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Phytochemical screening by HR-LCMS and *In-vitro* Antidiarrhoeal activity of *Gardenia arborea Roxb.*

Research Article

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Abstract

Objectives: This study aimed to investigate the phytochemical constituents and assess the *in-vitro* antidiarrhoeal activity of *Gardenia arborea Roxb.* leaves, specifically exploring the ethanolic extracts against common diarrhoea-causing pathogens, *Escherichia coli* and *Staphylococcus aureus*. Methods: Phytochemical profiling was conducted using high-resolution liquid chromatography-mass spectrometry (HR-LCMS), and the compounds were identified against the Metlin database. The antidiarrhoeal activity was evaluated through antibacterial assays, measuring zones of inhibition in millimetres, to determine the effectiveness of the extracts against the pathogens at various concentrations. Results: The HR-LCMS analysis identified several key compounds, including Isofraxidin, Arborinine, and Ustiloxin D, within the extracts. In vitro antidiarrhoeal tests demonstrated significant antibacterial activity. The ethanolic extract (GDET) at 500 µg/ml inhibited E. coli by 21.8±0.5 mm and S. aureus by 23.6±0.5 mm. The chloroform extract (GDCH) showed even greater efficacy, inhibiting E. coli by 24.2±0.6 mm and S. aureus by 25.7±0.7 mm, compared to the standard antibiotic ciprofloxacin, which inhibited E. coli by 25.8±0.3 mm and S. aureus by 26.4±0.6 mm at the same concentration. Conclusion: The findings confirm the potent antidiarrhoeal and antibacterial properties of *Gardenia arborea Roxb.*, supporting its traditional use and potential development into a natural therapeutic agent for treating diarrhoea. The distinct phytochemical profile and the dose-dependent antibacterial activity emphasise the plant's utility in pharmacological applications.

Keywords: *Gardenia arborea Roxb., Phytochemical profiling, HR-LCMS, Antidiarrhoeal activity, Antibacterial assay, Natural remedies*.

Introduction

Diarrhoea remains one of the leading causes of morbidity and mortality globally, particularly among children in developing countries.(1) Defined medically as the condition of having at least three loose or liquid bowel movements each day, diarrhoea drastically affects the body's ability to retain water and absorb nutrients.(2) The primary causes of this ailment include infections by bacteria, viruses, or parasites, which are predominantly spread through contaminated water or food.(3) The World Health Organisation (WHO) estimates that diarrhoea kills over 525,000 children under five annually, underscoring its severe public health impact.(4) Despite the availability of effective treatment modalities like oral rehydration solutions (ORS) and supplemental zinc therapy, the search for more accessible and natural treatments is crucial.(5) This need is driven by concerns over drug resistance and the side effects of synthetic drugs, which have prompted renewed interest in herbal remedies known for fewer side effects and long-standing use in traditional medicine.(6)

Gardenia arborea Roxb. Linn. (Family: Rubiaceae), commonly known as 'Dikamali' in various regional Indian languages, is a small tree or shrub native to the Indian subcontinent, specifically flourishing in the dry deciduous forests.(7) Traditionally used in Ayurvedic medicine to treat ailments ranging from skin disorders to inflammation, *G. gummifera* has been reported in folklore medicine to possess various therapeutic properties.(8) The leaves and the resin obtained from incisions made in the stem are especially valued in traditional practices.(9) Phytochemical screenings of *G. gummifera* have revealed the presence of several bioactive compounds, including flavonoids, terpenoids, and saponins, which are speculated to contribute to its medicinal capabilities.(10) Recent pharmacological studies have focused on these compounds, investigating their potential antioxidant, anti-inflammatory, and antimicrobial activities.(11) However, the antidiarrhoeal properties of *G. gummifera* leaves, despite their anecdotal endorsement in traditional medicine (12), have not been extensively

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studied.(13) This research aims to fill this gap by conducting a comprehensive phytochemical investigation and assessing the *in vitro* antidiarrhoeal activity of *G. gummifera* leaf extracts, thereby providing a scientific basis for its traditional use and potentially offering a new, effective natural remedy for diarrhoea.(14)

