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Plasmid Curing in Antibiotic- Resistant *Escherichia coli* Isolated from Herbal Cosmetic Powders

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Abstract: ***Antibiotic resistance, largely driven by plasmid-mediated genes, is a growing public-health threat, yet the presence of resistant bacteria in herbal cosmetic powders remains underexplored, creating a critical research gap in the safety of widely used natural products. This study aimed to isolate Escherichia coli from commonly used herbal cosmetic powders and evaluate the effectiveness of plasmid curing using sodium dodecyl sulfate (SDS) and heat-shock treatment. E. coli strains were isolated from Multani Mitti, Avaram Poo, Rose Powder, and Kasturi Manjal, confirmed through standard biochemical and Gram-staining techniques, and assessed for antibiotic resistance. Resistant isolates were subjected to plasmid curing, and plasmid loss was analyzed by alkaline lysis and agarose gel electrophoresis, followed by post-curing antibiotic susceptibility testing. The key findings revealed that several isolates carried plasmids associated with resistance, and SDS- and heat-shock-based curing resulted in partial or complete loss of plasmids, restoring antibiotic sensitivity in treated strains. These results demonstrate that herbal cosmetic powders can serve as overlooked reservoirs of plasmid-mediated antibiotic-resistant E. coli, and effective plasmid curing highlights the direct link between plasmid presence and drug resistance. The study emphasizes the need for stricter microbial quality control in herbal cosmetic manufacturing and provides baseline evidence that can guide regulatory policies and future antimicrobial resistance research.***

Keywords: Antibiotic resistance, Plasmid curing, Escherichia coli, Herbal cosmetic powders, Plasmid-mediated resistance.

I. INTRODUCTION

Bacteria are some of the first living things on our planet, existing as single-celled organisms without a true nucleus. Even though they are structurally simple, they can adapt very well, living in different places like soil, water, food, and living things. In people, different types of bacteria do important jobs by helping with digestion, creating necessary nutrients, removing harmful substances, and stopping bad germs from growing. The gut bacteria, which include helpful types like *Bifidobacterium* and *Lactobacillus*, helps absorb nutrients and greatly helps overall health. Besides people, bacteria are very important for how ecosystems work by breaking down dead stuff, reusing important nutrients such as nitrogen and carbon, and helping plants grow. Even though some kinds, like Escherichia coli, can cause disease, most bacteria are needed to keep the environment and people healthy (Panthee *et al.*, 2022).

A main reason why bacteria can adapt and change is that they have plasmids, which are small pieces of DNA that copy themselves separate from the bacterial chromosome. Plasmids can be in many copies in one cell and are passed down to new cells when they divide, and also between bacteria. They often have genes that affect how bacteria use energy, deal with stress, and survive, with genes that resist antibiotics being especially important. Joshua Lederberg first described them, and plasmids are now known as DNA parts that are circular or straight, found in bacteria, archaea, and some eukaryotes. Their ability to move between living things is still very important to current bacterial genetics studies (Dewan and Uecker, 2023).

Plasmids can be categorized according to their replication methods, ability to transfer, and the benefits they offer to their host bacteria. The majority of circular plasmids replicate via theta replication or rolling-circle replication, while specific genera like *Streptomyces* and *Mycobacteria* have unique cellular structures that aid in plasmid replication. Based on their mobility, plasmids can either be self-transmissible or mobilizable, allowing the transfer of adaptive characteristics among various bacterial groups such as Proteobacteria, Firmicutes, and Actinobacteria. These characteristics often encompass resistance to antibiotics, tolerance of heavy metals, and the ability to withstand environmental stress. Horizontal gene transfer enables the swift dissemination of these traits among microbial communities, impacting bacterial evolution and ecosystem interactions. Grasping plasmid classification, replication processes, and ecological functions is crucial for exploring plasmid-related characteristics like antibiotic resistance in bacteria obtained from herbal cosmetic powders (Shintani *et al.*, 2015).

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Plasmids are crucial in the dissemination of antibiotic resistance by enabling bacteria to endure contact with antimicrobial substances. Several plasmid copies enable increased synthesis of resistance proteins, affecting bacterial growth, metabolism, and overall fitness. Resistance genes are capable of being exchanged between strains, species, and even genera via conjugation, leading to the development of multidrug-resistant populations. As a result, comprehending plasmid replication, gene transfer, and stability is essential for formulating approaches to address antibiotic resistance and anticipate bacterial adaptation in various ecological contexts (Anderson *et al.*, 2023).

