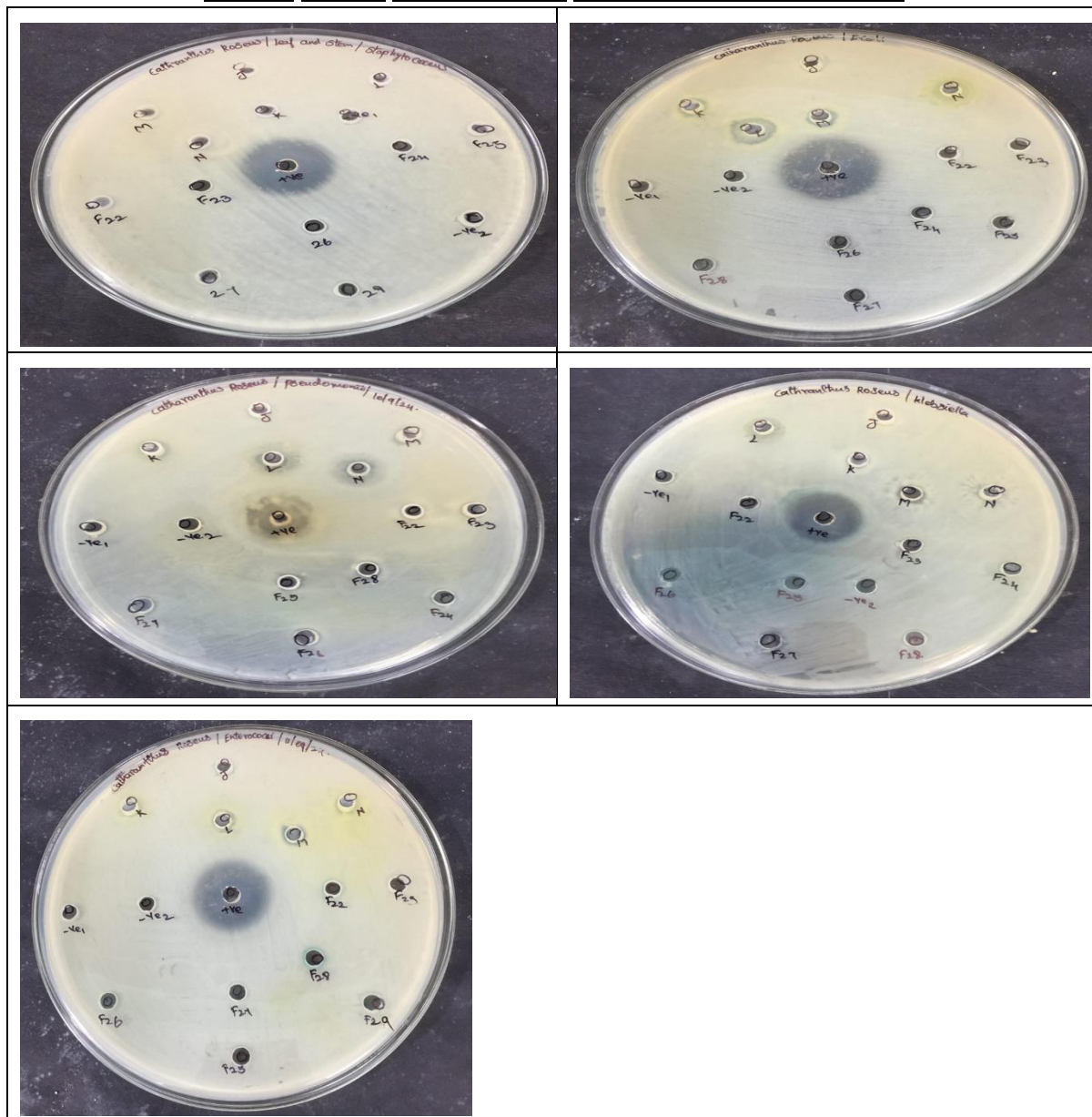


Photo plate showing zone of inhibition of *Catharanthus roseus* plant extract against *S. aureus*, *E.Coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*