High-resolution liquid chromatography-mass spectrometry (HR-LCMS) is a pivotal analytical technique for phytochemical investigations, allowing for the precise identification and quantification of bioactive compounds in plant extracts.(15) This method combines the separation capabilities of high-performance liquid chromatography (HPLC) with the mass accuracy and resolution of mass spectrometry (MS), making it exceptionally suitable for the complex mixtures found in herbal preparations.(15) HR-LCMS is instrumental in elucidating the chemical fingerprints of phytochemical constituents, which include secondary metabolites such as alkaloids, phenolics, glycosides, and terpenoids.(16) For *Gardenia arborea Roxb.*, which has a rich ethnobotanical history but limited scientific phytochemical data, HR-LCMS serves as a robust tool to explore its biochemical landscape thoroughly. (17) This approach not only aids in identifying specific compounds that may contribute to the plant's medicinal properties but also enhances the understanding of how these compounds may synergise to exert therapeutic effects.(18)

The primary objectives of this study are twofold. Firstly, to conduct a detailed phytochemical analysis of the leaves of *Gardenia arborea Roxb.* using high-resolution liquid chromatography-mass spectrometry (HR-LCMS) to identify and quantify the bioactive compounds present. Secondly, to evaluate the *in vitro* antidiarrhoeal activity of these leaf extracts. By achieving these goals, the study aims to scientifically validate the traditional uses of *G. gummifera* leaves and potentially develop a novel, effective natural therapy for diarrhoea. This research hopes to contribute to the broader field of ethnopharmacology by bridging traditional knowledge with modern scientific methodologies.

Materials and Methods

Materials

The antidiarrhoeal activity of the plant extracts was assessed using analytical standards of chloroform, ethyl acetate (Lneos Solvents Belgium), ferric chloride, HCl, Mayer-Wagner reagent (2.5 gm of iodine is dissolved in 12.5 gm of KI2 with 250 mL of distilled water), NaOH, sulphuric acid, potassium ferricyanide (K2Fe (CN6), dimethyl sulfoxide (DMSO) (Mettler-Toledo India Pvt. Ltd), Mueller Hinton Broth (Thermo ScientificTM), Ciprofloxacin (Bactigen FDC Limited), *Staphylococcus aureus* (ATCC43300), *Escherichia coli* (ATCC 25922), and sulphuric acid were obtained from Sciquaint Innovations (OPC) Private Limited, Pune.

Methods

Collection of plant material

The whole *Gardenia arborea Roxb.* plants were collected from field side of Pune, Maharashtra, India. The GD plant was authenticated by plant taxonomist from Sciquaint Innovations (OPC) Private Limited, Pune and the whole dried plant was preserved with the voucher specimen (SI/Outside/2023/40) in our department's herbarium. Thereafter, the separated said parts were used for further investigation.

Preparation of Plant Extract

A hundred grams of *Gardenia arborea Roxb.* weighed packet of powdered leaves was placed into the Soxhlet extractor's thimble. A measuring cylinder was used to measure 300 milliliters of the solvent (ethanol), which was then poured into the Soxhlet extractor's still pot. The apparatus was then coupled, and the condenser unit was connected to an overhead water tank to cool the rising solvent vapour. A Bunsen burner running at 68°C served as the heat source. The solvent condensed at the Soxhlet extractor's condenser unit after evaporating via the expansion adapter, thimble, and distillation path.(19) The sample was inside the thimble when the condensed vapour returned there in the form of liquid droplets. The entire contents of the thimble and siphon were emptied back into the still pot of the Soxhlet extractor when the solvent in the thimble reached the level of the siphon top. After roughly nine refluxes over the course of three hours, the extraction procedure was finished.(20)

Fractionation by Column chromatography

A 15 g ethanolic extract of *Gardenia arborea Roxb.* (GDET) was analysed using a glass column (6 cm × 110 cm) and silica as an adsorbent. Petroleum ether was used for wet packing of the column. Ethanol was added incrementally to the column to elute it. Chloroform was added in increments after 100% ethanol was reached. A mobile phase of 1000 mL was used to elute the column. TLC was used to monitor every fraction. Mixed fractions had comparable TLC profiles. Two fractions in all were gathered.(21)

Phytochemical screening

Phytochemical screening of *Gardenia gummifera Roxb.* leaves were conducted using standard qualitative methods as described by Sileshi Dubale *et al*. (22). The leaf extracts were tested for the presence of alkaloids (Dragendorff's, Mayer's, Hager's, Wagner's tests), carbohydrates (Molisch's, Fehling's, Benedict's tests), glycosides (Keller-Killiani, Borntrager's tests), steroids (Libermann-Burchard, Salkowski tests), flavonoids (Shinoda's, Alkaline Reagent, Lead acetate tests), saponins (Foam, Haemolytic tests), tannins and phenolic compounds (Lead acetate, Ferric chloride, Potassium dichromate tests), triterpenes (Sulphuric acid test), and phenols (Ferric chloride test). The results were recorded based on the presence or absence of characteristic reactions, such as colour changes or precipitate formation.