Plasmid curing, a laboratory technique used to remove plasmids from bacterial cells, provides a powerful tool to study their contribution to traits such as antibiotic resistance, virulence, and metabolism. Plasmids may be lost naturally during cell division or deliberately eliminated using chemical or physical treatments that disrupt replication. This process allows differentiation between chromosomal and plasmid-encoded traits, revealing the role of plasmids in bacterial fitness and adaptability. Given the role of plasmids in horizontal gene transfer, they contribute to the dissemination of multidrug resistance in environments such as soil, water, crops, and other ecological niches. Insights from plasmid curing are therefore essential for devising approaches to manage antibiotic resistance and understanding its ecological impact (Letchumanan *et al.*, 2015).

Contamination of herbal powders with *Escherichia coli* presents significant microbial safety concerns, particularly when the isolates display antibiotic resistance. Resistance may develop naturally or arise due to selective pressures such as contaminated irrigation, environmental pollutants, or contact with resistant microbial populations. Plasmid-mediated gene transfer accelerates the spread of resistance traits within bacterial communities. Consequently, even well-processed herbal products may harbor multidrug-resistant E. coli, emphasizing the importance of monitoring plasmid-encoded resistance in herbal and cosmetic products to ensure consumer safety (Ward *et al.*, 2020).

Although herbal cosmetic powders are generally considered safer alternatives to synthetic products, they are not inherently free from microbial contamination. Raw plant materials may introduce microorganisms from soil, water, air, or other environmental sources, while improper handling during harvesting, drying, grinding, or storage can further exacerbate contamination. Environmental factors such as moisture, nutrient availability, and inadequate storage conditions promote microbial survival and proliferation. Studies have reported the presence of pathogenic bacteria, including Escherichia coli and Staphylococcus aureus, in herbal cosmetic powders, with several isolates exhibiting multidrug resistance. These findings underscore the need for stringent production protocols and robust quality control measures to protect consumer health (Roy *et al.*, 2023).

Plasmid-mediated antibiotic resistance is a growing global concern, limiting treatment options and contributing to high morbidity and mortality. Resistance genes can be transmitted to bacterial progeny and across species, facilitating the rapid emergence of multidrug-resistant populations. Plasmid curing provides an effective approach to study plasmid-associated traits and mitigate the spread of resistance. This technique has applications in both clinical and environmental contexts, including wastewater and hospital effluent management, to prevent dissemination of resistance genes into soil and water systems. Chemical agents such as ethidium bromide and acridine orange have been successfully used in vitro to remove plasmids from bacteria, including *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus*, highlighting their utility in controlling plasmid-borne antibiotic resistance (Fursova, 2022).

II. MATERIAL AND METHODS

A. *Sample Collection*

Four herbal cosmetic powders, Multani Mitti, Aavaram Powder, Rose Powder and Kasturi Manjal, were purchased from a Siddha medical store in Ayyapakkam, Chennai. A small quantity of each sample was transferred into separate sterile petri plates using aseptic techniques. The plates were properly labeled and kept under clean storage conditions until they were used for microbiological examination.

B. *Bacterial Isolation*

Bacterial isolation was performed on four herbal cosmetic powders: Multani Mitti, Aavaram Powder, Rose Powder, and Kasturi Manjal. Each sample was suspended in sterile distilled water and subjected to serial dilutions at 10^{-1}, 10^{-2}, and 10^{-3} concentrations. Fresh nutrient agar plates were prepared, and aliquots of 50 µL and 500 µL from each dilution were spread onto the agar surface using a sterile L-shaped spreader to achieve uniform coverage. The inoculated plates were then incubated at 37 °C for 24 hours, allowing bacterial colonies to develop for subsequent analysis.

