DOI: 10.55522/jmpas.V14I4.6853 ISSN NO. 2320 – 7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Tridax procumbens (dagadipala) and *vitex negundo* (nirgudi) leaf extracts as plasmid curing agents

Suneeta Panicker* [1,2], **Mangesh Patil**[1], **Shubham Kude**[1]

[1] Dr. D.Y. Patil Arts, Commerce and Science College, Pune, Maharashtra, India
[2] Development of Mendine Pharmaceuticals Pvt. Ltd., Alipore, Kolkata, West Bengal, India

Corresponding author: Suneeta Panicker, ✉suneetapanicker@gmail.com, **Orcid Id**: https://orcid.org/0000-0002-3228-7718

Development of Mendine Pharmaceuticals Pvt. Ltd., Alipore, Kolkata, West Bengal, India

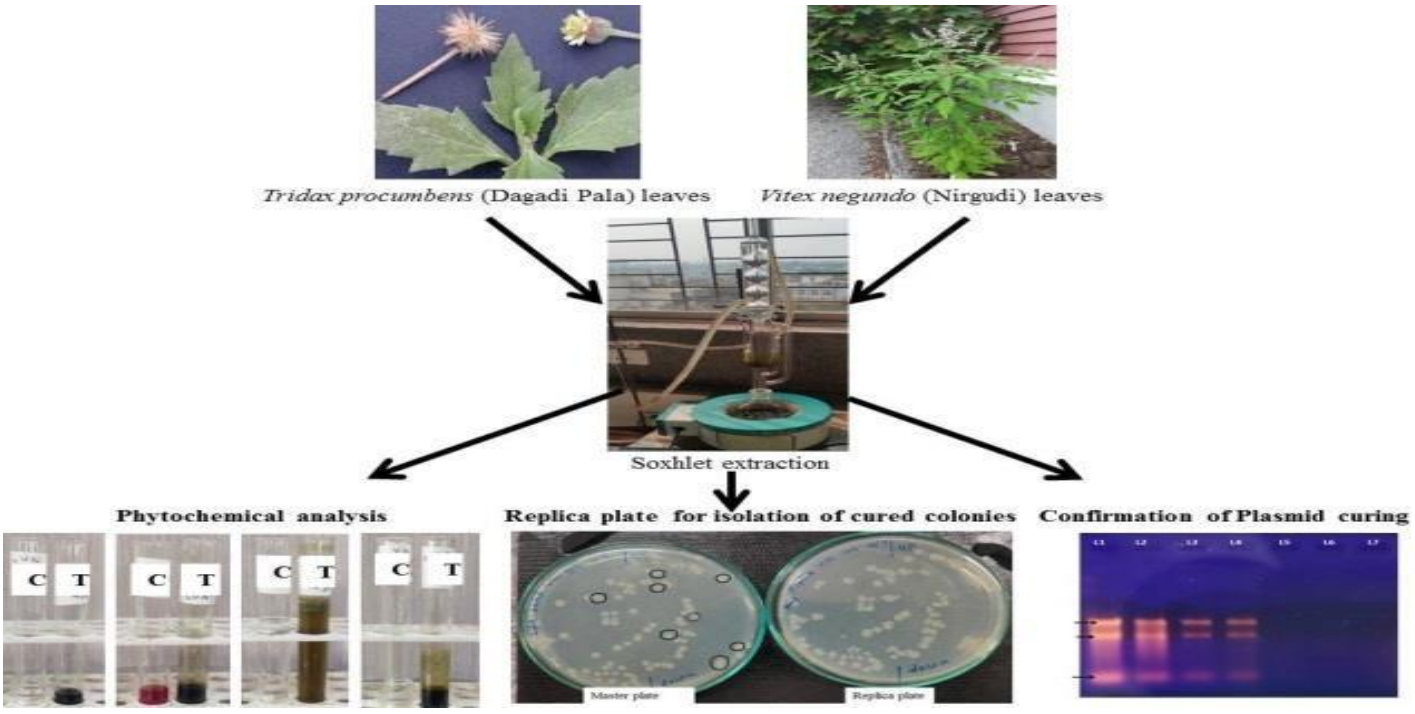
Received - 04-04-2025**, Revised** - 08-07-2025, **Accepted** - 30-08-2025 **(DD-MM-YYYY)**

Refer This Article

Suneeta Panicker, Mangesh Patil, Shubham Kude, 2025. *Tridax procumbens* (dagadipala) and *vitex negundo* (nirgudi) leaf extracts as plasmid curing agents. Journal of medical pharmaceutical and allied sciences, V 14 - I 4, Pages - 27 – 33. Doi: https://doi.org/10.55522/jmpas.V14I4.6853.

ABSTRACT

Plasmid-encoded antibiotic resistance transcends bacterial genus and species, as plasmid-encoded resistance has been observed clinically for various Gram-negative and Gram-positive pathogenic bacteria. Plasmid curing is the treatment of cells with a substance that interferes with plasmid replication. The present study was to investigate the plasmid curing activities of natural compounds over multidrug-resistant bacteria. *E. coli* strains known to have pUC19 and F plasmid were selected to assess the plasmid-curing efficacy of the compounds. The plasmid curing was done by overnight incubating the culture with the test compounds. Chloroform extracts of *Tridax procumbens* (Dagadi pala) and ethanol extract of *Vitex negundo* (Nirgudi) showed promising curing activity. Plasmid isolation and replica plate method confirmed the elimination of the plasmid. Eliminating the plasmid using the natural compound makes it vulnerable to any antibiotic without drug resistance.



