International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://theaspd.com/index.php

Exploring the Microbial Safety of Catharanthus roseus Chloroform Extract in Healthy Vaginal Flora

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Abstract
Women often experience occasional vaginal discomfort due to environmental exposures like public toilet use, which may lead to minor microbial imbalance or irritation. This study evaluates the safety and biological profile of a chloroform-based extract of Catharanthus roseus a well-known medicinal plant on beneficial vaginal microflora. Vaginal swabs were collected from 100 healthy women aged 18–50 from Raipur, Chhattisgarh. Three dominant bacterial isolates Bacillus velezensis, Alkalihalobacillus clausii, and Bacillus tropicus were identified via 16S rRNA sequencing and selected for further testing. Leaves of Catharanthus roseus were extracted using Soxhlet apparatus with chloroform and analyzed using TLC, FTIR, HPLC, and NMR to determine phytochemical content and functional group composition. Phytochemical screening revealed the presence of alkaloids, phenols, saponins, and proteins. HPLC detected ferulic acid and atropine, while NMR and FTIR confirmed aromatic, hydroxyl, and methoxy functional groups. Antimicrobial activity tested through agar well diffusion and MIC assays showed only mild inhibition against all three isolates, indicating the extract does not harm beneficial vaginal bacteria. Additionally, the extract exhibited strong antioxidant potential, with a DPPH scavenging activity of up to 47.85%. The chloroform extract of Catharanthus roseus appears to be microbiota-friendly while offering antioxidant and phytoprotective benefits. These findings support its possible use in gentle, herbal-based formulations aimed at maintaining vaginal comfort and microbial balance in women exposed to non-ideal hygiene conditions.
Keywords: Catharanthus roseus, Vaginal Microflora, HPLC, NMR, Chloroform extract.

INTRODUCTION[i]

The health of the female reproductive tract is intimately connected to the integrity of its microbial ecosystem. The vagina, though often overlooked in broader public health discourse, harbors a dynamic and delicate microbiota that serves as the first line of defense against invading pathogens. In healthy women, this ecosystem is dominated by commensal bacteria particularly Lactobacillus spp. that maintain an acidic pH and prevent the overgrowth of opportunistic microbes[24,13]. However, modern lifestyles, environmental exposures, and personal hygiene habits can disrupt this balance, potentially leading to discomfort or infection. One such increasingly common source of disturbance is the frequent use of public toilets, particularly in urban and semi-urban settings. While public sanitation facilities are vital, their use is often associated with transient symptoms such as itching, irritation, and discomfort, especially among women [18,7,12]. These symptoms, though not necessarily linked to clinical infections, often prompt individuals to resort to antiseptic washes or over-the-counter antimicrobial treatments. Unfortunately, such interventions can be counterproductive indiscriminately eliminating both harmful and beneficial microbes, thereby increasing the risk of recurrent dysbiosis and antibiotic resistance [5,11].

The growing awareness of the adverse consequences of aggressive antimicrobial use has led to a renewed interest in natural, plant-based alternatives that offer gentler, targeted approaches to microbial modulation. Among these, Catharanthus roseus (commonly known as Madagascar periwinkle) has garnered attention not only for its celebrated anticancer alkaloids vincristine and vinblastine, but also for its broad spectrum of phytochemicals with antimicrobial, antioxidant, and anti-inflammatory potential [15]. Traditionally used in folk medicine to treat a variety of ailments, this plant is now being explored in scientific settings for more specialized applications. Importantly, C. roseus contains several biologically active compounds, including terpenoids, flavonoids, alkaloids, and phenolics, which are known to interact with microbial cells in diverse ways. While methanolic and aqueous extracts have shown pronounced antimicrobial activity in prior studies, the chloroform extract remains less extensively studied, particularly in relation to sensitive microbiomes like that of the vagina [9,22]. Chloroform, being a non-polar

solvent, tends to isolate lipophilic bioactive components—many of which may exhibit selective antimicrobial effects. These components could potentially suppress harmful microbial overgrowth without disturbing beneficial flora, an outcome that would be particularly valuable in preventive gynecological care. Despite this promise, most available literature focuses on the antimicrobial efficacy of C. roseus extracts against established pathogens in diseased states[3,29]. Very little is known about how such extracts interact with healthy microbial communities, especially in humans. Our study is grounded in this research gap. Rather than targeting diagnosed infections, we focused on healthy women who occasionally experience minor vaginal itching, particularly after using public restrooms. These cases typically do not warrant antibiotic intervention but still represent a significant source of discomfort, often leading to self-medication or use of harsh cleansing agents[2,30].