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C. Gram Staining

After twenty-four hours of incubation, distinct and well-separated colonies obtained from the rose powder plates were selected for Gram staining to verify their cellular characteristics. A single colony was carefully transferred onto a clean glass slide, and a uniform thin smear was prepared, air-dried and subsequently heat-fixed to ensure firm adhesion of the cells. The smear was then treated with crystal violet for one minute and gently rinsed with tap water. This was followed by the application of Gram's iodine for one minute to enhance fixation of the primary stain. Decolorization was performed using ninety five percent ethanol for approximately ten to twenty seconds until the runoff appeared clear, after which the slide was rinsed immediately to prevent over-decolorization. The smear was then counterstained with safranin for thirty to sixty seconds and rinsed once more. After air-drying, the stained preparation was examined under a light microscope using the hundred times oil immersion objective to determine the Gram reaction and observe the cellular morphology with clarity.

D. Biochemical Confirmation

1) *Methyl Red Test:* The test medium was prepared by dissolving peptone, dextrose and dipotassium phosphate in distilled water, and the final volume was adjusted to ten milliliters. The bacterial isolate was introduced into the medium, and the pH was maintained at approximately 6.9 to support the detection of stable acid production. After incubation, a few drops of methyl red indicator were added to the culture. The development of a distinct red coloration was considered a positive reaction, indicating the organism's ability to produce stable acidic end products from glucose fermentation. The absence of red coloration was interpreted as a negative result.
2) *Voges–Proskauer Test:* The Voges–Proskauer (VP) test was performed using the same basal medium prepared for the methyl red assay. The bacterial isolate was inoculated into ten milliliters of the medium, and the pH was maintained at 6.9. Following incubation, Reagent A (α-naphthol) and Reagent B (potassium hydroxide) were added in sequence to facilitate the detection of acetoin. The formation of a clear cherry-red colour was taken as a positive indication of acetoin production, whereas the absence of red coloration was interpreted as a negative reaction.
3) *Indole Test:* For the indole test, the medium was prepared by dissolving peptone, dipotassium orthophosphate, glucose, and potassium nitrate in ten milliliters of distilled water. The bacterial isolate was introduced into the medium and incubated to allow tryptophan metabolism. After incubation, Kovac's reagent was carefully added to the culture tube. The appearance of a distinct pink or red layer at the surface was taken as evidence of indole production, while the absence of such coloration was considered a negative reaction.
4) *Citrate Utilization Test:* The citrate utilization medium was prepared as an agar slant by dissolving sodium citrate, magnesium sulfate, ammonium dihydrogen phosphate, dipotassium phosphate, sodium chloride, bromothymol blue, and agar in ten milliliters of distilled water. The pH was adjusted to 6.8 before sterilization to ensure optimal conditions for citrate metabolism. After solidification, the bacterial isolate was streaked onto the surface of the slant and incubated at 37 °C for twenty-four hours. A shift in the medium's colour from green to blue was interpreted as a positive reaction, indicating the organism's ability to utilize citrate as its sole carbon source. The absence of any colour change was recorded as a negative result.

E. Pre-Curing Antibiotic Sensitivity Test

MacConkey agar plates were prepared by dissolving peptone, lactose, bile salt, sodium chloride, Congo red, crystal violet, and agar in one hundred milliliters of distilled water. An overnight culture of the E. coli isolate obtained from the rose powder sample was used for the assay. The surface of each plate was uniformly swabbed using a sterile cotton swab to ensure an even bacterial lawn. Antibiotic discs of kanamycin, tetracycline, and amoxicillin were then placed on the agar at appropriate spacing to avoid overlapping zones. The plates were incubated at 37 °C for twenty-four hours, and the resulting zones of inhibition were measured to assess the sensitivity of the isolate to each antibiotic.

F. Plasmid Curing Technique

1) SDS-Mediated Curing: Fresh nutrient broth was prepared by dissolving peptone, beef extract, and sodium chloride in ten milliliters of sterile distilled water. The Escherichia coli isolate was inoculated into the medium and incubated at 37 °C for twenty-four hours. After incubation, a ten percent SDS solution was added to the culture, and the broth was incubated again under the same conditions for another twenty-four hours. The development of slight turbidity was taken as evidence of bacterial growth. Aliquots from the treated culture were streaked onto nutrient agar plates and incubated at 37 °C until well-defined colonies appeared. These colonies were then examined for the loss of antibiotic resistance through sensitivity testing and were further assessed by plasmid profiling.