High-Resolution Liquid Chromatography-Mass Spectroscopy (HR-LCMS)

The metabolomics data was analysed using an HR-LCMS, specifically the Agilent 6550 Q-TOF MS instrument from Agilent Technologies in Santa Clara, California. Version B.06.01 of the MassHunter LC/MS Data Acquisition software was utilised to manage the equipment and gather the data. The data was assessed using MassHunter Qualitative and Quantitative Analysis software (version B.07.00). Prior to injection, each sample was passed through a nylon membrane filter with a pore size of 0.2 µm. Chromatographic separation was performed using a Zorbax Eclipse C18 column with a 2.1 ¼ 150 mm diameter and a 5-micron thickness. 10% water and 0.1% formic acid in acetonitrile and water in water was used as a gradient solvent system. It took 2–20 minutes for A 95% to separate, 5 minutes for B 5%, 20–25 minutes for A 5%, 5 minutes for B 5%, and 26–30 minutes for A 95% and 5 percent. A constant pressure of 1,200 bar was maintained at a flow rate of 0.2 milliliters per minute. Positive mode electro spraying was used to obtain the mass spectral data. The presence of the positive mode was indicated by the maintenance of the capillary voltage, source cone voltage, and extraction cone voltage at 3.25 kV, 30 V, and 4 V, respectively. In this investigation, nitrogen was utilised as the desolvation gas, and 900 litres of nitrogen per hour was supplied. 120°C was kept as the source temperature and 550°C was kept as the desolvation temperature. Mass spectra were obtained using the mass-to-charge ratio (m/z) range of 100–1,200, with a mass resolution of 22,000 FWHM (full-width half).(23)

Identification of compounds

The Metlin library database was used to interpret the mass spectrum after the components were identified through retention index analysis. There are about 62,000 records of recognised chemicals in the collection. The standard mass spectra of recognised constituents found in the Metlin library were compared with the spectra of the unidentified constituents of the *Gardenia arborea Roxb.* fraction.(24)

***In-Vitro* Antidiarrhoeal activity**

Mueller Hinton Agar (MHA) plates were used for the agar well diffusion method of antimicrobial assay for extracts from various plants. After being injected into Nutrient Broth, the test organisms were incubated for a full night at 37°C to achieve a turbidity of 0.5 McFarland standards, resulting in a final inoculum of 1.5 × 108 CFU/ml. Standardised microbial culture broth was used to lawn cultivate MHA plates.(25) Plant fractions, including those from *G. gummifera*, were prepared in Dimethyl Sulfoxide (DMSO) at concentrations of 125, 250, and 500 µg/ml. Using a sterile 6-mm cork borer, three wells of 6 mm diameter were made in the inoculated MHA plates.(26) A volume of 125, 250, and 500 µg/ml of the positive control (ciprofloxacin) was added to each well. After allowing diffusion at room temperature for approximately 30 minutes, the plates were incubated at 37°C for 18 to 24 hours.(27) Post-incubation, plates were examined for clear zones of inhibition (ZOI) around the wells, indicating antimicrobial activity. The diameter of the ZOI was measured in millimetres to quantify the antimicrobial efficacy of the compounds under test, including *Gardenia arborea Roxb*.(28)

Statistical analysis

GraphPad Prism and Microsoft Office Excel 2016 were used to statistically analyse the outcomes of all the experiments. After calculating the means, all of the mean values from various instances were compared using a one-way analysis of variance (ANOVA) test. The standard deviation (SD) was used to calculate the mean differences at a significance threshold of 0.05.