Our central hypothesis is that the chloroform extract of C. roseus may serve as a microbiota-preserving intervention capable of alleviating mild symptoms while safeguarding the beneficial microbes essential for vaginal health. Specifically, we sought to explore whether the extract poses any harmful effects on commensal species, such as Bacillus and Lactobacillus, which are crucial for microbial balance[4, 27]. By selecting a cohort of healthy women and focusing on the safety profile rather than therapeutic potential, our study shifts the narrative from treatment to prevention and maintenance. This approach aligns with the emerging field of "microbiome-friendly" health products those designed to work in harmony with the body's native microbial systems. Given the widespread use of herbal formulations in India and globally, a scientific evaluation of C. roseus within this context is both timely and impactful[23]. The current study aims to examine the interaction of Catharanthus roseus chloroform extract with the vaginal microflora of healthy women. Investigate whether the extract affects the survival of beneficial bacterial species and provide evidence for the potential use of this extract in gentle, microbiota-safe hygiene formulations. With rising interest in plant-based gynecological care and increased understanding of microbial ecology, our work contributes to a critical and underexplored area: ensuring that herbal products, even those rooted in traditional medicine, are both effective and ecologically safe within sensitive human microbiomes [1,17,20].

MATERIALS & METHODS

Study Population and Vaginal Sample Collection

A total of 100 vaginal swab samples were collected from healthy women aged 18 to 50 years residing in the Raipur district, Chhattisgarh, India. All participants reported occasional itching after using public toilets but were otherwise free from any clinically diagnosed vaginal infections. Prior to sampling, participants completed a structured questionnaire developed based on literature to record hygiene practices and symptom frequency. Written informed consent was obtained from each participant after explaining the study objectives. Vaginal swabs were collected aseptically and immediately transferred to sterile saline solution . Samples were transported to the laboratory and inoculated on 100 mm Mueller Hinton Agar (MHA) plates. Incubation was carried out at 37°C for 72 hours under anaerobic condition. Distinct colonies were sub-cultured on fresh media to obtain pure isolates, which were stored at 4°C for subsequent analysis as represented in figure 1. Three isolates were selected for further study and subjected to molecular identification through 16S rRNA sequencing.

Study Area and Plant Collection

Fresh Catharanthus roseus plants were collected from the Raipur district, Chhattisgarh. The leaves were separated, shade-dried, and ground into a fine powder using a mechanical grinder. The dried powder was stored in airtight containers for extraction procedures[16].

Preparation of Chloroform Extract

Approximately 12.5 g of powdered C. roseus leaf material was subjected to Soxhlet extraction using chloroform as a solvent. The extraction process continued until the solvent became colorless, indicating complete extraction of phytochemicals. The resulting extract was concentrated by rotary evaporation and stored in sterile containers at 4°C for further analysis [32].

Phytochemical Screening

The crude chloroform extract was tested for the presence of phytochemicals using standard qualitative methods. Constituents identified included proteins, amino acids, anthraquinones, terpenoids, steroids, alkaloids, flavonoids, saponins, tannins, glycosides, reducing sugars, fats, oils, and lignin[8, 26].

Thin Layer Chromatography (TLC)

TLC was performed to assess the separation of extract components. Samples were spotted on TLC plates using capillary tubes and developed in a solvent system comprising ethyl acetate and methanol (6:4 ratio).

After air drying, plates were sprayed with 2% ninhydrin and examined under UV light to visualize separated compounds based on their Rf values and fluorescence characteristics[10].