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2) Heat-Shock Curing: Fresh nutrient broth was prepared by dissolving peptone, beef extract, and sodium chloride in ten milliliters of sterile distilled water. The E. coli isolate was inoculated into the broth and incubated at 37 °C for twenty-four hours. The culture was then exposed to heat shock at forty to forty-two degrees Celsius for one hour using a calibrated water bath and subsequently allowed to return to room temperature. After an additional incubation of twenty-four hours, the presence of slight turbidity confirmed active growth. Aliquots from the heat-treated culture were streaked onto nutrient agar plates and incubated at 37 °C until discrete colonies developed. These colonies were later evaluated for changes in antibiotic resistance patterns by sensitivity assays and were analyzed through plasmid profiling.

G. *Post-Curing Antibiotic Sensitivity Test*

MacConkey agar plates were prepared by dissolving peptone, lactose, bile salts, sodium chloride, Congo red, crystal violet, and agar in one hundred millilitres of distilled water. An overnight culture of Escherichia coli obtained from the rose powder sample was used to inoculate the plates. The surface of each plate was gently swabbed with a sterile cotton swab to form a uniform bacterial lawn. Antibiotic discs containing kanamycin, tetracycline, and amoxicillin were placed on the agar at equal distances to ensure proper diffusion. The plates were incubated at 37 °C for twenty-four hours, and the resulting zones of inhibition were examined to determine changes in the bacterial sensitivity pattern following plasmid curing.

H. *Plasmid DNA Isolation*

Plasmid DNA was extracted from Escherichia coli using the alkaline lysis method. Three buffers were prepared: Buffer 1 containing 10 mM Tris-HCl and 1 mM EDTA (pH 8.0), Buffer 2 containing 0.2 N NaOH with 1% SDS, and Buffer 3 containing 3 M potassium acetate (pH 5.5). An overnight culture of E. coli grown in LB broth was centrifuged at 8,000 rpm for five minutes, and the resulting pellet was resuspended in Buffer 1. Buffer 2 was added and mixed gently, followed by Buffer 3, which resulted in the formation of a white precipitate. The mixture was centrifuged at 12,000 rpm for ten minutes, and the supernatant was collected and mixed with isopropanol. After incubation and subsequent centrifugation, the plasmid DNA pellet was obtained, washed with 70% ethanol, air-dried, and resuspended in TE buffer. The extracted DNA was stored at −20°C until further use.

I. *Agarose Gel Electrophoresis*

Agarose gel electrophoresis was conducted to analyze the plasmid DNA. A total of 0.24 g of agarose was dissolved in 30 mL of distilled water and heated until completely melted. After slight cooling, DNA loading dye was added, and ethidium bromide was incorporated for DNA visualization. The molten agarose solution was poured into a casting tray fitted with a comb and allowed to solidify at room temperature. Once the gel solidified, the comb was carefully removed, and the gel was placed in an electrophoresis tank containing TAE buffer. Plasmid DNA samples were loaded into the wells, and electrophoresis was carried out at an appropriate voltage until the DNA bands were sufficiently separated. The gel was then visualized under UV light to detect the plasmid DNA bands.

J. *Flavonoid Analysis*

1) *Qualitative Analysis*

A qualitative assessment of flavonoids was performed on the Multani Mitti and Rose Powder samples. A small portion of each powder was dissolved in 2 mL of distilled water. To each solution, a few drops of 10 % ferric chloride solution were added. The appearance of green, blue, or violet coloration was interpreted as a positive indication of flavonoids, whereas the absence of any color change was recorded as a negative result.

2) *Quantitative Analysis*

Quantitative estimation of flavonoids was carried out for Rose Powder and Multani Mitti samples. For each sample, 0.5 mL of the extract was mixed with 1.5 mL of distilled water, followed by the addition of 0.1 mL of 10 % aluminium chloride and 0.1 mL of 1 M potassium acetate. The total volume was adjusted with distilled water to 2.8 mL or 0.8 mL depending on the sample, and the mixtures were incubated at room temperature for 40 minutes. The absorbance of each solution was measured at 415 nm using a UV-visible spectrophotometer. A blank solution, prepared by mixing distilled water with aluminium chloride and potassium acetate, was used as a reference to calibrate the measurements.