Keywords: Antibiotic resistance, Plasmid-curing, pUC19 and F plasmid, *Tridax procumbens*, *Vitex negundo*.

INTRODUCTION

Antimicrobial resistance is recognized as a significant public health concern worldwide, as effective infection treatment is hindered and the cost and duration of treatment are increased. One primary mechanism by which resistance is acquired by bacteria is the uptake of plasmids that encode resistance-mediating proteins. Antibiotic-resistance genes are often carried by plasmids, and their transfer is promoted, leading to the emergence of multidrug-resistant bacteria or "superbugs." Multidrug-resistant bacteria are prevalent in hospitals and communities, creating an urgent need for novel antibacterial agents. Resistance encoded by plasmids is observed for the major classes of antibiotics (including cephalosporins, fluoroquinolines, and aminoglycosides), transcending bacterial genera and species. The elimination of these plasmids from bacteria could make infections treatable with antibiotics to which resistance had previously been exhibited by the bacteria [1].

Many resistance plasmids can be conjugated, with the necessary functions being carried to facilitate DNA transfer between bacterial cells. Mobilizable resistance plasmids tend to be relatively small, with only a few genes being carried. In contrast, conjugative plasmids are bigger, typically 30 kb or more, and substantial DNA is required to encode the conjugation functions necessary for cell-to-cell coupling, particularly in Gram-negative bacteria [2].

Plasmids, which are DNA segments that replicate themselves, are often considered to be home to antimicrobial resistance genes (ARGs). Some of them have been expanded around the world and are frequently transmitted amongst microorganisms. The process of removing plasmid DNA from bacterial isolates is known as plasmid curing to ascertain how plasmid DNA and multidrug resistance are related.

Plasmids from bacterial or eukaryotic cells are eliminated by plasmid curing, a method that interferes with plasmid replication. The primary reason for plasmid curing is the removal of antibiotic resistance from bacterial cells. Traditional plasmid curing agents like Ethidium Bromide (EB) or Acridine Orange (AO) are limited due to their toxicity, which makes them unsuitable for medical applications. Therefore, an investigation into the effects of natural plant-based compounds that can effectively cure plasmids without being toxic or mutagenic is needed. Herbal extracts are considered to be more helpful in this regard, as a safe and effective means of suppressing antibiotic resistance in various pathogenic bacteria is offered by them [3].

Vitex negundo L. (Verbenaceae) is grown widely throughout India, and its leaves and bark are used in traditional medicine. It has been shown by studies that various medicinal properties are present in the plant, including antiulcerogenic, antiparasitic, antimicrobial, and hepato-protective potentials [4]. Mosquito repellent [5] and insecticidal effects [6] are also shown. The aim of this experiment was to evaluate the antibacterial activity of different extracts (petroleum ether, chloroform, ethanol, methanol, and aqueous) against eight prominent Gram-positive and Gram-negative human pathogenic bacteria and to determine the MIC and MBC of the extracts. Additionally, phytochemical screening of the extracts was carried out to assess the presence of different phytochemicals.

Tridax procumbens L. is a common weed found worldwide. Traditionally, it is used by practitioners to treat bronchial catarrh, dysentery, malaria, stomach ache, diarrhoea, high blood pressure, promote blood clotting in cuts, bruises, and wounds, and prevent hair loss. Additionally, insecticidal, antiseptic, parasiticidal, and hepato protective properties are possessed by it, and respiration is significantly depressed by it [7]. Insights into the genetic diversity, gene expression, and secondary metabolite production of *T. procumbens* are provided by these studies, which could have implications for its conservation, management, and potential use in medicine and agriculture.

A green chemistry methodology was used to create a natural plasmid curing agent using a *V. negundo* herbal plant extract. Plant extracts were prepared by the researchers using the Soxhlet technique, and the resultant plant extract was characterized through phytochemical tests, antimicrobial tests, and Minimum Inhibitory Concentration (MIC) assays. The synthesized plant extract was subsequently employed by researchers as a plasmid curing agent against *Escherichia coli* strains, which contained the plasmids pUC19 (Ampicillin resistant) and F plasmid (Tetracycline resistant).

MATERIALS AND METHODS

Collection of plant material (*Tridax procumbens*)

The herbal plant *Tridax procumbens* was collected from a local agricultural land in Moshi, Pune, India.

Collection of plant material (*Vitex negundo*)

The herbal plant *Vitex negundo* was collected from a local agricultural land in Charholi, Pune, India.

Preparation of plant extract (*T. procumbens*)

To extract the bioactive compounds from *T. procumbens*, 10 g of dried leaf powder was taken in a thimble, placed in a Soxhlet apparatus, and 250 ml of chloroform was added. The extraction process was carried out by the researchers for 35 cycles at 64.63°C. The resulting extract was then dried for 1 day to obtain the bioactive components. After the sample was dried, 9.52 g of dried extract was obtained, which was dissolved in 5 ml of Dimethyl sulfoxide (DMSO 99%) [8].