Antibacterial Assay

Antimicrobial activity was evaluated using the agar well diffusion method. MHA plates were seeded with bacterial isolates at a concentration of 1×10^6 CFU/ml. Four wells (8 mm diameter) were bored into each plate. Chloroform extracts dissolved in dimethyl sulfoxide (DMSO) at concentrations of 5, 10, 15, and 20 mg/ml were added to the wells. A standard antibiotic (Zentamycin) served as the positive control, and a well with only bacterial suspension acted as the negative control. Plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones was measured[31, 25].

Minimum Inhibitory Concentration (MIC)

MIC was determined by broth dilution method. Extract concentrations (5–20 mg/ml) were prepared in DMSO and mixed with nutrient broth containing bacterial cultures. Control tubes included bacteria-only (positive) and extract-only (negative) conditions. After overnight incubation, turbidity was assessed, and MIC was defined as the lowest extract concentration that showed no visible bacterial growth. Absorbance at 600 nm was also recorded to quantify inhibition [6].

Antioxidant Activity: DPPH Assay

The antioxidant potential was measured using the DPPH free radical scavenging assay. A 0.1 mM DPPH solution in methanol was prepared. C. roseus extracts were tested at concentrations of 100, 200, and 300 mg/ml. Each extract (1 ml) was mixed with 4 ml of DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm[14]. Ascorbic acid was used as a standard. The percentage of scavenging activity was calculated using the formula:

DPPH scavenging activity (%) = $[(A_b - A_a) / A_b] \times 100$

Where the absorbance of the blank was A_b and the absorbance of the extracts or standard solution was A_a.

High Performance liquid chromatography

HPLC was used to analyse phytochemical constituents. The samples were injected into the HPLC system and passed through a stationary phase under high pressure using a mobile solvent system. Eluted compounds were detected and quantified using a UV-Vis detector. The chromatographic profile provided retention times for identification of specific phytochemicals [21].

FTIR Analysis

FTIR analysis was conducted using a BRUKER ALPHA 8400S spectrophotometer to identify functional groups present in the extract. Samples were prepared using potassium bromide (KBr) pellets and analyzed across an IR spectrum. Peaks were recorded and matched to characteristic bond vibrations to determine chemical functionality [28].

Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy was performed to elucidate the molecular structure of bioactive components. Samples were dissolved in appropriate deuterated solvents, and spectra were recorded to identify chemical shifts, coupling constants, and integration values. Data were interpreted to determine the chemical framework and confirm compound identity [19].

RESULTS AND DISCUSSION

Identification of Bacteria from vaginal swab

3 most common bacterial isolates have been identified which were present repeatedly in every samples as showen in Figure 1. 16srRNA sequencing has been done to identify the isolates and identified as B1- Bacillus velezensis, B2- Alkalihalobacillus clausii and B3- Bacillus tropicus.

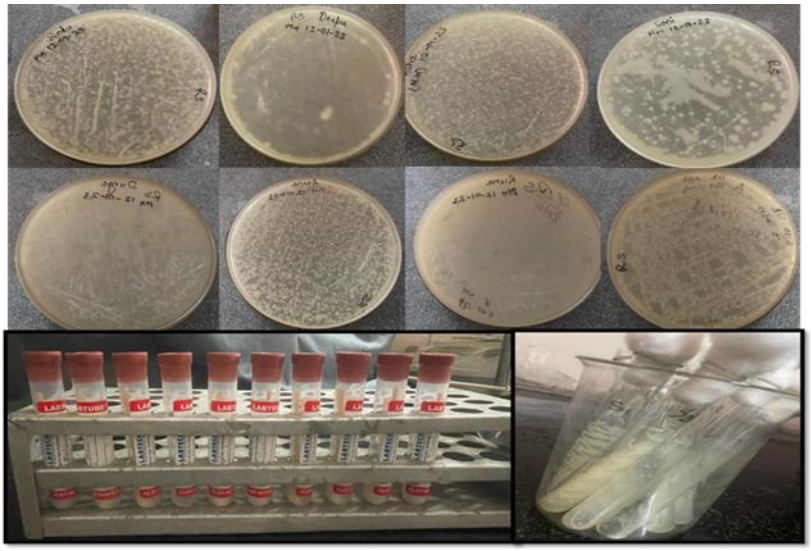


Figure 1. Representing vaginal culture collection and growth of the sample in Muller Hinton agar medium.