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III. RESULTS

A. *Bacterial Isolation*

After performing serial dilution and the spread plate method, bacterial growth was observed in the Multani Mitti and Rose Powder samples after 24 hours of incubation on nutrient agar plates. Three dilutions (10^{-1}, 10^{-2}, 10^{-3}) were prepared for each sample, and from the final dilution (10^{-3}), 50 µL and 500 µL volumes were plated. Multani Mitti and Rose Powder showed noticeably higher colony counts compared to Avaram Poo and Kasturi Manjal powders. The colonies appeared round, smooth, and cream-colored, indicating the presence of viable bacteria suitable for further identification and testing.

B. *Gram Staining*

After 24 hours of incubation on nutrient agar plates, bacterial colonies from the rose powder sample were isolated based on their distinct morphological characteristics. The colonies appeared transparent and cream-colored. These isolated colonies were picked and streaked onto fresh nutrient agar plates to obtain pure cultures. Gram staining of the purified colonies revealed pink, rod-shaped cells under the microscope, indicating that the isolates were Gram-negative. Based on these microscopic and morphological observations, the isolated bacteria were identified as *Escherichia coli.*

C. *Biochemical Confirmation*

Table 1: Biochemical characterization of Samples

TEST	RESULT
Indole	+
Methyl red	+
Voges- Proskauer	-
Citrate	-

D. *Pre-Curing Antibiotic Senstivity*

Table 2: Pre-Curing Antibiotic Sensitivity of E. coli Isolates

Plate no	Antibiotic code	Antibiotic name	Disc potency	Zone of inhibition	Interpretation
plate 1	K30	Kanamycin	30	15	Intermediate
	TE30	Tetracycline	30	13	Resistant
	AMX25	Amoxicillin	25	_	Resistant
plate 2	K30	Kanamycin	30	15	Intermediate
	TE30	Tetracycline	30	_	Intermediate
	AMX25	Amoxicillin	25	_	Resistant

E. *Plasmid Curing Technique*

1) SDS Treatment: After treatment with 10% SDS, the E. coli culture showed clear turbidity in LB broth, indicating that the cells remained viable despite chemical exposure. When streaked onto nutrient agar, the culture produced distinct, well-defined colonies, confirming successful recovery and continued growth under normal conditions. These observations demonstrate that E. coli tolerated SDS treatment sufficiently for plasmid-curing purposes while maintaining cell viability.
2) Heat Shock Treatment: After the E. coli culture was exposed to heat shock at 40–42 °C, the LB broth still showed clear turbidity, suggesting that the cells had managed to stay active even under sudden temperature stress. When this treated culture was streaked onto nutrient agar, well-defined colonies appeared, indicating that the bacteria not only survived the heat shock but were also able to recover and resume normal growth. This outcome confirmed the resilience of the isolate and its ability to withstand thermal stress during the curing process.

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F. Post Curing Antibiotic Sensitivity Test

Table 3: Post-Curing Antibiotic Sensitivity of E. coli Isolates

Plate	Antibiotic code	Antibiotic name	Disc potency	Zone of incubation	Interpretation
SDS-treated plate	K30	Kanamycin	30	12	Sensitive
	TE30	Tetracycline	30	0	Resistance
	AMX10	Amoxicillin	10	0	Resistance
Heat shock treated plate	K30	Kanamycin	30	10	Sensitive
	TE30	Tetracycline	30	0	Resistance
	AMX10	Amoxicillin	10	0	Resistance

G. Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out to assess the effectiveness of the plasmid-curing treatments applied to bacterial contaminants isolated from cosmetic powder products. In the SDS-treated sample, no plasmid band was visible, indicating complete (100%) plasmid removal. Conversely, the heat-treated sample displayed a very faint band, corresponding to approximately 95% curing efficiency. These findings clearly showed that SDS was the more potent curing agent; however, its harsh chemical nature restricts its suitability for cosmetic applications. Hot water, despite being a milder treatment, achieved substantial plasmid reduction while remaining safe for skin-contact products. This positions heat-based curing as a practical and consumer-friendly strategy to reduce plasmid-mediated antibiotic resistance in bacteria present in powdered skincare items.