Results and Discussion

Results

Results of Phytochemical screening

Table 1: Results of Phytochemical screening of *Gardenia arborea Roxb. Leaves*

Phytochemicals	Test/reagent	*Gardenia arborea Roxb.*
Alkaloids	Dragendorff's test Mayer's test Hager's test Wagner's test	+ + + +
Carbohydrates	Molisch's test Fehling's test Benedict's test	+ + +
Glycosides	Keller-Killiani test Borntrager's test	+ +
Steroids	Libermann-Burchard test Salkowski test	+ +
Flavonoids	Shinoda's test Alkaline Reagent Test Lead acetate test	+ + +
Saponins	Foam test	+
	Haemolytic Test	+
Tannins and phenolic compounds	Lead acetate test. Ferric chloride test Potassium dichromate test	+ + +
Triterpenes	Sulphuric acid test	-
Phenols	Ferric chloride Test	-

+present, -absent

Identification of compounds using the Metlin library:

The HR-LCMS data obtained from the metabolite screening were compared to the Metlin library, a comprehensive database of known metabolites, for compound identification. The compounds were identified based on accurate mass measurements and fragmentation patterns. In the HR-LCMS report, compounds with higher abundance were selected from *Gardenia arborea Roxb.*, where 20 compounds were selected from the ethanol fraction and 16 compounds in the chloroform fraction.

Figure 1: HR-LCMS report of ethanolic fraction (GDET) of *Gardenia arborea Roxb.*

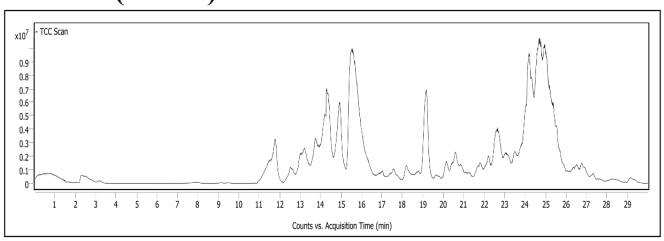


Table 2: List of the compounds identified in the ethanolic fraction (GDET) of *Gardenia arborea Roxb.*

Sr. No.	Compound Name	Retention Time	Molecular Weight (MASS)	Score
1	Isofraxidin	2.297	222.05	97.64
2	(Ac)2-L-Lys-D-Ala-D-Ala	12.509	372.20	95.30
3	Licoricesaponin B2	13.768	808.42	93.10
4	4-Oxoglutaramate	14.269	145.03	96.26
5	N-trans-p-Coumaroyloctopamine	16.966	299.11	95.61
6	5-Methylbarbiturate	17.015	142.03	96.49
7	Koeniginequinone B	17.031	271.08	93.93
8	Skimmianine	17.382	259.08	94.11
9	(10)-Dehydroshogaol	17.596	330.21	90.91
10	Arborinine	18.125	285.10	93.30
11	Quinacridone	18.284	312.08	91.24
12	Elaterinide	18.728	718.35	92.73
13	Dehydroxymethylflazine	19.153	278.06	94.44
14	Lamtidine	19.748	344.23	96.80
15	Juzirine	20.156	281.10	94.08
16	7-(Methylthio)heptanenitrile	20.235	157.09	91.54
17	alpha-Amylcinnamyl isovalerate	20.386	288.20	91.67
18	4-Nerolidylcatechol	21.566	314.22	Varied
19	Lamtidine	20.100	344.23	95.62
20	Tributyl phosphate	24.734	266.16	98.36

Figure 2: HR-LCMS report of chloroform fraction (GDCH) of *Gardenia arborea Roxb.*

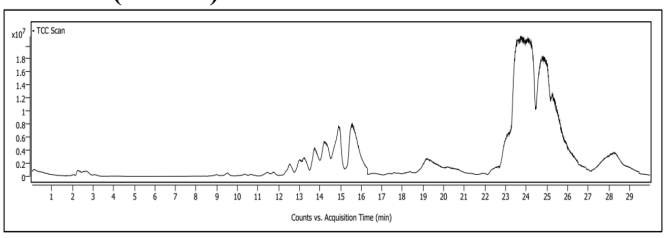
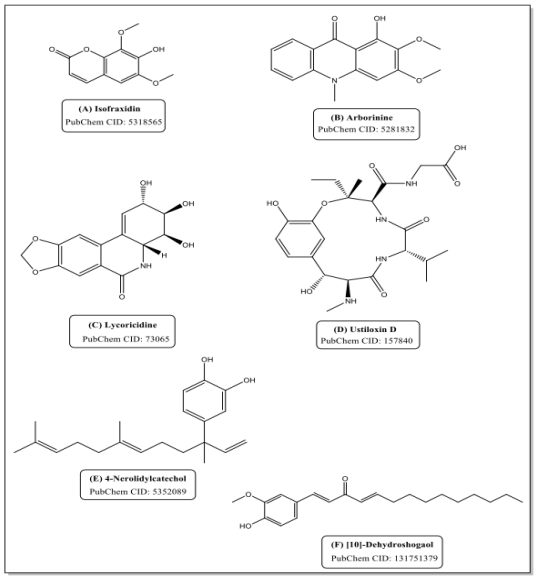