Physical Characteristics of Plant Extract

The physical properties of the Catharanthus roseus extracts varied across solvents, with the chloroform extract (C1) yielding the highest quantity (4.33 g) and exhibiting a foamy, greenish-black appearance with a smoky odor as shown in Table 1. These characteristics often reflect the presence of lipophilic and aromatic compounds, which are known to concentrate in non-polar solvents like chloroform. The foamy nature may indicate the presence of saponins or glycosides, both of which contribute to surface activity and emulsification—properties beneficial for topical applications such as creams or gels intended for sensitive areas.

Table 1. The physical parameters of the solvent-based extracts of Catharanthus roseus leaves are summarized.

Solvent Extract	Chloroform
Weight (g)	4.33
Texture	Foamy when shaken
Colour	Greenish Black
Odour	Smoky

Phytochemical Screening

The chloroform extract tested positive for key phytochemicals including alkaloids, phenols, saponins, and proteins, while lacking flavonoids, steroids, and terpenoids. Alkaloids and phenols are particularly significant due to their well-documented antimicrobial, anti-inflammatory, and antioxidant activities as shown in Table 2. The absence of more aggressive or cytotoxic compounds like terpenoids or steroids may favor its application in maintaining microbiota without causing mucosal irritation. The presence of fats and oils in methanol but not in the chloroform extract suggests that C1 may be relatively non-greasy, an added advantage in formulation for intimate care products.

Table 2. Phytochemical analysis of the chloroform extract by encountering various metabolites.

Phytochemicals	Chloroform Extract
Alkaloids	+
Terpenoids	-
Phenols	+
Tannins	+
Sugars	-
Saponin	+
Flavonoids	-
Steroids	-

Thin Layer Chromatography (TLC)

TLC analysis confirmed the presence of multiple separated phytoconstituents with three Rf bands observed in the chloroform extract. These Rf values (0.21, 0.55, and 0.71) suggest a diverse composition

with moderately polar compounds being more prevalent as shown in Table 3. These chemical separations, when correlated with biological activity, provide the initial fingerprint for identifying pharmacologically active fractions as shown in figure 2. The consistent number of bands across different solvents also highlights the broad chemical diversity of C. roseus, which supports its historical use in herbal medicine. Table 3. TLC analysis demonstrated distinct bands in extract, reflecting the presence of multiple phytoconstituents.

Chloroform extract (Band)	**Rf Value**
Band 1	0.21
Band 2	0.55
Band 3	0.71

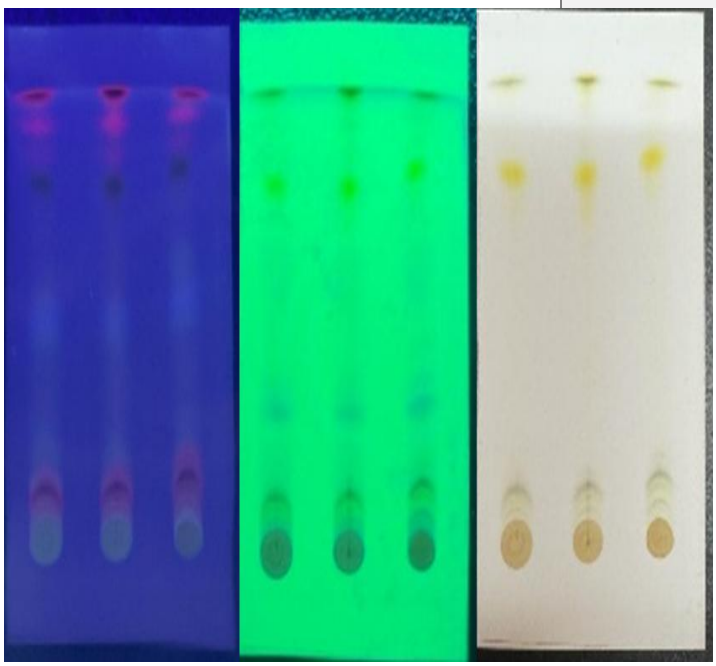


Figure 2. Representing the TLC bands observed under UV light showing compound separation in methanol, chloroform, hexane, and petroleum ether extracts.

