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RESEARCH ARTICLE

# Unveiling the medicinal potential of dadima as a bioactive compound in Dadimashtaka choornam for exploring the antimicrobial properties against bacterial pathogens

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## Abstract

Ayurvedic formulations have long served as traditional remedies and Dadimashtaka churna known for its anti-diarrheal and anti-ulcer properties is now being explored for its antimicrobial potential. Considering rising antimicrobial resistance this study investigates the antibacterial activity and phytochemical profile of Dadimashtaka churna against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Methanolic and aqueous extracts were analysed through organoleptic, physicochemical, thin layer chromatography (TLC) and high-performance thin layer chromatography (HPTLC) methods. Phytochemical screening confirmed the presence of flavonoids, alkaloids, terpenoids, tannins and phenolics. The disc diffusion assay showed a maximum inhibition zone of 28±0.37 mm for *Staphylococcus aureus*, followed by 22 ± 0.23 mm for *Escherichia coli,* while *Pseudomonas aeruginosa* exhibited the smallest inhibition zone of 14 ± 0.21 mm. High performance thin liquid chromatography revealed the consistent presence of Dadima in all 3 samples, with retention factor (Rf) values of 0.74, 0.79 and 0.74, respectively and a peak area eight times higher than that of the other ingredients. This aligns with its dominant quantity of 8 phala in the formulation, while the other ingredients are present in 1 phala, half, or quarter proportions. Heavy metal content was within WHO safety limits. These findings validate Dadimashtaka choornam potential as a safe and effective antibacterial agent and its comparative study reveals that sample C closely follows the classical Ayurvedic proportions, which is also supported by its higher total peak area.

**Keywords:** antibacterial activity; Dadimashtaka churna; fluorescent analysis; high performance thin layer chromatography; inductively coupled plasma-atomic emission spectroscopy; thin layer chromatography

## Introduction

Ayurvedic formulations have been traditionally used in healthcare for their medicinal properties, offering natural remedies for various ailments (1). Despite their widespread use, scientific validation of their therapeutic effects remains limited. Among these formulations Dadimashtaka churna is a well-known Ayurvedic preparation with a significant impact on the gastrointestinal system possessing anti-diarrheal and anti-ulcer properties (2). This formulation contains various phytoconstituents derived from different herbs, which make them suitable for treating gastric disorders, especially for digestive problems (3). Any ailment affecting in the gastrointestinal tract is broadly categorized under gastrointestinal disorders. As per classical Ayurvedic text, Sahasra Yogam this formulation is indicated for digestive disorders, diarrhoea, irritable bowel syndrome and amoebic dysentery.In Ashtanga Hrudyam Chikitsasthanam, the formulation is explained in Atisara chikitsa (treatment of gastrointestinal conditions) plays a significant role when administered along with other medications to address the before mentioned ailments. The text further explains that diarrhoea and emesis are 2 disorders with similar characteristics. When food in took as such or digested halfway or after digestion becomes harmful or toxic to the body. The body itself tries to expel it from the body via the *urdhwaadhomarga* leading to pathogenesis of diarrhoea and emesis. In both cases the site affected are the stomach and the intestines where in which the digestion is impacted. The malabsorptive disorders in Ashtanga Hrudyam are categorised into mild, moderate and severe forms. Dadimashtaka churna is advised in the milder and moderate conditions. An infectious gastrointestinal disorder like dysentery

causes inflammation of the intestines, the causative agents reach the body through contaminated food, water and unhygienic conditions. However, formulation's potential antibacterial properties against bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as well as its phytochemical profile remain relatively unexplored in modern scientific literature. This highlights the potential of Ayurvedic medicine in addressing microbial infections which is becoming increasingly relevant in the face of growing antibiotic resistance (4). The global acceptance of natural medicine increases day by day due to their property to exert lesser side effects as compared to synthetic medicines (5). Dadimashtaka churnam can be administered at a dose of 5–10 g as directed by physician. For paediatric use, the recommended dose ranges from 500 mg to 2 g, depending on the age of the child (6). Dadimashtaka churna enhances digestive enzyme secretion and improves appetite. This effectiveness is attributed to the presence of tannins, flavonoids, essential oils and alkaloids (7). Its high phenolic content contributes to the protection of the gut mucosa (8).