Table 3: List of the compounds identified in the chloroform fraction (GDCH) of *Gardenia arborea Roxb.*

Sr. No.	Compound Name	Retention Time	Molecular Weight (MASS)	Score
1	L-Arginine phosphate	2.260	254.0789	96.25
2	Thalassemine	2.319	298.1029	90.37
3	Isofraxidin	2.454	222.0530	97.05
4	Maculosin	8.963	260.1168	91.28
5	1,2-Benzisothiazol-3(2H)-one	9.547	151.0092	92.28
6	3-Carboxy-2,3,4,9-tetrahydro-1H-pyrido(3,4-b)indole-1-propanoic acid	9.601	288.1107	92.86
7	AminoDAHP	10.640	287.0405	93.08
8	1-(9H-Pyrido(3,4-b)indol-1-yl)-1,4-butanediol	11.735	256.1207	93.36
9	1-O-(2-(L-Cysteinamido)-2-deoxy-alpha-D-glucopyranosyl)-1D-myo-inositol	11.773	444.1400	93.67
10	Lycoricidine	12.317	291.0756	93.35
11	2-Oxo-8-methylthiooctanoic acid	13.337	204.0816	91.95
12	Sanguisorbin E	13.760	792.4642	90.60
13	(10)-Dehydroshogaol	17.624	330.2182	90.16
14	Lycaconitine	18.062	668.3299	92.69
15	Linalool oxide D 3-(apiosyl-(1->6)-glucoside)	20.101	464.2269	95.57
16	Ustiloxin D	20.629	494.2369	97.55

Figure 3: Structure of compounds identified in fraction of GDET and GDCH responsible for showing antidiarrhoeal activity.



(A) Isofraxidin, (B) Arborinine, (C) Lycoricidine, (D) Ustiloxin D, (E) 4-Nerolidylcatechol, and (F) (10)-Dehydroshogaol.

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Results of *In-Vitro* antidiarrhoeal activity

Table 4: Results of antidiarrhoeal activity of fractions of *Gardenia arborea Roxb.*

Compound	Code No.	Concentration (µg/ml)	Zone of Inhibition (mm)	
			E. coli	*S. aureus*
GDET	F1	125	9.8±0.5	11.6±0.8
	F2	250	17.9±0.4	20.5±0.3
	F3	500	21.8±0.5	23.6±0.5
GDCH	F1	125	12.3±0.2	13.1±0.7
	F2	250	20.2±0.4	22.3±0.3
	F3	500	24.2±0.6	25.7±0.7
MS (Ciprofloxacin)	F1	125	13.4±0.6	14.5±0.6
	F2	250	18.7±0.5	20.3±0.9
	F3	500	25.8±0.3	26.4±0.6

Values are expressed in mean±SD (n=3), GDET (Ethanolic fraction of *Gardenia arborea Roxb*), GDCH (Chloroform fraction of *Gardenia arborea Roxb*).