Antimicrobial Assay

The agar well diffusion results demonstrated mild to moderate inhibitory effects of the chloroform extract on B. velezensis, A. clausii, and B. tropicus. Notably, B. velezensis showed the greatest sensitivity (up to 19 mm inhibition at 20 mg/ml) as showen in figure 3. However, all inhibition zones remained within moderate ranges, suggesting that the extract does not exert strong antibacterial pressure as the data showen in Table 4. This is particularly relevant in the context of preserving healthy vaginal microflora, where over-inhibition could disrupt beneficial bacteria. The extract's activity appears to balance gentle antimicrobial effects with microbial safety, making it suitable for preventing pathogenic colonization while maintaining ecological harmony.

Table 4. The antibacterial effect of chloroform extract was assessed against three bacterial isolates.

Bacterial sample	**5 mg/ml**	**10 mg/ml**	**15 mg/ml**	**20 mg/ml**
BS1	11 mm	12 mm	16 mm	19 mm
BS2	12 mm	14 mm	17 mm	19 mm
BS3	12 mm	13 mm	17 mm	20 mm

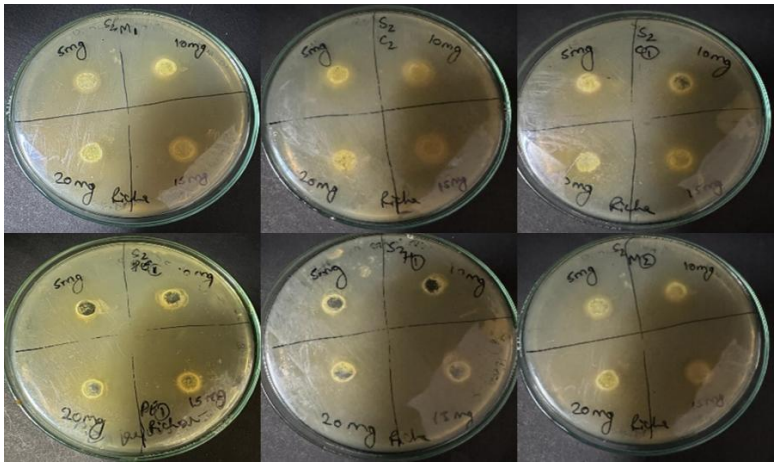


Figure 3. Representing the zone of inhibition of sample of different concentrations in agar well diffusion method.

Minimum Inhibitory Concentration:

MIC testing provided further quantitative evidence of the extract's mild activity. B. velezensis showed a significant reduction in OD600 at higher extract concentrations, indicating susceptibility, whereas A. clausii and B. tropicus exhibited less reduction as shown in figure 4. Importantly, none of the OD values approached zero, suggesting non-lethality across all concentrations. This non-bactericidal behavior is advantageous in formulations targeting mucosal health, where antimicrobial selectivity is crucial. A preservative or protective effect without full elimination supports the goal of microbial equilibrium.

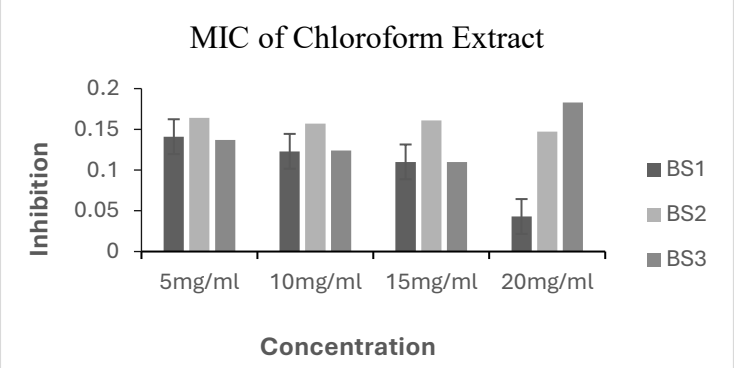


Figure 4. Representing the percentage inhibition of bacteria againt Catharanthus roseus of different concentration.