In recent years, antibiotic resistance has emerged as a critical challenge in modern medicine, reducing the efficacy of conventional drugs against bacterial infections. The overuse and misuse of antibiotics have accelerated this problem making it imperative to explore alternative solutions. Herbal medicine presents a promising approach; its formulations possess natural antimicrobial properties that could help combat resistant pathogens (9). Additionally, Ayurvedic formulations offer a holistic approach to one's health, by strengthening the body's natural defence mechanisms unlike targeting specific pathogens. Integrating these remedies into current healthcare could contribute to more sustainable and effective treatment strategies. Herbal medicine is considered a safer alternative to synthetic drugs. Synthetic drugs target specific microorganisms that causes infections but at the same time impacts the gut flora (10).

Phytoconstituent such as phenols, flavonoids in Dadimashtaka churna play a major role in providing health benefits (8). Phenolic compounds have a strong affinity for different molecular structures, such as proteins or glycoproteins, which contribute to their significant role in bacterial resistance (11).

Additionally, herbal medicines have the potential to interact with prescription drugs, either diminishing their effectiveness or increasing the likelihood of adverse effects. Therefore, it is crucial to use these remedies under proper medical supervision and with a well-researched understanding of their benefits and risks. A balanced approach, combining traditional medicine while ensuring safety and efficacy (12). Through this comprehensive study efforts are made to synthesis traditional approach backed by scientific evidence, that "Dadimashtaka churna" is a safe and effective antibacterial agent. This formulation comprises 14 ingredients, with Dadima serves as a principal component. It is rich in carbohydrates, terpenoids, flavonoids and tannins. Calcium oxalate crystal of Dadima, siliceous crystals due to the presence of Maranta arundinacea and oil globules of *Piper nigrum L.*. The presence of essential oil and flavonoids that are antispasmodic in nature also proves the effectivity of the formulation (13). Dadima which is the main component has significant antibacterial activity (14). Given the context, the present study was aimed to evaluate the medicinal potential of dadima (*Punica granatum L.*) as a key bioactive component of Dadimashtaka churnam, emphasising on its antibacterial efficacy against selected bacterial strains. Additionally, the study seeks to characterise the phytochemical profile of the formulation and establish a correlation between its bioactive constituents and the observed antimicrobial activity.

## Materials and Methods

### Collection of ayurvedic formulation

The Ayurvedic formulation, Dadimashtaka churna, was procured from three reputable sources: Amala Ayurvedic Hospital and Research Centre, Thrissur District, Kerala. Kottakkal Arya, Vaidya Sala, Kerala & Oushadi, Kuttanellur, Kerala.

### Bacterial strains

Bacterial samples *S. aureus* (MTCC 6571) *P. aeruginosa* (MTCC 1688), *E. coli* (MTCC 1687) was procured from MTCC, Chandigarh. The isolates were sub-cultured on nutrient agar plates, to obtain pure culture and were maintained at 5 °C.

### Extract preparation

Dadimashtaka churna was finely powdered to ensure uniformity. For extraction, 3 g of the powdered formulation from each of the 3 samples were separately suspended in 30 mL of methanol. Among the various solvents used for extraction, methanol showed higher activity compared to others. The suspensions were allowed to stand for 24 hr to facilitate thorough extraction. After extraction, the mixtures were filtered and the filtrates were concentrated on a boiling water bath at 40 °C until the volume reduced to 20 mL (15).

### Organoleptic evaluation

The colour, odour and taste of the churna were examined through organoleptic evaluation (16). The results are presented in Table 1

### Preliminary phytochemical tests

Various phytoconstituents were examined through preliminary phytochemical screening (16). The various tests carried out are explained and the observations of 3 samples are recorded and tabulated in Table 2.

#### Test for carbohydrates

Churna was heated with Fehling's solution A and B produced a red colour, confirming the presence of carbohydrate (17).