Figure 4: Antidiarrhoeal activity of fraction GDET (Ethanolic fraction of *Gardenia arborea Roxb*) with *E. coli* and *S. aureus.*

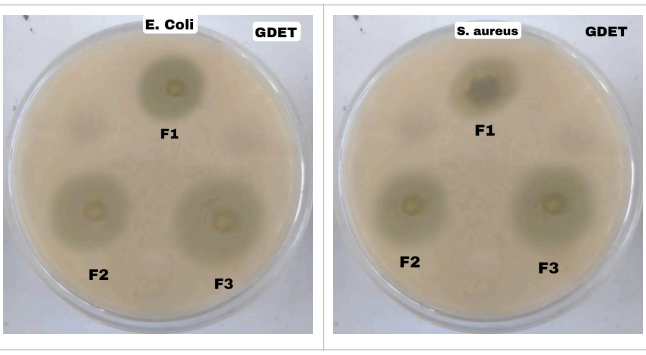


Figure 5: Antidiarrhoeal activity of fraction GDCH (Chloroform fraction of *Gardenia arborea Roxb*) with *E. coli* and *S. aureus.*

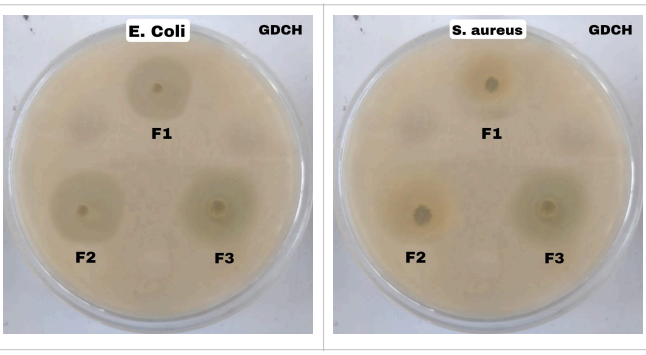


Figure 6: Antidiarrhoeal activity of MS (Marketed standard-Ciprofloxacin) with *E. coli* and *S. aureus.*

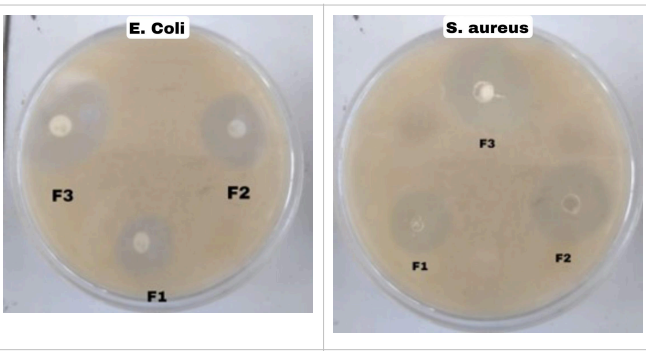
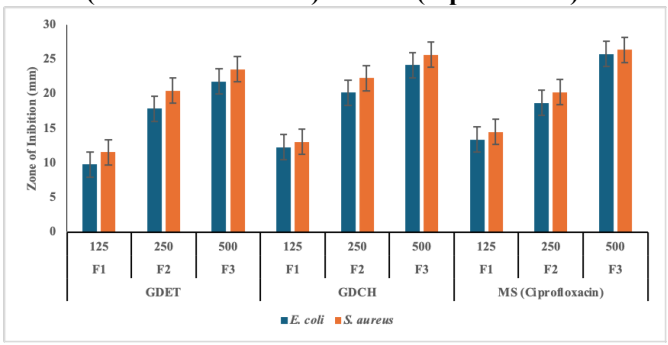


Figure 7: Graphical representation of *In-Vitro* Antidiarrhoeal activity of fraction GDET (Ethanolic fraction), GDCH (Chloroform fraction) and MS (Ciprofloxacin).



Discussion

The comprehensive phytochemical screening of *Gardenia arborea Roxb.* leaves, as summarised in Table 1, reveals a rich presence of a variety of bioactive compounds, suggesting a significant therapeutic potential in line with its traditional uses. The positive outcomes across multiple tests for alkaloids, carbohydrates, glycosides, steroids, flavonoids, saponins, and tannins and phenolic compounds indicate a complex phytochemical matrix that could contribute to the plant's medicinal properties (29). Notably, the presence of flavonoids and saponins, detected through multiple respective tests, aligns well with the known antidiarrhoeal activities of these compounds as reported in other medicinal plants. Flavonoids, in particular, are renowned for their antioxidant and anti-inflammatory properties, which can mitigate intestinal inflammation often associated with diarrhoea. The presence of saponins, with their surfactant-like properties, could also contribute to protective effects on the mucosal lining of the gut, thereby potentially reducing the fluid secretion that typifies diarrhoeal episodes (30, 31).