The results revealed that the antimicrobial activities of *Catharanthus roseus* plant extract prepared in different extraction solvents showed inhibitory activity against the bacteria, viz. *S. aureus*, *E. coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*. The maximum antimicrobial activity against bacteria was recorded in *E. coli* 21mm with positive control, followed by *Enterococci* 19mm, *Klebsiella* 18mm, and *Pseudomonas* 17mm, and also with plant extract

showed 10 and 7 mm in *E. coli* followed by *Pseudomonas* 12, 10, 9, and 7mm, respectively shown in Table no.4. Shanmugaraju and Bhakayaraj in 2016 recorded the same results from *Catharanthus roseus* leaf extracts showed maximum antibacterial activity against all the pathogenic microorganisms. Among the solvents tested, ethanol showed maximum antibacterial activity of plant extracts, viz., *Catharanthus roseus* leaf, when compared to acetone and chloroform extracts, viz., *Catharanthus roseus* leaf. *Staphylococcus sp* were found to be more susceptible to *Catharanthus roseus* leaf extracts tested, followed by *E. coli*, *Pseudomonas sp*, and *Streptococcus sp*.

Table No. 5. Antimicrobial activity of *Gloriosa superba* L:

S N o	Culture	+ve	- v e 1	- v e 2	U	V	W	X	Y	Z	F3 4	F 35	F 36	F 37	F3 8	F3 9
		Zone of Inhibition														
1.	<i>Staphylococcus</i>	19 mm	—	—	6m m	—	—	—	—	—	—	—	—	—	—	—
2.	<i>E. Coli</i>	21 mm	—	—	—	—	6m m	6m m	—	6m m	—	—	—	—	—	—
3.	<i>Pseudomonas</i>	20 mm	—	—	—	—	—	—	6m m	—	—	—	—	—	—	6m m
4.	<i>Klebsiella</i>	23 mm	—	—	—	—	—	—	—	—	6m m	—	—	—	6m m	—
5.	<i>Enterococcus</i>	19 mm	—	—	—	—	—	—	6m m	—	—	—	—	—	—	6m m

The graphical representation showing the zone of inhibition of *Gloriosa superba* L. plant extract against *S. aureus*, *E. Coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*.