Preparation of plant extract (*V. negundo*)

To prepare *V. negundo* extract, dried *V. negundo* leaves were used. 15.0 g of the dry powder was taken, placed in a thimble, and added to the Soxhlet apparatus while 100 ml of 70% (w/v) ethyl alcohol was added to a round bottom flask. The extraction process was carried out for 35 cycles at 78.37°C. Afterward, the resulting extract was dried for 1 day to obtain the bioactive components. After drying, 12.48 g of dried extract was obtained, which was dissolved in 10 ml of 99% DMSO [4].

Phytochemical analysis

Phytochemical screening tests were conducted by the researchers to detect the presence of both primary and secondary metabolites in the extracts. The protocols for qualitative analyses of alkaloids, flavonoids, steroids, tannins, saponins, carbohydrates, and proteins are listed in Table 1 [9].

Table 1: Methods for qualitative analysis of phytochemical in both extracts

Phytochemical test	Protocol
Flavonoids	1000 µl extract + 1 ml 2% NaOH + 100 µl HCL (diluted)
Saponin (Foam Test)	500 µl extract + 500 µl H_2O + mix thoroughly for 1 min.
Steroids	500 µl extract + 500 µl Chloroform + 500 µl H_2SO_4 (conc.)
Tannins	500 µl extract + 500 µl H_2O + 1 drop $FeCl_3$ (5%)
Alkaloids (Wagner reagent test)	2 ml of filtrate + 1 ml of dil. HCL & Wagner reagent is added
Proteins	2 ml extract + few drops of conc. Nitric acid solution
Carbohydrates	500 µl extract + 500 µl Benedict's reagent + heat

Antimicrobial assay

Bacterial cultures

Test organisms (*Escherichia coli*, *Staphylococcus sp*., *Staphylococcus sp*., *Klebsiella sp*., *Candida sp*.) were procured from the Microbiology Department, Dr. D.Y. Patil ACS College, and the bacterial cultures were maintained on Luria Bertani (LB) agar plates and slants. At the same time, Sabouraud Dextrose Agar (SDA) was used for the yeast.

Preparation of inoculums

A single colony of *Escherichia coli*, *Staphylococcus sp*., and *Klebsiella sp*. was inoculated into 5 ml of sterile LB broth, and a colony of *Candida sp*. was inoculated into sterile Sabouraud Dextrose Broth (SDB) and then the cultures were incubated at 37°C for 18 hours.

Antimicrobial assay for both extracts

Antimicrobial activity was determined by performing a well diffusion assay [10]. Briefly, 0.1 ml of each overnight-grown test culture (*Escherichia coli*, *Staphylococcus sp*., *Staphylococcus sp*., and *Klebsiella sp*.) was spread on Muller Hinton (MH) agar plates. A sterile PDA plate was spread with Candida sp. culture (0.1 ml). 8 mm wells were made in these plates using a cork borer. 100 µl of extract, Tetracycline (100 mg/ml) as positive control, and DMSO as negative control were added to the respective wells. The plates were then incubated at 37°C for 16-18 hrs, and the zone of inhibition was measured.

Minimum inhibitory concentration (MIC)

5 test tubes, each containing 1 ml sterile LB broth and 100 µl of different concentration plant extract (For *T. procumbens* - 0.62, 0.70, 0.77, 0.85, 0.95 µg/ml and *V. negundo* - 0.16, 0.18, 0.20, 0.22, 0.25 µg/ml), were added with 100 µl overnight grown culture of E. coli. These tubes were incubated for 24 hrs at 37°C. The first concentration which did not show growth was recorded as the MIC of that extract [10].

Plasmid curing with *V. negundo* as curing agent

E. coli culture (0.1 ml) containing pUC19 plasmid was inoculated in 1 ml of sterile LB broth containing ranges of curing agent concentrations (0.22µg/ml). Treatment was carried out overnight at 37°C. The culture flask displaying observable turbidity was serially diluted up to 10^{-6}. The last 4 dilutions were spread on a sterile LB agar plate. Plates were incubated overnight at 37°C. After growth on LB agar plates was observed, a replica plate technique was carried out on LB with ampicillin agar plates using the plate with isolated colonies as a master plate. Replica plates were incubated overnight at 37°C. LB agar (Master plate) and LA+ ampicillin (Replica plate) were compared to find the cured colonies. The colonies that were present on the master plate, but not the replica plate, were marked as cured [11].

Plasmid curing with *T. procumbens* as curing agent:

E. coli culture (0.1 ml) containing F plasmid was inoculated with 1 ml of sterile LB broth containing 100 µl of curing agent concentration 0.85 µg/ml. Overnight incubation was performed for the cultures at 37°C. Culture flasks displaying observable turbidity were used for serial dilutions up to 10^{-6}. The last 4 dilutions were spread on the LB agar plate. Overnight incubation was performed for the plates at 37°C. After growth on LB agar plates was observed, a replica plate technique was carried out on LB + Tetracycline agar plates. Overnight incubation was performed for the plates at 37°C. Both LB agar and LB with Tetracycline agar plates were observed and compared to find a missing colony on the antibiotic plate [11].