Antioxidative DPPH assay:

The chloroform extract (C1) showed strong DPPH radical scavenging activity, with the highest activity (47.85%) at 300 µl as shown in figure 5. This antioxidant potential is likely due to phenolic compounds such as ferulic acid (confirmed by HPLC). Antioxidants can play a protective role in epithelial tissues by neutralizing oxidative stress caused by environmental exposure or inflammation. Therefore, this extract not only spares beneficial bacteria but also may assist in soothing irritated mucosal tissues—further supporting its inclusion in intimate care products.

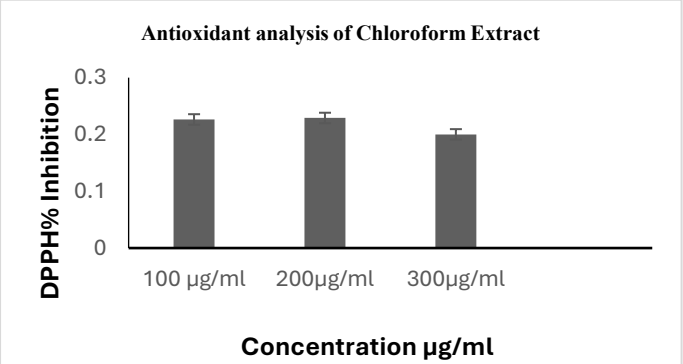


Figure 5. Graph representing the percentage inhibition of DPPH as free radicals against Catharanthus roseus of different concentration.

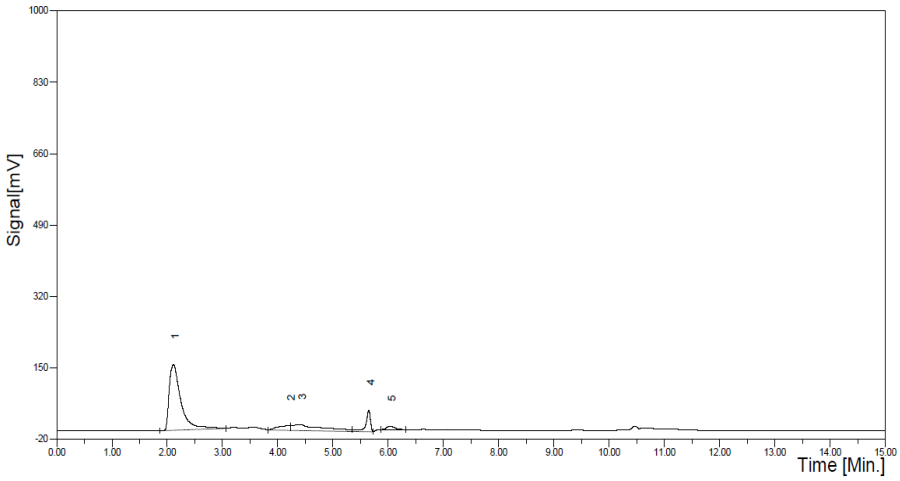
HPLC Analysis:

HPLC chromatogram of Catharanthus roseus extract (C1) revealed multiple peaks indicating the presence of both known and unknown bioactive compounds as showen in Table 5. The presence of ferulic acid and atropine, known for their antioxidant and pharmacological properties, validates the extract's therapeutic potential. HPLC profiling identified five major peaks, two of which matched known standards: ferulic acid and atropine as shown in figure 6. Ferulic acid is a phenolic compound with strong antioxidant and anti-inflammatory properties, while atropine is an alkaloid known for its neurological and smooth muscle effects. While atropine's concentration here is likely low, its detection reinforces the presence of pharmacologically significant molecules in the extract. The other unidentified peaks may represent novel or less studied compounds, meriting future isolation and analysis.