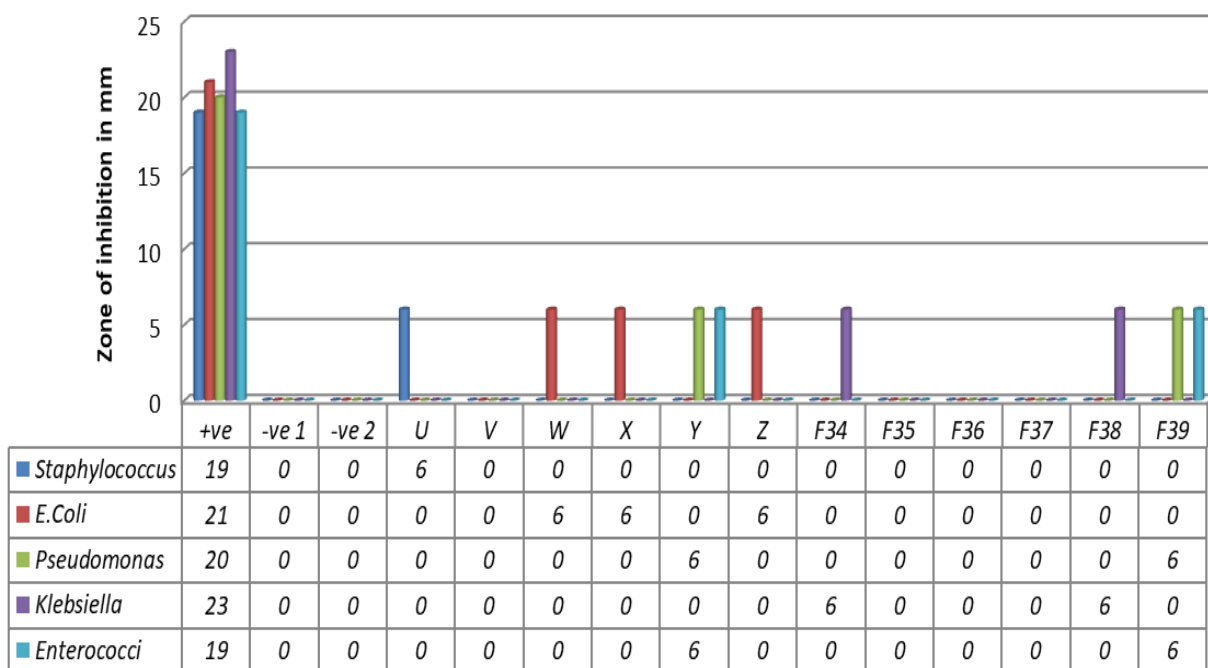
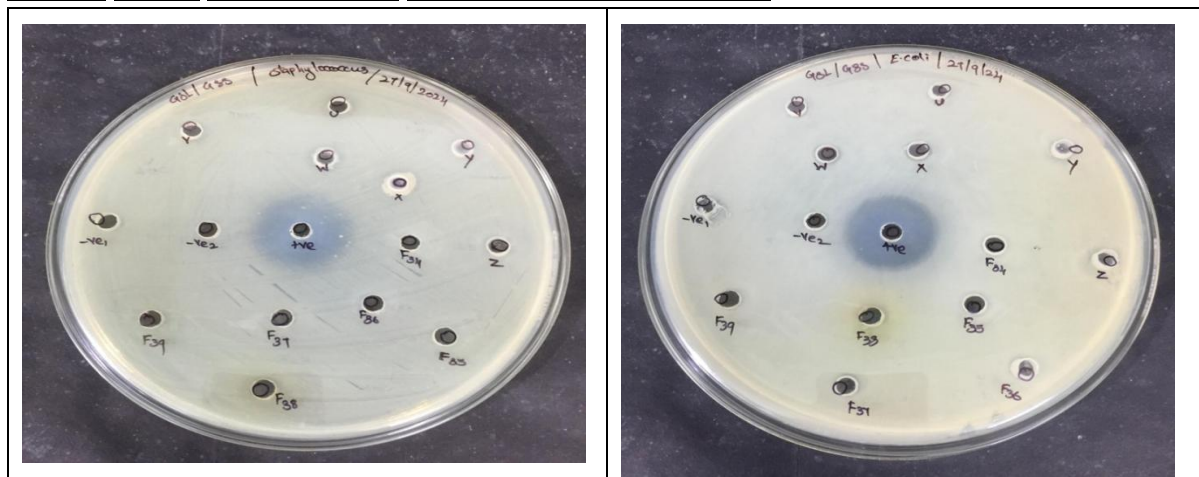
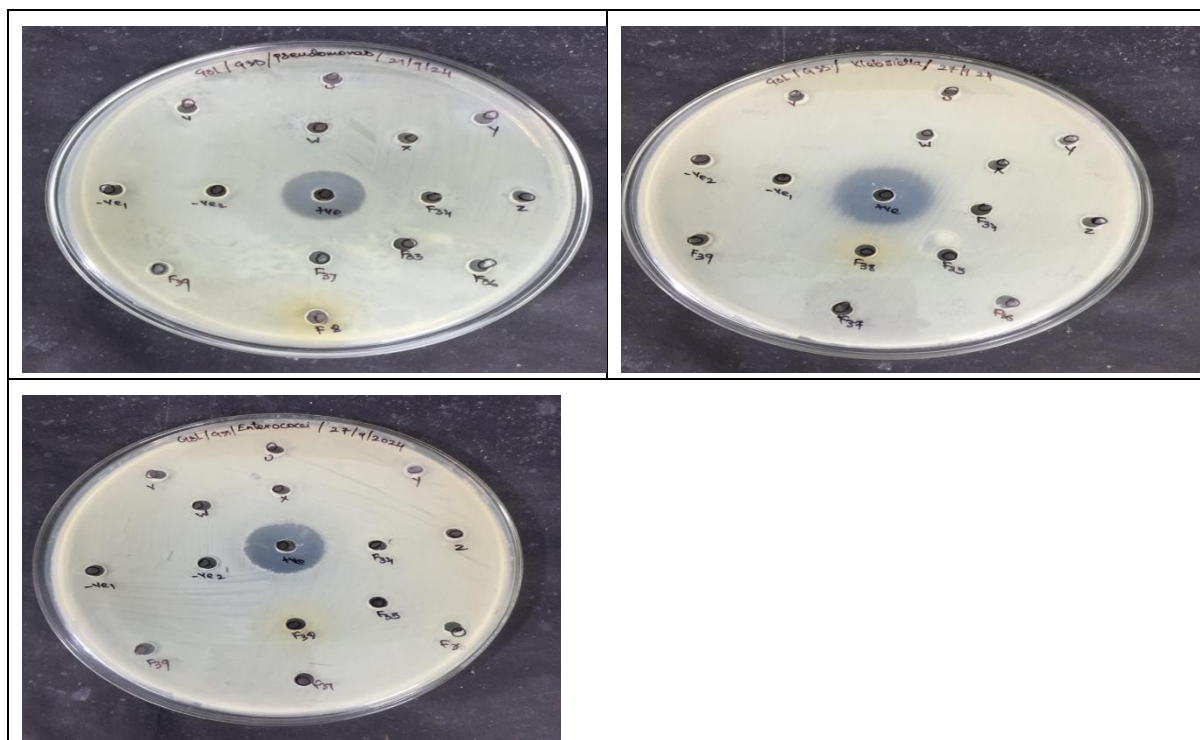