Comparatively, the absence of triterpenes and phenols in *Gardenia arborea Roxb.*, as indicated by the negative results in sulphuric acid and Ferric chloride tests respectively, sets it apart from other antidiarrhoeal plants where these compounds are typically present. This distinctive phytochemical profile suggests that the antidiarrhoeal mechanism of *G. gummifera* may rely more heavily on the synergistic effects of its other active components rather than triterpenes and phenols, which are often credited with antimicrobial and anti-inflammatory properties. This observation could guide future studies towards exploring the specific bioactivities of the identified compounds and their interactions, thereby providing insights into the therapeutic efficacy and safety of *G. gummifera* leaf extracts as an antidiarrhoeal agent. Such studies could also illuminate how the unique combination of phytochemicals in *G. gummifera* compares to more conventionally used treatments, potentially positioning this plant as a novel resource in managing diarrhoea.

The identification of compounds in the ethanolic and chloroform fractions of *Gardenia arborea Roxb.* using HR-LCMS and subsequent comparison against the Metlin database highlights a diverse phytochemical composition, reflecting a complex interaction of

metabolites with potential therapeutic implications. The successful matching of 20 compounds from the ethanol extract (GDET) underscores the sensitivity and precision of HR-LCMS in detecting and quantifying phytochemicals based on accurate mass measurements and fragmentation patterns, as depicted in Figure 1 and detailed in Table 2. Notable compounds such as Isofraxidin, Licoricesaponin B2, and Tributyl phosphate, which have high match scores, indicate a high confidence in their identification. These compounds are associated with various biological activities; for example, Isofraxidin is known for its anti-inflammatory and antioxidant properties, which could contribute to the antidiarrhoeal efficacy of the extract.

The presence of diverse compounds like N-trans-p-Coumaroyloctopamine and (10)-Dehydroshogaol further enriches the therapeutic profile of the plant extract, suggesting mechanisms that could involve modulation of gut motility or interactions with gut flora. The detailed profiling and identification process not only validate traditional uses but also open pathways for new therapeutic explorations. Comparatively, the identification and functional analysis of these compounds could provide insights into how the ethanolic fraction of *G. gummifera* might offer benefits over other traditional remedies, primarily through a combination of anti-inflammatory, antimicrobial, and direct antispasmodic effects. This comparative analysis fosters a deeper understanding of *G. gummifera's* potential as a multifaceted herbal remedy, positioning it as a candidate for further pharmacological studies aimed at exploiting its full medicinal potential.

The identification of compounds in the chloroform fraction (GDCH) of *Gardenia arborea Roxb.*, as outlined in Table 3 and visualised in Figure 2, expands our understanding of the phytochemical diversity within this medicinal plant. This fraction, characterised by HR-LCMS, showcases a range of bioactive compounds with significant implications for therapeutic applications. The presence of Isofraxidin in both the ethanolic and chloroform fractions, each showing high match scores, suggests a pervasive role for this compound across different solvent extractions, possibly contributing to the plant's overall efficacy in traditional medicine due to its known pharmacological activities. The unique compounds identified in the chloroform fraction, such as Ustiloxin D and Linalool oxide D 3-(apiosyl-(1->6)-glucoside), with high confidence scores, indicate the specificity of solvent extraction in isolating distinct compounds with potentially different modes of action. Ustiloxin D, in particular, is intriguing due to its rare occurrence and significant biological activity, which might offer unique interactions within the biological system, especially concerning gastrointestinal health. The comparison between the ethanolic and chloroform extracts in terms of their compound profiles and associated bioactivities could be crucial for understanding the varying effects of different extracts from the same plant. This comparative approach allows for a nuanced view of how different solvents influence the solubility and recovery of phytochemicals, thereby impacting their biological effectiveness and potential therapeutic utility. Such detailed phytochemical profiling aids in rationalising the traditional use of *Gardenia arborea Roxb.* and sets a foundation for further targeted studies exploring each compound's specific contributions to the plant's medicinal properties.