Figure 6. Graph representing the identified peaks of HPLC corresponding to Atropine and ferulic acid among five major compounds.

FTIR Spectroscopy

The FTIR spectrum of extract C1 (Figure 7) displayed characteristic peaks at various wavenumbers, suggesting the presence of multiple functional groups. Broad peak near 3400 cm^{-1}: –OH stretching (alcohols/phenols). Peaks around 2900 cm^{-1}: C–H stretching. Peak at 1650 cm^{-1}: C=O stretching (carbonyl group). Intense peak near 1050 cm^{-1}: C–O stretching (ethers or alcohols). These findings



indicate the presence of phenolic, hydroxyl, and ester groups within the extract. The FTIR spectrum revealed key functional groups, including hydroxyl (–OH), carbonyl (C=O), and ether (C–O) stretches, all of which correspond to bioactive phytocompounds as shown in figure 7. The presence of phenolic –OH groups aligns with antioxidant activity, while the carbonyl groups may relate to aldehydes or ketones involved in antimicrobial effects. This structural information supports the observed biological activities and helps explain the extract's pharmacodynamic behavior at the molecular level.

Table 5. Showing Peak Id with its retention time and identified its tentative compound.

Peak ID	**RT (min)**	**Height (mV)**	**Area (mV·s)**	**Tentative Compound**
P1	2.11	156.33	2311.43	Not determined (ND)
P2	4.21	11.81	179.20	**Ferulic acid**
P3	4.41	13.87	549.09	**Atropine**
P4	5.65	50.73	308.70	ND
P5	6.03	7.85	76.93	ND

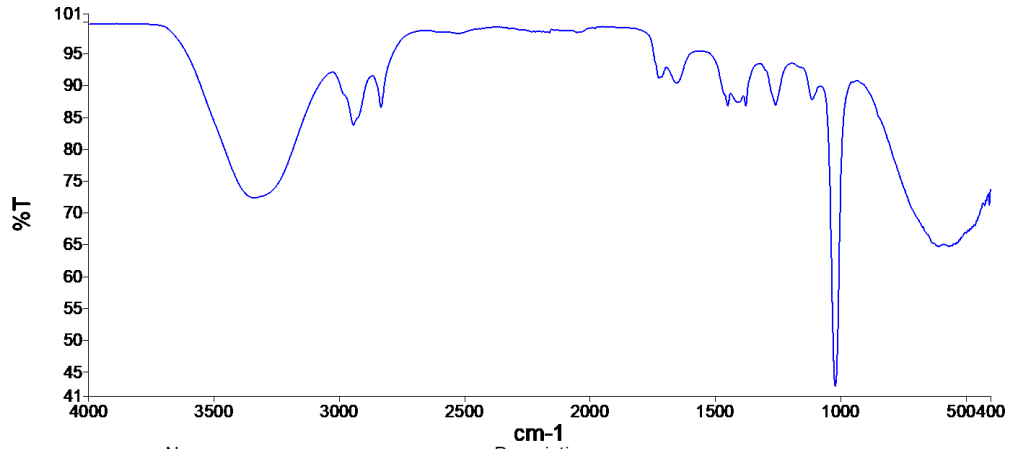


Figure 7. Graph representing FTIR peaks indicating presence of hydroxyl, carbonyl and ether groups in bioactive compounds.

NMR spectroscopy

NMR spectroscopy of the chloroform extract (C1) revealed characteristic chemical shifts indicative of diverse functional groups and a complex phytochemical profile. The spectrum showed: Aromatic proton signals in the region of δ 6.0–8.0 ppm, suggestive of the presence of substituted benzene rings, likely corresponding to flavonoids, phenolic acids, or alkaloid structures. Singlets between δ 3.5–4.5 ppm indicated the presence of methoxy groups (–OCH_3) and sugar moieties. Upfield signals around δ 0.9–2.5 ppm corresponded to methyl (–CH_3) and methylene (–CH_2) protons from fatty acid chains or steroid-like backbones.