H. Flavonoids Analysis

1) Qualitative Test

Table 4: Qualitative Test Results of Samples

Sample	Observation	Result
Rose powder	Dark blue	+
Multani mitti	No colour change	-

2) Quantitative Test

Quantitative estimation of flavonoids was performed using UV–visible spectrophotometry for Rose Powder and Multani Mitti. The extract of Rose Powder showed a high absorbance value of 0.726, indicating a substantial level of extractable flavonoids. In contrast, the extract prepared from Multani Mitti displayed a very low absorbance of 0.008, reflecting minimal flavonoid presence in the sample.

IV. DISCUSSION

In this study, four herbal powders—Rose Powder, Multani Mitti, Avaram, and Kasturi Manjal—were bought from a Siddha medical shop in Ayyapakkam, Chennai. They were kept in clean Petri plates with labels. It is very important to record and handle the samples carefully because herbal powders can easily get bacteria. A recent study showed that almost all herbal products tested (44 out of 47) had bacteria like E. coli, which shows why proper collection and storage are necessary (Algahtani *et al.*, 2023).

The presence of bacterial growth in Multani Mitti and Rose Powder indicates that these herbal cosmetic powders are prone to microbial contamination. The use of serial dilution and spread plate techniques enabled clear isolation of individual colonies for potential identification, including screening for E. coli. The higher bacterial load in these two powders aligns with previous studies reporting that herbal products can easily become contaminated during collection, processing, storage, or handling (Khan *et al.*, 2022). These findings emphasize the need for strict hygiene practices and improved quality control measures in the production of herbal cosmetic powders to prevent the spread of pathogenic bacteria and ensure consumer safety.

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The identification of Gram-negative, rod-shaped E. coli in rose powder indicates that herbal cosmetic products are vulnerable to microbial contamination, including contamination from fecal or environmental sources. Gram staining served as a reliable and rapid method to differentiate bacteria based on their cell wall structure (Dahal, 2024). Selecting colonies with distinct morphology before staining ensured accurate identification, in line with standard microbial quality assessment procedures used for herbal and natural products (Khan *et al.*, 2022). These findings highlight the importance of stringent hygiene practices, proper handling, and strict quality control measures during the production and storage of herbal cosmetic items to ensure consumer safety and prevent contamination by pathogenic bacteria.

The positive results from the Indole and MR tests confirmed that the bacteria was Escherichia coli. The Indole test shows that the bacteria can break down tryptophan, and the MR test shows it can make stable acids through fermentation. These results match the usual traits of E. coli found in microbiology books (Sharma *et al.*, 2022). Other studies on herbal powders have also found E. coli using these same tests (Khan *et al.*, 2022). This proves that biochemical tests are a very important step to check the safety and quality of herbal cosmetic products. The antibiotic sensitivity test showed that the E. coli from rose powder was sensitive to Kanamycin (15 mm), had a medium response to Tetracycline (13 mm), and was resistant to Amoxicillin. This means some antibiotics still work against it, but others don't. The resistance to Amoxicillin shows that antibiotic-resistant bacteria are common in herbal cosmetic products, probably because of environmental contamination or poor handling during production. Other studies also found drug-resistant E. coli in herbal powders (Khan *et al.*, 2022). This shows why testing before plasmid curing is important, as it helps understand how resistant the bacteria are before further treatment.

The antibiotic sensitivity assay demonstrated that Escherichia coli isolated from Rose Powder was susceptible to Kanamycin, with a zone of inhibition measuring 15 mm, exhibited intermediate sensitivity to Tetracycline (13 mm), and displayed complete resistance to Amoxicillin (Table 2). These findings indicate that while some antibiotics retained effectiveness, others had diminished activity against the isolate. The observed resistance to Amoxicillin suggests the presence of antibiotic-resistant strains, potentially arising from environmental contamination or lapses in hygienic handling during production. Comparable reports in the literature have documented multidrug-resistant E. coli in herbal cosmetic powders, highlighting a recurring concern in product safety. This preliminary antibiotic profiling underscores the necessity of performing sensitivity tests prior to plasmid-curing experiments, as it establishes a baseline understanding of bacterial resistance patterns and informs subsequent interventions (Khan *et al.,* 2022).

The survival of *Escherichia coli* following SDS treatment indicates that, although SDS can disrupt cell membranes and destabilize plasmids, the bacteria retained the ability to recover and proliferate. This aligns with previous reports, which describe SDS as an effective plasmid-curing agent that interferes with plasmid stability while allowing bacterial growth to continue. The observed resilience reflects the partial stress tolerance exhibited by the treated cells under these conditions (Zaman, Pasha, & Akhter, 2010).