Confirmation of plasmid Curing:

For confirmation of plasmid curing, plasmid isolation was performed by us using the alkaline lysis method as described by Ehret and Schnapp Inger [12]. Finally, the plasmid DNA pellet was dissolved in 50 µL of TE buffer and agarose gel electrophoresis was then carried out. Confirmation was also achieved by spot inoculation of the cured colonies on antibiotic-containing plates.

RESULT

Preparation of plant extract

The leaves of *Tridax procumbens* were dried and powdered. The active compounds from it were extracted using the soxhlet extraction method and chloroform as a solvent. The solvent in the extract was evaporated, and then 5 ml Dimethyl sulfoxide (DMSO)

was used to resuspend 9.5 gm of dried extract. Similarly, an extract of *Vitex negundo* was prepared using ethanol as a solvent, and 10 ml DMSO was used to resuspend 12.48 gm dried extract.

In the phytochemical test of *V. negundo*, positive results were obtained for the alkaloid, flavonoids, steroid, tannin, and saponin tests, except for the carbohydrate and protein tests, which were found to be negative, as seen in Table 2 above.

Positive results for the alkaloid, flavonoid, steroid, tannin, and saponin tests were indicated by the phytochemical analysis of *V. negundo*. However, negative results were yielded by the carbohydrate and protein tests, suggesting the absence of carbohydrates and proteins in the extract, and these results are observed in Table 3.

Phytochemical analysis

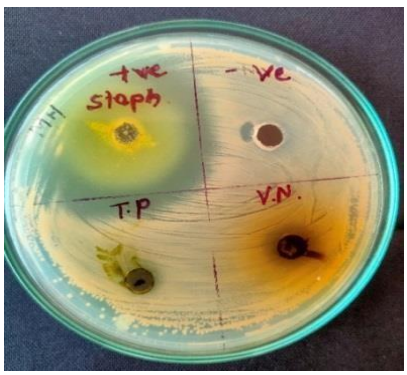
Table 2: Phytochemical Test for *Vitex negundo* (Dagadi Pala)

Phytochemical Tests	Observation	Result
Alkaloid Test	Formation of yellow color	+
Flavonoids Test	Disappearance of yellow color	+
Steroid Test	Upper layer turned reddish brown and lower H_2SO_4 showed yellow color	+
Carbohydrate Test	Nobrown red color observed	-
Protein Test	No yellow color observed	-
Tannin Test	Blackish blue color showed presence of gallic tannins	+
Saponin Test	Formation of foam	+

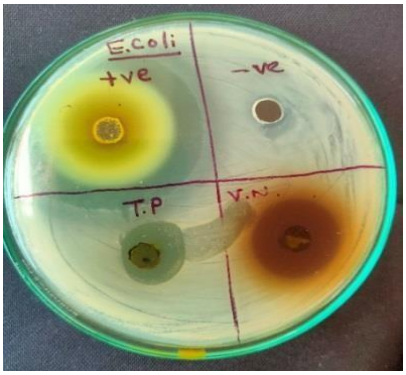
Table 3: Phytochemical Test for *Tridax procumbens* (Dagadi Pala)

Phytochemical Tests	**Observation**	**Result**
Alkaloid Test	Formation of yellow color	+
Flavonoids Test	Disappearance of yellow color	+
Steroid Test	Upper layer turned reddish brown and lower H_2SO_4 showed yellow color	+
Carbohydrate Test	Nobrown red color observed	-
Protein Test	No yellow color observed	-
Tannin Test	Blackish blue color showed presence of gallic tannins	+
Saponin Test	Formation of foam	+

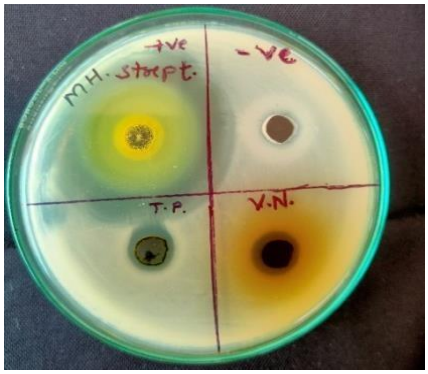
Figure 1: Antimicrobial Activity of both plant extracts; T.P.: *Tridax procumbens*, V.N.: *Vitex negundo*, Positive control: Antibiotic (tetracycline), Negative Control: DMSO



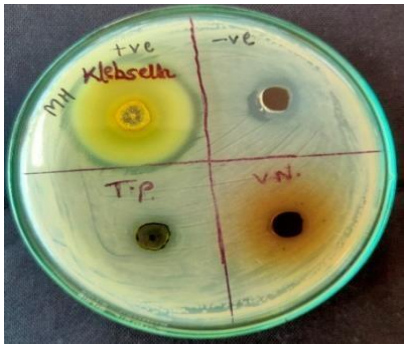
Staphylococcus sp.