#### Test for alkaloids

The test substance was treated with few drops of acetic acid, followed by Dragendroff's reagent and mixture were shaken well, yielding an orange-red precipitate that indicates the presence of alkaloids. When treated with dilute hydrochloric acid and Mayer's reagent, a white precipitate was observed. Both reactions confirm the presence of alkaloids (16).

#### Test for glycosides

Churna was added with little amount of anthrone and one drop of concentrated sulphuric acid was added and made into a paste,

**Table 1.** Organoleptic evaluation of Dadimashtaka churna

| Characteristics | Sample A | Sample B | Sample C |
|---|---|---|---|
| Colour | Brownish yellow | Brownish yellow | Brownish yellow |
| Odour | Aromatic and spicy | Aromatic | Aromatic |
| Taste | Bitter | Bitter | Bitter |

**Table 2.** Preliminary phytochemical screening of the extract- sample A, B and C

| Sl. No | Screening test | Water extract A | Water extract B | Water extract C | Methanol extract A | Methanol extract B | Methanol extract C |
|---|---|---|---|---|---|---|---|
| **1** | **Test for carbohydrate** | | | | | | |
| A | Molisch's test | ++ | ++ | ++ | ++ | ++ | +++ |
| B | Fehlings test | ++ | + | ++ | ++ | ++ | +++ |
| **2** | **Test for alkaloids** | | | | | | |
| A | Drangendroff's method | - | - | ++ | ++ | - | - |
| **3** | **Test for flavanoids** | | | | | | |
| A | Shinoda test | ++ | ++ | - | +++ | ++ | +++ |
| **4** | **Test for steroids** | | | | | | |
| A | Salkowsky test | ++ | - | ++ | +++ | - | ++ |
| **5** | **Test for phenols** | +++ | + | +++ | ++ | - | + |
| **6** | **Test for tannins** | | | | | | |
| A | Braemer's test | +++ | ++ | +++ | +++ | ++ | ++ |
| B | Lead acetate test | ++ | ++ | ++ | ++ | ++ | ++ |
| **7** | **Test for glycosides** | | | | | | |
| A | Benedict's test | ++ | ++ | ++ | ++ | ++ | ++ |
| **8** | **Test for terpenoids** | | | | | | |
| A | Lieberman-burchard test | +++ | +++ | +++ | +++ | ++ | +++ |
| **9** | **Test for saponins** | - | - | + | - | - | - |

A, B and C represent different samples of Dadimashtaka churna. Preliminary phytochemical screening was carried out using aqueous and methanolic extracts "+" = Weak presence, "++" = Moderate presence, "+++" = Strong presence, "–" = Absent.

gently warmed on a water bath. The appearance of dark green colour indicates the presence of glycosides.

**Test for terpenoids**

Dissolve 2 mL of the ethanolic extract in a few drops of chloroform. Carefully add 3 mL of concentrated $H_2SO_4$ along the side of the test tube. The formation of a reddish-brown interface indicates the presence of terpenoids (18).

**Test for flavonoids: Shinoda's test**

Take a small amount of extract in a test tube and add a piece of magnesium ribbon. Carefully add concentrated HCL along the sides of the test tube, red colour indicates the presence of flavonoids.

**Test for tannins**

The extract was mixed with 10 % lead acetate solution forms a white precipitate indicates the presence of tannins.

**Test for steroids: Liebermann–Burchard test**

Dissolve the extract in chloroform in a test tube. Add 2–3 mL of acetic anhydride and carefully add a few drops of concentrated $H_2SO_4$along the sides of the tube. The presence of steroids is indicated by a bluish green colour.