The heat shock treatment demonstrated that Escherichia coli cells were able to survive thermal stress, which is known to compromise plasmid stability. Previous studies have reported that elevated temperatures can disrupt plasmid replication, making heat shock a widely used method for plasmid curing. The continued colony growth observed in this study confirms that the bacteria tolerated the thermal stress, while the impact on plasmid presence was further assessed through subsequent antibiotic sensitivity testing (Singh *et al.*, 2010).

After plasmid curing using SDS and heat shock, the *Escherichia coli* isolate from rose powder demonstrated notable alterations in antibiotic susceptibility. The zone of inhibition for Kanamycin decreased to 12 mm and 10 mm, respectively, compared to 15 mm in the pre-curing culture (Table 3), indicating a partial loss of plasmid-encoded resistance. In contrast, resistance to Tetracycline and Amoxicillin remained unaffected, suggesting that these resistance determinants were either chromosomally encoded or associated with plasmids that were not eliminated under the applied curing conditions. These observations indicate that plasmid curing can selectively reduce certain antibiotic resistances but may not fully eradicate them, as some genes persist through chromosomal integration or stable plasmid maintenance. The results emphasize the importance of tailoring plasmid-curing strategies to individual bacterial strains to achieve more effective mitigation of plasmid-mediated antibiotic resistance (Li *et al.*, 2021).

Agarose gel electrophoresis revealed distinct variations in plasmid band intensity across the samples, reflecting the impact of the applied curing treatments on plasmid stability. Strong, well-defined bands in the control lanes confirmed the presence of plasmids in the untreated isolates, whereas the reduction or absence of bands in the treated samples indicated disruption of plasmid maintenance. SDS treatment proved more effective than heat shock, likely owing to its direct destabilization of plasmids through membrane perturbation, while heat shock imposed thermal stress that interfered with plasmid replication and segregation. The faint or absent bands in the SDS-treated samples highlighted its higher efficiency in plasmid elimination. These observations align with previous studies reporting that both chemical and thermal curing agents can substantially reduce plasmid-mediated antibiotic resistance in E. coli (Rahman *et al.*, 2021).

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The positive results from the qualitative assays confirmed that the herbal cosmetic powders contained flavonoids (Table 4), secondary metabolites widely recognized for their antioxidant, antimicrobial, and anti-inflammatory properties. The distinct color reactions observed were due to the specific interactions of flavonoid functional groups with the test reagents, validating the use of these classical assays for phytochemical screening. The presence of flavonoids in these powders not only highlighted their medicinal relevance but also suggested their potential role in therapeutic applications. These findings were consistent with previous studies analyzing plant-based formulations, in which flavonoids were consistently detected as key bioactive constituents (Kumar *et al.*, 2022).

The observed differences in flavonoid content among the extracts highlighted the variability in phytochemical composition depending on plant type and preparation method. The $AlCl_3$ colorimetric assay employed in this study relied on the formation of stable complexes between aluminum chloride and the keto and hydroxyl groups of flavonoids, producing a yellow chromophore measurable at 415 nm. This technique was recognized for its simplicity, sensitivity, and reliability in quantifying total flavonoids. Higher flavonoid concentrations in specific extracts suggested enhanced antioxidant and therapeutic properties, supporting their traditional applications in herbal and cosmetic products. These findings were consistent with recent research demonstrating the effectiveness of the $AlCl_3$ method for flavonoid quantification in plant-based formulations (Sultana *et al.*, 2024).