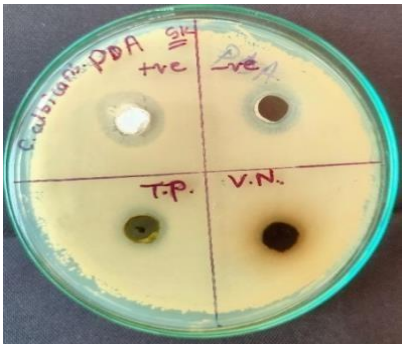
E. coli



Streptococcus sp.



Klebsiella sp.



Candida sp.

Table 4: Curing efficiency of *V. negundo* and *T. procumbens* extracts

Curing Agent	**Concentration of extract used (µg/ml)**	**Total number of colonies**	**Number of colonies cured**	**Curing Efficiency percent (%)**
V. negundo	0.22	47	8	17.02
T. procumbens	0.85	69	19	27.53

Antimicrobial assay

Both plant extracts were shown to be effective against *E. coli* and *Streptococcus sp*., and a zone of inhibition was observed after 18 hours of incubation at 37°C (Figure 1). More prominent activity against *E. coli* than *Streptococcus* sp. was shown by the extracts of both plants.

Minimum inhibitory concentration (MIC)

The *E. coli* culture, when inoculated in different concentrations of *T. procumbens* leaf extract, was observed to show no growth or turbidity after 18 hrs of incubation at 37°C at 0.85 µg/ ml concentration. Therefore, the MIC value of *T. procumbens* was calculated to be 0.85 µg/ ml. The *E. coli* culture, when inoculated in different concentrations of *V. negundo* leaf extract, was observed to show no growth or turbidity after 18 hrs of incubation at 37°C at 0.22 µg/ ml concentration. Therefore, the MIC value of *V. negundo* was calculated to be 0.22 µg/ ml.

Plasmid curing by plant extracts (checking by replica plate method).

The log phase cultures of the test organisms were treated with *V. negundo* (0.22 µg/ml) as a curing agent. A replica plate technique was performed to identify the cured colonies. 8 colonies, which could not grow in the presence of antibiotics, were identified as cured isolates. Thus, the pUC19 plasmid could be cured by 0.22 µg/ml of *V. negundo* extract with an efficiency of 17.02% (Table 4). Similarly, when a curing agent was used in the form of *T. procumbens* (0.85 µg/ml), 19 colonies which could not grow in the presence of antibiotics were identified as cured isolates. Thus, the F plasmid could be cured by 0.85 µg/ml of *V. negundo* extract with an efficiency of 27.53 % (Table 4).

Confirmation of plasmid curing

The presence of plasmid was checked in the cured colonies from the replica plated technique to confirm the plasmid loss from these cells. The traditional alkaline lysis method was followed for plasmid isolation. No presence of plasmid DNA was shown by the cured colonies on the agarose gel electrophoresis (Figure 2 and Figure 3), confirming the curing activity of both plant extracts.

Figure 2: Agarose gel (1.2%) electrophoresis: plasmid isolation from cured colonies using *V. negundo* extract