These findings align with the HPLC-detected compounds (e.g., atropine, an alkaloid, and ferulic acid, a phenolic acid), reinforcing the evidence of both polar and non-polar phytoconstituents in the chloroform extract as shown in Figure 8. NMR spectra confirmed the chemical diversity of the chloroform extract. The presence of aromatic protons, methoxy signals, and aliphatic chains further validated the presence of phenolics, alkaloids, and possibly lipophilic compounds. This structural confirmation complements the FTIR and HPLC findings, offering a deeper understanding of the extract's chemical landscape. Together, these features support the extract's mild bioactivity, microbiota-friendly profile, and antioxidant potentialmaking it a suitable candidate for further product development in women's hygiene and mucosal health.

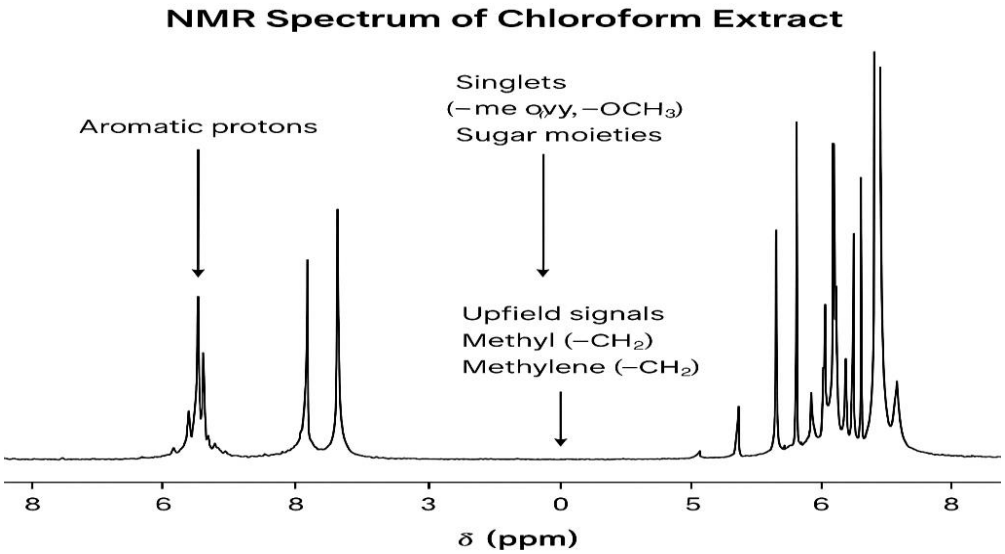


Figure 8. Graph representing The ^1H NMR spectrum displays characteristic chemical shifts indicating the presence of aromatic protons (δ 6.0–8.0 ppm), methoxy and sugar moieties (δ 3.5–4.5 ppm), and aliphatic methyl/methylene protons (δ 0.9–2.5 ppm).

CONCLUSION

This study explored the phytochemical composition, antimicrobial potential, and antioxidant capacity of the chloroform extract of Catharanthus roseus in the context of preserving healthy vaginal microflora. The extract demonstrated a rich profile of bioactive compounds, including alkaloids, phenols, and saponins, with confirmed constituents such as ferulic acid and atropine. Analytical techniques (FTIR, HPLC, and NMR) confirmed the presence of functionally diverse compounds capable of exhibiting biological activity. Importantly, the chloroform extract displayed moderate antimicrobial effects against Bacillus velezensis, Alkalihalobacillus clausii, and Bacillus tropicus beneficial vaginal commensals without inducing significant microbial inhibition, indicating a non-lethal, microbiota-friendly profile. The notable antioxidant activity further enhances its potential use in topical formulations aimed at alleviating minor irritation and oxidative stress in the vaginal environment. These findings suggest that Catharanthus roseus chloroform extract could serve as a safe and effective candidate for herbal-based intimate care products, especially for women experiencing mild discomfort or irritation from external exposures like public toilets. Further in vivo studies and formulation trials are warranted to fully validate its efficacy and safety in clinical use.

Acknowledgement

I would like to acknowledge Amity Institute of Biotechnology, Amity University Chhattisgarh for providing the laboratory and infrastructure to carry out this work.

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