**Determination of ash**

Accurately weigh 2 g of churna and transfer it into a previously ignited and tared silica crucible. The material was then ignited, gradually increasing the temperature to 500–600 °C, until it appeared white, indicating absence of carbon. After ignition, the crucible is allowed to cool in a desiccator. The total ash content is then calculated in milligrams per gram of air-dried material. The powdered sample is subjected to high temperatures using a muffle furnace to determine its ash value accurately. At this high temperature all the organic material was burned (19) and calculate the ash value of the powdered drug (Eqn. 1).

$$\text{Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of crude drug taken}} \times 100 \quad \text{(Eqn. 1)}$$

## Moisture content

The moisture content of the sample is determined using a hot oven. The accurately weighed sample is placed in the oven and heated. After 4 hr, the sample is removed, cooled and weighed again. Finally, the percentage of moisture content is calculated using the equation (Eqn.2) (19).

$$\text{Percentage loss of drying} = \frac{\text{Lossin weight in sample}}{\text{Weight of the Sample}} \times 100 \quad \text{(Eqn. 2)}$$

## Inductively coupled plasma-atomic emission spectroscopy (ICP – AES)

The given samples were digested using 5 mL of $HNO_3$ and 2 mL $HCLO_4$ and made up to 50 mL using HPLC grade water. The filtered solution analysed with Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) system. The results were recorded (20).

## Identification of plant components using thin layer chromatography and high-performance thin layer chromatography analysis

Thin layer chromatography (TLC) approximately 1–2 µL of each of the 3 sample was spotted onto precoated Silica gel-G aluminium plates of uniform thickness (0.5 mm), which served as a stationary phase. And mobile phase of toluene: ethyl acetate: formic acid (5:4:1) (21). Following thin layer chromatography band were observed in a UV chamber with a 254 nm wavelength. Phyto compound screening using high-performance thin layer chromatography (HPTLC) was carried out from CARe- Keralam Ltd, Department of Ayush, CPCSEA, Koratty, Ernakulam, Kerala. By comparing the mass spectra of the elements with the spot of the reference standard and percentage peak areas to the total peak area were used to express the relative amounts of each component.

## Antimicrobial screening

Antimicrobial assay was evaluated using the agar well diffusion method. The 20 mL of sterilised nutrient agar was poured into sterilised Petri dishes and allowed to solidify for a few minutes.

Bacterial stock suspensions $10^6$ colony forming units per mL were evenly swabbed onto the respective plates. Wells (6 mm in diameter) were cut using a sterilised well borer and the agar discs were discarded. Each well was filled with the sample and allowed to diffuse at room temperature, followed by incubation at 37 °C for 24 hr. Microbial growth was assessed by measuring the zone of inhibition around each well using a translucent scale. Each extract was analysed in triplicate and the mean value was recorded (22).

### Statistical analysis

The results are shown as the mean with a standard error of the mean calculated from 3 replicates. The analysis was done with an analysis of variance (ANOVA) and then Tukey's multiple comparisons ($p$<0.05) with GraphPad Prism (GraphPad, La Jolla, CA, USA) (23).

## Results

The present study aimed to scientifically evaluate Dadimashtaka hurna through physicochemical, phytochemical, chromatographic and antibacterial analyses. Organoleptic analysis showed a brownish-yellow colour, a spicy aromatic Odour and a slightly bitter taste (Table 1). Phytochemical screening confirmed the presence of flavonoids, alkaloids, terpenoids, tannins and phenolic compounds (Table 2) (16). Physicochemical parameters showed that the total ash content was 2.34 % for sample A, 4.37 % for Sample B and 3.38 % for Sample C. Water-soluble ash values were 2.43 %, 4.2 % and 2.63 % for Samples A, B and C respectively, while acid-insoluble ash was 2.2 %, 4.4 % and 4.3 % respectively (Table 3) Alcohol-soluble extractive values were found to be 30.06 % (Sample A), 29.27 % (Sample B) and 31.60 % (Sample C). The obtained value for acid insoluble ash was 2.2 %, 4.4 % and 4.3 % respectively Water-soluble extractive values were 27.46 %, 29.00 % and 29.47 % for Samples A, B and C, respectively. Moisture content was low in all samples 2.03 % (A), 3.08 % (B) and 2.79 % (C) indicating good stability and shelf life (Table 3).