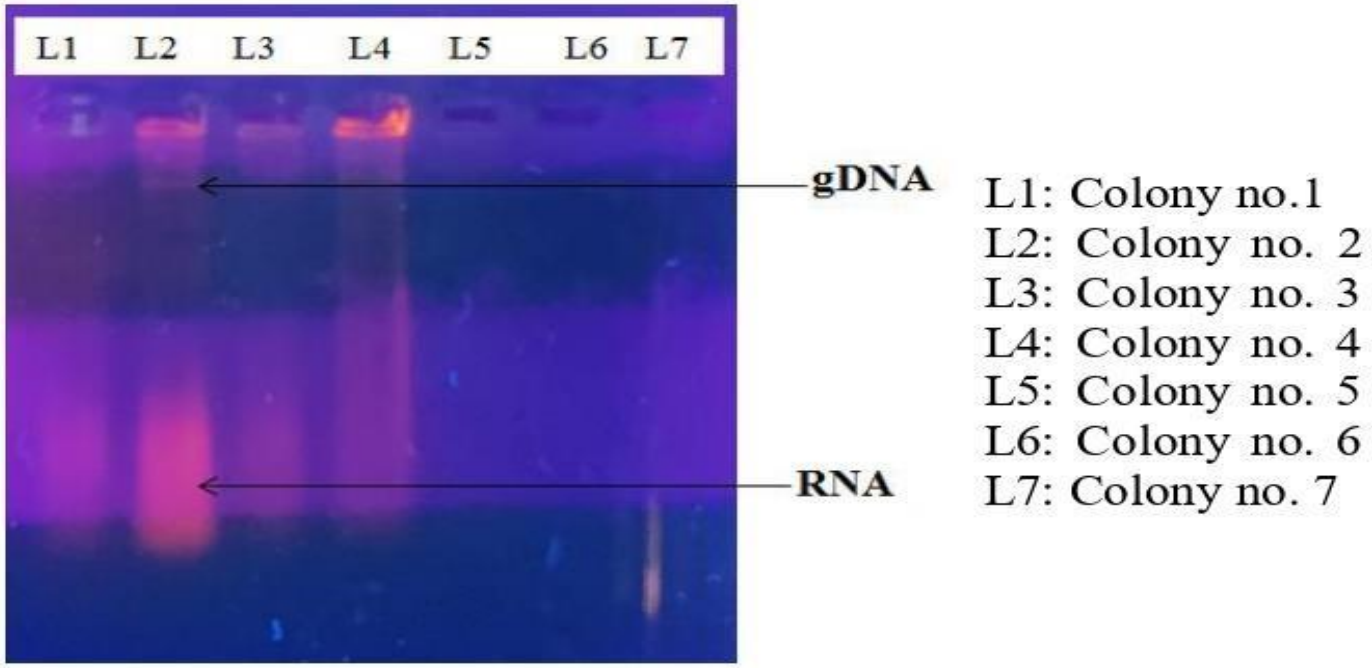


Figure 3: Agarose gel (1.2%) electrophoresis: F plasmid (L 1, 2), pUC19 plasmid (L3, 4) and cured colonies in replica plate of *T. procumbens* (L 5, 6, 7)

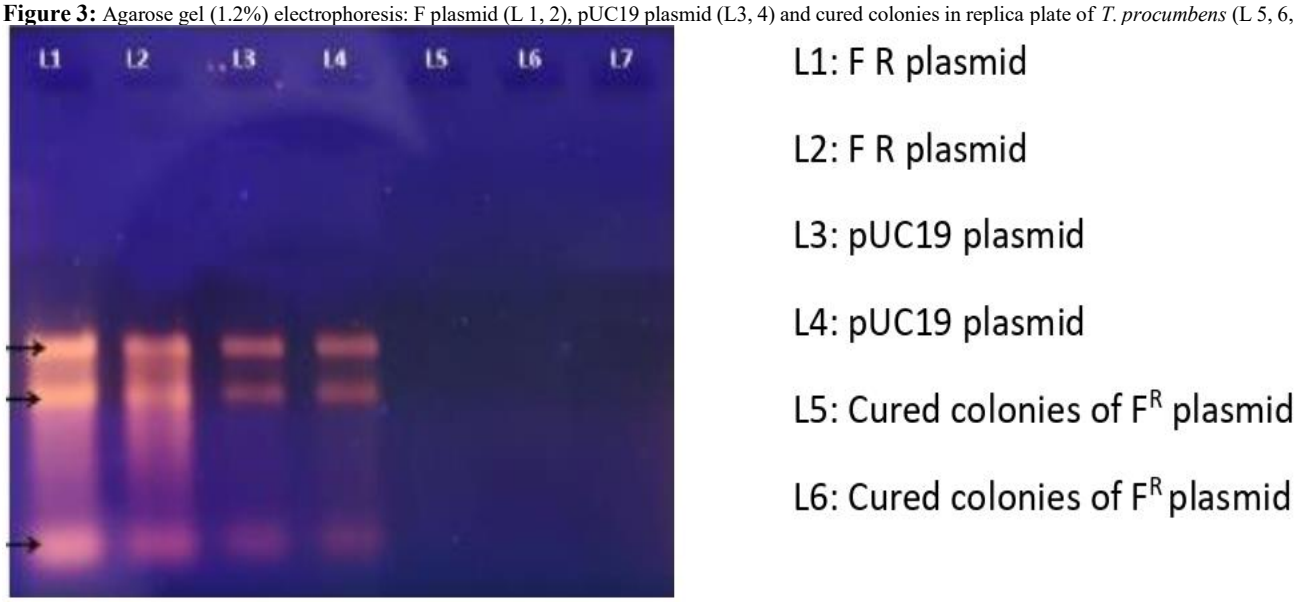


Figure 4: Cured colonies by *V. negundo* and *T. Procumbens* in presence of antibiotic: No growth observed