Photo plate showing zone of inhibition of *Gloriosa superba* L. plant extract against *S. aureus*, *E. coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*





The plant extract of *Gloriosa superba* Linn was primarily screened against different pathogens, including *S. aureus*, *E. coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*. The results showed that the highest inhibitory activity showed in *Klebsiella* 23 mm with Gentamycin (50 µg/mL) concentration as a positive control followed by *E. coli* 21 mm, *Pseudomonas* 20 mm and least in *Pseudomonas* 19 mm and *S. aureus* 19mm in positive control and also *Gloriosa superba* L showed moderate antimicrobial activity shown in table no.5.

Conclusion

In the present investigation of screening five plant species extract showed antimicrobial activity against human pathogens *S. aureus*, *E. coli*, *Pseudomonas*, *Klebsiella*, and *Enterococci*. The results confirmed that the plants have potential for therapeutic use in traditional medicine. The Ayurvedic and Unani Pharmacopoeia of India has been documented. The results of the present study support the folkloric usage of the studied plant and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation. Screening of *Vitex negundo*, *Holarrhena antidysenterica* and *Adhatoda vasica*, *Catharanthus roseus*, and *Gloriosa superba* L, having natural organic compounds and identifying active agents, is the need of the hour, because successful prediction of lead molecule and drug-like properties at the onset of drug discovery will pay off later in drug development.

Whole plant (shoot, flower, and tuber) extracts showed antibacterial and antifungal activity with maximum inhibition against to selected

microorganisms. ETOHs of tuber exhibited maximum antibacterial and antifungal activity. Phytochemical screening revealed that alkaloids, triterpenoids, phenols, saponins, and flavonoids could be responsible for the antimicrobial activities of the *G. superba* whole plant extracts. It is evident from the current results that compounds of *G. superba* can be used as antimicrobial agents and ingredients in the human pathogenic diseased formulations in the different pharmaceutical fields

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