Thin TLC and HPTLC analysis confirmed the consistent presence of Dadima in all three samples, with retention factor (Rf) values ranging between 0.74 and 0.79 (Fig. 1). The HPTLC profiling at 254 nm showed 10 Rf values for sample A, 7 for sample B and 9 for sample C (Fig. 2 and 3; Table 4). Sample A exhibited the highest total peak area. Similar chromatographic patterns were observed at 366 nm. Heavy metals were analysed using inductively coupled plasma-atomic emission spectroscopy (ICP– AES) revealed that lead, mercury and arsenic were within WHO-permissible limits, confirming the safety of the formulation. Antibacterial activity assessed by the disc diffusion method demonstrated significant zones of inhibition. The methanolic extract of all three samples showed the highest activity against *Staphylococcus aureus* (28 ± 0.87mm), followed by *E. coli* (22 ± 0.29mm) and *Pseudomonas aeruginosa* (14 ± 0.21mm), suggesting broad-spectrum antibacterial activity (Table 5).

## Discussion

Recently the global acceptance of botanical medicines has increased substantially, underscoring the need for systematic scientific validation and authentication of Ayurvedic formulations. The organoleptic characteristics of Dadimashtaka Churna (as per Table 1) were found to be consistent as mentioned in classical Ayurvedic texts, thereby confirming

**Table 3.** Physicochemical parameters

| Sample | Total ash (%) | Water insoluble ash (%) | Acid insoluble ash (%) | Water soluble extractives (%) | Alcohol soluble extractive (%) | Moisture content (%) |
|---|---|---|---|---|---|---|
| Sample A | 2.34±0.12$^a$ | 2.43±0.10$^a$ | 2.2±0.10$^a$ | 30.06±0.21$^b$ | 27.46±0.16$^a$ | 0.203±0.006$^a$ |
| Sample B | 4.37±0.15$^c$ | 4.2±0.10$^c$ | 4.4±0.02$^c$ | 29.27±0.06$^a$ | 29±0.15$^b$ | 0.308±0.003$^c$ |
| Sample C | 3.38±0.10$^b$ | 2.63±0.15$^b$ | 4.3±0.10$^b$ | 31.06±0.21$^c$ | 29.47±0.21$^b$ | 0.279±0.004$^b$ |

Values are expressed as mean ±standard deviation (SD) of 3 independent determinations (n=3). Different superscript letters within the same column indicate significant difference ($p$<0.05) by one way ANOVA followed by Tukey's multiple comparison test.

**Table 4.** retention factor (Rf) value of different samples at 254 nm

| PEAK | Rf values – wavelength 254 | | | |
|---|---|---|---|---|
| | Sample A | Sample B | Sample C | Dadima |
| 1 | 0.94±0.02 | 0.88±0.09 | 0.94±0.00 | 0.94±0.02 |
| 2 | 0.76±0.06 | 0.73±0.02 | 0.74±0.02 | |
| 3 | 0.50±0.03 | 0.50±0.05 | 0.52±0.07 | |
| 4 | 0.47±0.05 | 0.46±0.03 | 0.48±0.04 | |
| 5 | 0.42±0.07 | - | 0.43±0.03 | |
| 6 | 0.35±0.11 | - | - | |
| 7 | 0.31±0.08 | 0.31±0.05 | 0.37±0.01 | |
| 8 | 0.25±0.00 | 0.25±0.01 | 0.26±0.03 | |
| 9 | 0.13±0.06 | 0.12±0.03 | 0.13±0.05 | |
| 10 | 0.07±0.01 | 0.06±0.02 | 0.08±0.02 | |

Rf value 0.94 is common to Sample A, Sample C and the reference standard Dadima, indicating the possible presence of a similar or identical compound in all 3. Sample A: Shows the highest number of Rf peaks (10), indicating a diverse phytochemical profile. Sample B: Lacks peaks at Rf 0.42±0.07 & 0.35±0.11 suggesting fewer or different constituents compared to the other samples. Sample C: Shares several peaks with both Sample A and Dadima, but lacks the peak at 0.35±0.11, indicating minor compositional variation despite overall similarity.