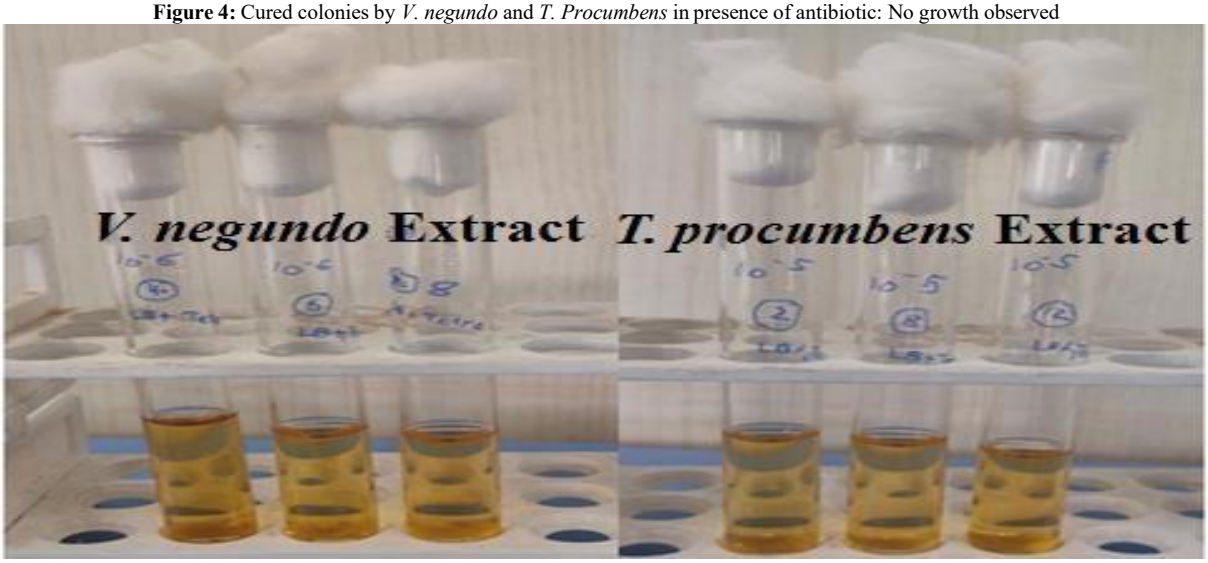
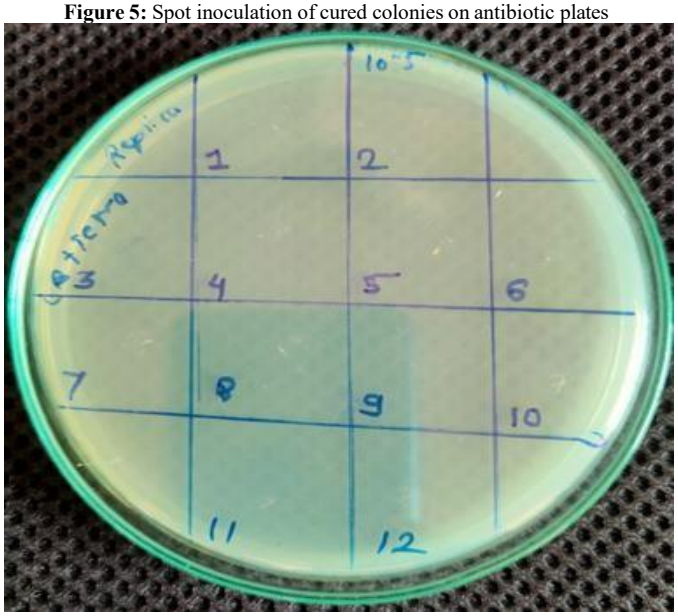


Figure 5: Spot inoculation of cured colonies on antibiotic plates



To confirm cured colonies, plasmid isolation was performed and its presence was checked by agarose gel electrophoresis (Figure 2, 3). Plasmid DNA bands were not shown by both pUC19 and F plasmid-cured colonies. The original plasmid-containing culture was processed for plasmid isolation along with these cured colonies and served as an internal control. Cured colonies were also checked by being grown in LB broth, which had an antibiotic.

However, after incubation at 37°C for 24 hours, no visible growth was observed (Figure4). A spot inoculation was performed on an antibiotic containing plate with isolated cured colonies from the replica plate to reconfirm the curing of their plasmid, and no growth was observed which assured the loss of their plasmid (Figure 5).

DISCUSSION

In our study, the plasmid curing potential of *T. procumbens* and *V. negundo* on *E. coli* strains carrying pUC19 and F plasmids was investigated. Previous research by Shriram et al.13 explored the use of methanol and aqueous extracts from Alpinia galangal rhizomes for plasmid curing in various bacterial strains. It was found that the methanol extract of Alpinia galangal rhizomes exhibited curing efficiency against *Enterococcus faecalis* (VRE), *Staphylococcus aureus* (VRSA), *Salmonella typhi*, and *Shigella sonnei.*

However, curing efficiency was not demonstrated by the methanol extract when it came to *Escherichia coli* (RP4) and *Bacillus subtilis* (pUB110). Additionally, plasmid isolation was adopted as the chosen method for confirming the successful elimination of plasmids after the replica plate technique, differentiating our study from Shriram et al. [13], which relied on the replica plate method. Plasmid isolation is a widely used and reliable technique for confirming plasmid curing.

In our study, the potential of *T. procumbens* and *V. neugendo* was aimed to be evaluated in curing *E. coli* strains harboring pUC19 and F plasmids. Previous research by Patwardhan et al. [11] has utilized lawsone, a compound derived from henna leaves, for the curing of plasmid from *E. coli* (pRK 2013) and *A.*

baumannii (pUPI281) strains.

To confirm the effectiveness of the plasmid curing, plasmid isolation was conducted and the replica plate method was employed using LB Agar. At the same time, confirmation was made by Soman et al. [14] through replica plate using MH agar, and it was noted that the plasmid curing activity of *Coriandrum sativum* against *Pseudomonas sp*. was found to be relatively low compared to the other tested strains. In the present study, conclusive evidence of the cured colonies was provided by both methods.

The importance of considering multiple factors and approaches when exploring plasmid-curing strategies is underscored by the divergence in methodology between our study and the previous research. Valuable insights into the potential applications of *Tridax procumbens* and *Vitex negundo* in plasmid elimination were gained by our focus on antimicrobial testing, MIC determination, and plant extracts. On-going efforts in developing novel approaches to combat antibiotic resistance are contributed to by this study, and valuable insights for future research are provided.