The identification of compounds such as Isofraxidin, Arborinine, Lycoricidine, Ustiloxin D, 4-Nerolidylcatechol, and (10)-Dehydroshogaol in the extracts of *Gardenia arborea Roxb.*, as shown in Figure 3, provides critical insights into the plant's antidiarrhoeal potential. Isofraxidin, with its anti-inflammatory and antioxidant properties, could modulate gastrointestinal disturbances, while the alkaloids Arborinine and Lycoricidine might interact with neuroreceptors or ion channels, affecting gut motility and secretion. Ustiloxin D, a cyclic peptide, potentially disrupts enzymatic pathways within pathogens or host cells, contributing further to antidiarrhoeal effects. Additionally, 4-Nerolidylcatechol's antioxidant and antimicrobial activities could target oxidative stress and microbial imbalances in the gut, and (10)-Dehydroshogaol, known for its gastrointestinal benefits, might modulate gut functions through enzyme and receptor interactions. Together, these compounds illustrate a multifaceted therapeutic approach, combining anti-inflammatory, antimicrobial, antispasmodic, and antioxidant actions, validating the traditional use of *Gardenia arborea Roxb.* and highlighting its potential as a source for developing multi-targeted antidiarrhoeal therapies.

The *in-vitro* antidiarrhoeal activity of *Gardenia arborea Roxb.,* as depicted in Figures 4, 5, and 7 and detailed in Table 4, reveals that both the ethanolic (GDET) and chloroform (GDCH) fractions exhibit significant antibacterial activity against *E. coli* and *S. aureus*, common pathogens associated with gastrointestinal infections. The zone of inhibition increases with concentration for both fractions, indicating a dose-dependent response. For instance, the ethanolic fraction at 500 µg/ml inhibits *E. coli* by 21.8±0.5 mm and *S. aureus* by 23.6±0.5 mm, while the chloroform fraction at the same concentration inhibits *E. coli* by 24.2±0.6 mm and *S. aureus* by 25.7±0.7 mm. These results are comparable to, and in some cases exceed, those of the standard antibiotic ciprofloxacin (MS), which shows inhibition zones of 25.8±0.3 mm and 26.4±0.6 mm against *E. coli* and *S. aureus* respectively at 500 µg/ml. This indicates that *Gardenia arborea Roxb.* fractions possess potent antimicrobial properties that could contribute to their antidiarrhoeal effects by reducing pathogen-induced gut disturbances. Comparatively, the GDCH fraction shows slightly higher efficacy than the GDET fraction at equivalent concentrations, suggesting that the chloroform solvent may extract more potent or a higher concentration of bioactive compounds effective against these bacteria, as evidenced by the zone of inhibition values presented in Figure 5 and 7. The consistency and significance of these results across different concentrations and bacterial strains emphasise the potential of *G. gummifera* as an effective natural remedy for diarrhoea,

supporting its traditional use and opening avenues for further pharmacological development. This comparative analysis also underscores the importance of solvent selection in maximising the extraction and efficacy of phytochemical constituents from medicinal plants, pointing to the need for further studies to optimise extraction techniques and fully characterise the bioactive compounds responsible for these observed effects.

Conclusion

The comprehensive study on *Gardenia arborea Roxb.* validates its significant therapeutic potential, corroborated through detailed phytochemical profiling and in-vitro antidiarrhoeal activity assessments. Phytochemical investigation via HR-LCMS revealed a rich array of bioactive compounds including Isofraxidin, Arborinine, and Ustiloxin D, supporting the plant's reputed medicinal benefits. The in-vitro tests demonstrated potent antibacterial effects against *E. coli* and *S. aureus*, showcasing efficacy comparable to or exceeding that of standard antibiotics such as ciprofloxacin. These findings not only substantiate the traditional use of G. gummifera in treating diarrhoea but also highlight its promise as a source for developing natural antidiarrhoeal agents. The observed dose-dependent effectiveness of the extracts indicates that the active components within the plant are capable of significantly reducing pathogen-induced gastrointestinal disturbances, suggesting a viable basis for future pharmacological advancements and therapeutic applications.

Abbreviations

HR-LCMS: High-Resolution Liquid Chromatography-Mass Spectroscopy; *E. coli*: *Escherichia coli*; *S. aureus*: *Staphylococcus aureus*; GDET: Ethanolic fraction of *Gardenia arborea Roxb.*; WHO: World Health Organisation; ORS: oral rehydration solutions; *G. gummifera*: *Gardenia arborea Roxb.*; DMSO: dimethyl sulfoxide; MHA: Mueller Hinton Agar; ZOI: zone of inhibition.

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Author Contribution

All Authors Contributed Equally.

Competing Interests

The authors declare that they have no competing interests.

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