**Table 5.** Antimicrobial activity of 3 different samples of churna against bacterial strains

| Test organism | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Sample A | Sample B | Sample C |
| *Escherichia coli* | 22±0.23$^a$ | 21±0.31$^b$ | 21±0.59$^b$ |
| *Staphylococcus aureus* | 28±0.37$^a$ | 27±0.56$^b$ | 27±0.61$^b$ |
| *Pseudomonas aeroginosa* | 14±0.21$^a$ | 14±0.58$^a$ | 13±0.45$^b$ |

Values are mean of the independent analyses ± standard deviation (n = 3). Values in a row with different superscript letters differs significantly at $p$<0.05.

**Fig. 1.** Thin layer chromatography.

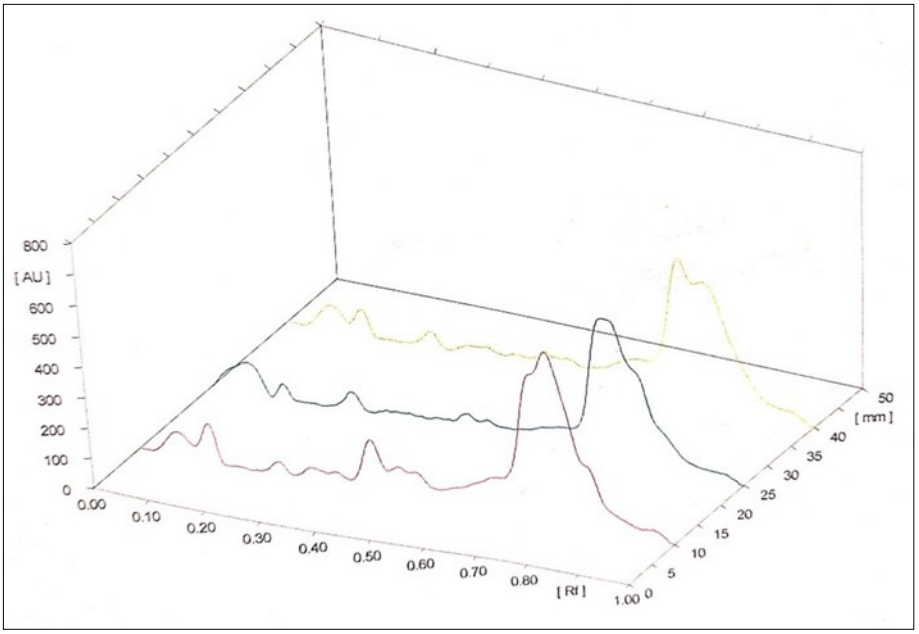


**Fig. 2.** 3D overlay of high-performance thin layer chromatography chromatogram of all tracks, at all wavelengths.

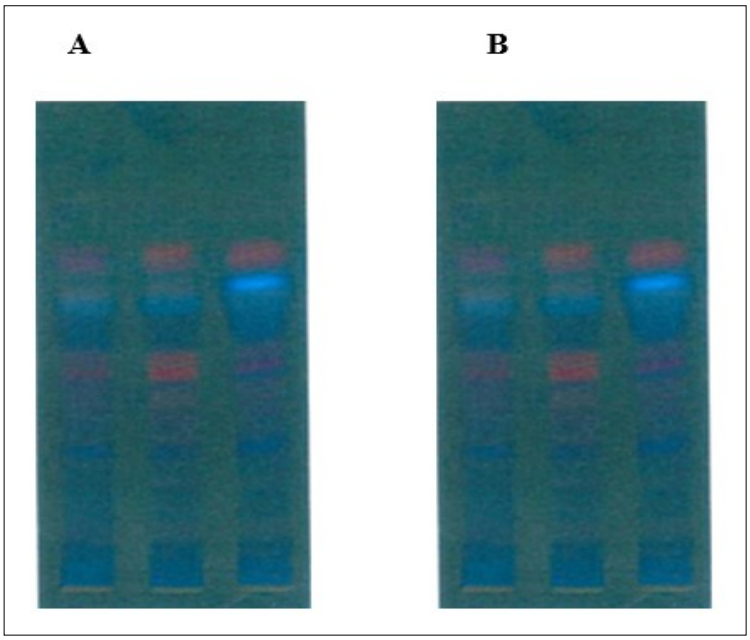


**Fig. 3.** High performance thin layer chromatography chromatogram of 3 samples is visualised at (A) 254 nm and (B) 366 nm. Multiple colour spots indicate the presence of different phytoconstituents. The Rf range of 0.88-0.94 corresponds to the major component, Dadima, present in all samples.

proper formulation and adherence to traditional standards (8). This preliminary evaluation serves as a fundamental step in quality control, ensuring authenticity and minimising the risk of substitution or adulteration.