Additional investigations should be conducted to investigate the mechanisms underlying plasmid curing and the specific compounds responsible for the observed effects. By refining our understanding of these processes, more targeted and efficient strategies can potentially be developed to combat antibiotic resistance mediated by plasmids in bacteria.

CONCLUSION

In conclusion, the growing knowledge of plasmid curing strategies is contributed to by our study. The plasmid curing potential of *Tridax procumbens* and *Vitex negundo* on *E. coli* strains is explored, providing valuable insights into the development of alternative approaches to combat antibiotic resistance. Furthermore, robustness is added to our study's findings and the reliability of the observed results is strengthened by using plasmid isolation to confirm plasmid elimination.

ACKNOWLEDGMENT

Authors acknowledge the Management of Dr. D.Y. Patil Arts, Commerce and Science College, Pimpri, Pune for supporting and providing the opportunity to carry out this research work.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Buckner MMC, Ciusa ML, Piddock LJV, 2018. Strategies to combat antimicrobial resistance: anti-plasmid and plasmid curing. FEMS Microbiol Rev. 42(6), Pages 781-804. Doi: https://doi.org/10.1093/femsre/fuy031.

2. Smillie C, Garcillán-Barcia MP, Francia MV, et al, 2010. Mobility of plasmids. Microbiol Mol Biol Rev. 74(3) Pages 434-52. Doi: https://doi.org/10.1128/mmbr.00020-10.

3. Oludare TO, Agbi M, Thonda OA, et al, 2019. Pre/Post Plasmid Curing and Killing Kinetic Reactivity of DiscoreaBulbifera Linn Against Multiple Antibiotics Resistant Clinical Isolates, Using Escherichia Coli as A Case Study. Int. J. Cell Sci & Mol. Bio. 6(2), Pages 46-56. Doi: https://doi.org/ 10.19080/ IJCSMB.2019.06.555685.

4. Subramanian A, Mahalingam S, 2015. Antibacterial behaviour of Vitex negundo extract assisted ZnO nanoparticles against pathogenic bacteria, J. Photochem. Photobiol. B. 146, Pages 52-57. Doi: https://doi.org/10.1016/j.jphotobiol.2015.02.020.

5. Hebbalkar DS, Hebbalkar GD, Sharma RN, et al, 1992. Mosquito repellent activity of oils from Vitex negundo Linn leaves. Ind. J. Med. Res. 95, Pages 200-203.

6. Prajapati V, Tripathi AK, Khanuja SPS, et al, 2003. Anti-insect screening of medicinal plants from Kukrail Forest, Lucknow, India. Pharma. Biol. 41, Pages 166-170. Doi: https://doi.org/10.1076/phbi.41.3.166.15092.

7. Jhample S, Gajdhane S, Kasabe P, et al, 2015. Phytochemical screening and in vitro antimicrobial activity of Tridax procumbens L. Life Sci. Info. Pub. 1(1), Pages 44-53. Doi: https://doi.org/10.26479/2015.0101.05.

8. Musale A, Bhandwakar S, Ghodke D, et al, 2023. A Phytochemical Constituents and Pharmacological Properties of plant Tridaxprocumbeans. Research J. Pharmacogn. Phytochem. 15(4), Pages 288-2. Doi: https://doi.org/10.52711/0975-4385.2023.00045.

9. Yadav M, Chatterji S, Gupta SK et al, 2014. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int. J. Pharm. Pharm. Sci. 6(5), Pages 539-542.

10. Panda SK, Thatoi HN, Dutta SK, 2009. Antibacterial activity and phytochemical screening of leaf and bark extracts of Vitex negundo l. from similipal biosphere reserve. Orissa J. Med. Plants Res. 3(4), Pages 294-300.

11. Patwardhan RB, Dhakephalkar PK, Chopade BA, et al, 2018. Purification and Characterization of an Active Principle, Lawsone, Responsible for the Plasmid Curing Activity of Plumbago zeylanica Root Extracts. Front. Microbiol. 9, Pages 2618. Doi: https://doi.org/10.3389/fmicb.2018.02618.

12. Ehrt S, Schnappinger D, 2003. Isolation of Plasmids from E. coli by Alkaline Lysis. In: Casali, N., Preston, A. (eds) E. coli Plasmid Vectors. Methods Mol. Biol. 235, Pages 75-78. Doi: 10.1385/1-59259-409-3:75.

13. Shriram V, Kumar V, Javed M, et al, 2013. Curing of Plasmid-Mediated Antibiotic Resistance in Multi-Drug-Resistant Pathogens Using Alpinia galanga Rhizome Extract Adv. Bio. Tech. 13(01), Pages 01-05. Doi: 10.1016/j.jep.2009.03.028.

14. Soman Y, Mohite J, Thakre S, 2015. Plasmid Curing Activity by Seed Extracts of Cuminum cyminum, Coriandrum sativum and Myristica fragrans Houtt and Fruit Peel Extracts of Orange, Banana and Pineapple Against Gram Negative Bacteria Int. J. Curr. Microbiol. App. Sci. Special Issue-2, Pages 302-316.