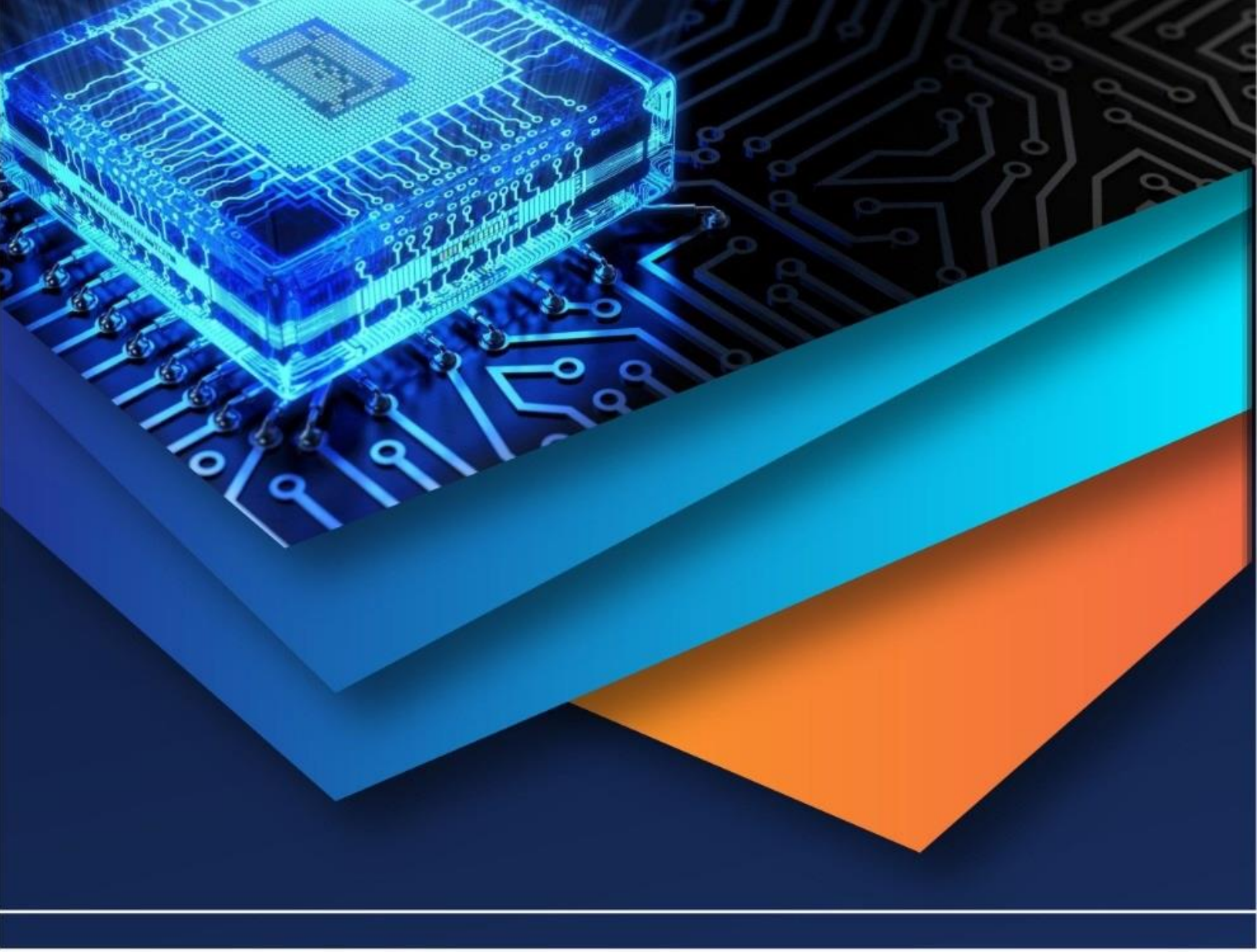
V. CONCLUSION

In this study, *Escherichia coli* was successfully isolated from selected herbal cosmetic powders, and its antibiotic resistance was determined to be plasmid-mediated. Plasmid curing experiments revealed that SDS was more effective than heat shock in eliminating plasmids, confirming the role of plasmids in carrying resistance traits. Phytochemical screening of Rose Powder and Multani Mitti showed the presence of valuable flavonoids, indicating their potential antioxidant and therapeutic properties. The detection of antibiotic-resistant bacteria highlights the importance of strict hygiene and quality control during the preparation and handling of herbal cosmetics. Simultaneously, the presence of bioactive compounds underscores the medicinal potential of these powders. Overall, this study demonstrates the dual nature of herbal cosmetic powders: while they may harbor plasmid-mediated antibiotic-resistant bacteria, they also contain beneficial phytochemicals. The findings emphasize the need for integrated approaches that ensure consumer safety while preserving the natural therapeutic value of herbal products.

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