Phytochemical screening as per Table 2 revealed presence of flavonoids, alkaloids, tannins and phenolic compounds, all of which are known to possess significant biological activities. As per Table 4, of the 14 ingredients of Dadimashtaka churna, dadima (*P. granatum*) has the most flavonoid content observed which can be corroborated with Duysak (24). In this study, agar well diffusion method employed on the 3 samples exhibited zone of inhibition which can be interpreted as antimicrobial activity (as shown in Table 5). This was confirmed by previous researchers stating that flavonoids such as pelargonidin-3-glucoside, pelargonidin-3,5-diglucoside, cyanidin-3-glucoside and cyanidin-3,5-diglucoside have been reported in *P. granatum* and are associated with antimicrobial and antioxidant properties (25). These compounds contribute to membrane disruption, protein denaturation and interference with microbial metabolic pathways, by which supports the antibacterial potential observed in the present study.

Ash values are critical indicators of purity and quality as they measure the amount of inorganic content and help verify a formulation's purity and detect adulteration (26). Acid-insoluble ash particularly indicates the contamination with siliceous material e.g. earthy matter and sand. Alcohol-soluble extractive values, indicates the presence of semi-polar organic compounds, while water-soluble extractive values reflect the number of water-soluble substances such as sugars, glycosides and tannins present in the formulation (27). A low value of either extractive may suggest poor quality or possible adulteration. In the present study, both water-soluble and alcohol-soluble extractives in all 3 samples were found to be within the permissible range, further confirming good quality and the absence of adulteration. Moisture content, which influences the product's microbial stability, was low in all samples, suggesting good stability and shelf life.

Chromatographic fingerprinting using thin TLC and HPTLC confirmed the presence and proportional dominance of Dadima, which exhibited a peak area approximately eight times higher than other ingredients, consistent with its 8-phala proportion in the classical formulation (28). Sample A closely matched the classical Ayurvedic proportions, as evidenced by its higher number of Rf values and total peak area at both 254 nm and 366 nm. Minor variations observed among samples may be attributed to differences in raw material sourcing, geographical origin and processing methods. Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP - AES) was used to analyse heavy metal which confirmed that the formulation is safe for consumption, as all tested metals were within WHO-recommended limits

The antibacterial activity observed in the study may be associated with phenolic compounds and essential oil constituents present in the formulation (29). The synergistic interaction of multiple phytoconstituents in the polyherbal formulation may further enhance its therapeutic efficacy.

Overall, the present findings substantiate the quality, safety and therapeutic potential of Dadimashtaka churna. By integrating traditional Ayurvedic principles with modern phytochemical and chromatographic standardization approaches, this study provides scientific validation for its continued use that underscores the importance of systematic quality control in Ayurvedic formulations.

## Conclusion

Using chromatographic analysis, the study clearly establishes that dadima, a primary component of Dadimashtaka churnam, contains important bioactive components, including flavonoids that exhibit antimicrobial action against the selected bacterial strains. The safety standard parameters assessed by heavy metals analysis are within the permissible range as per norm. The formulation meets standard quality parameters, with its extractive values, moisture content and ash content aligning with traditional and modern quality assessment criteria. The anti-microbial activity observed in the methanol extract supports the fact it's an effective remedy for treating gastrointestinal infections. Future studies focusing on stability assessments will further validate its therapeutic potential and contribute to its standardisation as an effective Ayurvedic formulation.

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## Authors' contributions

SJD participated in the design of study and developed the methodology. SKS collected and analysed the Data. DMD wrote the manuscript and prepared the visualisations. SN and AKK conceptualised and supervised the study. NJ and VKV reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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