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Phytochemical Analysis and Antimicrobial Activity of *Bergenia ligulata* and its Ayurvedic Formulation

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Abstract: Paashanbheda (which means "to dissolve stones"), or *Bergenia ligulata*, is a critically important medicinal plant native to the Indian states of Uttarakhand and Himachal Pradesh. Phenols, flavonoids, tannins, lactones, etc. are some of the many secondary metabolites which are responsible for its pharmacological applications. According to the physicochemical analysis, the alcohol and water-soluble extractive value of *Bergenia ligulata* was found to be 20± 0.5% w/w and 16±6% w/w, respectively. Gallic acid is a major component of *Bergenia ligulate* which is confirmed by chromatographic analysis. Optimized solvent system ethyl acetate: toluene:formic acid (6:6:1 v/v) was found to be the most effective for HPTLC fingerprint separation in both ethanolic and aqueous extract of *Bergenia ligulata* and its herbal formulation (GBD tablets). Antimicrobial activity of aqueous extracts of *Bergenia ligulate* and formulation (GBD tablet) was analysed on *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* which showed satisfactory effects. Present study revealed that *Bergenia ligulate* and its formulations are rich source of antioxidant and beneficial for medication.

Keywords: *Bergenia ligulata*, Antibacterial, Agar well diffusion, Minimum inhibitory concentration, Gallic acid, Phytochemicals

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Introduction

Bergenia ligulata commonly known as the Fringed Bergenia or Ciliated Bergenia, is a perennial flowering plant belonging to the family Saxifragaceae. For centuries, the rhizomes of *Bergenia ligulata* plant have been used in Indian herbal medicine to aid in the passing of kidney and bladder stones, giving the plant the moniker 'Paashanbheda' (meaning 'to dissolve stones') (Gurav *et al*., 2014). In addition to its Sanskrit and English names, this plant is known as Amabhedaka, Silpbheda, Dhoklumbo, Patharchat, Laoo-patra in Hindi, and Pashanbheda in Marathi (Ahmad *et al*., 2018). It is a clump-forming herbaceous plant that averages 12-24 inches in height. The undersides of its broad, spherical leaves have a crimson or purple color, while the top surfaces are bright green. The leaves of this plant have fringed edges, earning it the popular name "Fringed Bergenia". At blooming season (March-May), the leaves are either elliptical or spherical and may grow to be 5-15 cm in length (Kirtikar and Basu, 2005; Gurav *et al*., 2014). *Bergenia ligulata* has several use in traditional medicine. Numerous Ayurvedic and Unani medicines rely heavily on its rhizomes and leaves (Singh *et al*., 2007). It has medicinal properties and are used in herbal remedies for treating ailments such as diarrhoea, dysentery, and respiratory disorders. Diuretic, antidiabetic (Singh *et al*., 2009a, b; Saijyo *et al*., 2009), antitussive, insecticidal, antiviral (Kour *et al*., 2019), anti-inflammatory (Singh *et al*., 2021, Ghosh *et al*., 2021), antipyretic, anti-bradykinin, antimalarial, antibacterial (Agnihotri *et al*., 2015), hepato-protective (Haritha *et al*., 2021), antiulcer, anticancer, antioxidant (Uddin *et al*., 2014), free radical scavenging (Shirsat *et al*., 2008), anti-obesity, are just a few of the biological activities (Patel *et al*., 2015; Kahkeshani *et al*., 2019; Yang *et al*., 2020) displayed by *Bergenia* species. About 30 different genera and 580 different species make up this group. Its natural habitats include Afghanistan, southern Tibet, and Bhutan. It inhabits the 900-3000 m altitude range over the temperate Himalayas, from Kashmir to Bhutan (Handa *et al*., 1997). *Bergenia ligulata* shows the presence of different chemical constituents like coumarins, Sterols such as β-sitosterol and stigmasterol, flavonoids such as quercetin, kaempferol, and their glycosides. Flavonoids are known for their antioxidant and anti-inflammatory properties, Phenolic acids including gallic acid, ferulic acid, and caffeic acid. These compounds contribute to plant's antioxidant and antimicrobial activities (Chandrareddy *et al*., 1998; Sadat *et al*., 2015; Khan *et al*., 2016)

Gallic acid is a naturally occurring organic compounds that belongs to family of phenolics acids. It is widely distributed in various plants, fruits and beverages and is known for its pharmacological properties (antioxidant and anti-inflammatory). It is chemically classified as a trihydroxy benzoic acid, exhibit strong antioxidant activity, which makes it useful in various applications, including food preservation, pharmaceuticals, and cosmetics.

The present study used the HPTLC technique to analyse the aqueous and ethanolic extract of commercially available *Bergenia ligulata* powder and its polyherbal formulation (GBD tablets) for the presence of gallic acid. Phytochemical and physicochemical evaluation were also performed. Studies on the antimicrobial properties of an aqueous extract of *Bergenia ligulata* and an ayurvedic preparation of the plant were also conducted (Indian Herbal Pharmacopoeia, 2002; Singh *et al*., 2011).

Materials and Methods

Plant materials and Chemicals

For this study standard gallic acid was purchased from Yuca Enterprises, Mumbai, India. Powder of *Bergenia ligulata* was purchased from Manakarnika Aushadhalaya, Chinchwad, Pune, India. GBD Tablets of Inducare Pharma Pvt. Ltd Jejuri, Pune was used for comparative study.

Preparation of Standard solution

By mixing 10 mg of accurately measured Gallic

acid into 100 ml methanol, a solution of Gallic acid (100 µg/ml) was prepared and referred as stock solution. Calibration of the HPTLC plate was performed by applying a standard solution between 100 and 600 ng per band.

Preparation of sample

Using the solubility of marker compounds, 10 g of *Bergenia ligulata* powder, 10 g of GBD tablet was added into 100 ml of water and ethanol, respectively, and extracted using the maceration procedure over the course of 24 h. After filtering, concentrating, and using the solution, HPTLC analysis was performed.

HPTLC instrumentation and experimental conditions

The methanol was used to clean the plates beforehand. After that, chromatography plates were heated to 100 °C for 10 min to activate them. Bands 6 mm wide contained the drug standard and samples. The Camag Linomat 5 applicator was used to spot the bands using a Camag microlitre syringe onto silica gel-coated aluminium plates of dimension 60 RP-18 F254 S. The scanning rate was set at 20 mm/sec. Toluene, ethyl acetate, and formic acid (by volume) (6:6:1) made up the mobile phase. We did a linear rising growth in a 20 x 10 cm twin-trough glass box (Camag, Muttenz, Switzerland) that was filled with the mobile phase. At room temperature, 15 min was the optimal period for mobile phase saturation in the optimization chamber. The chromatogram run was around 80 mm in length. Following development, the HPTLC plates were dried using an air drier and a flow of air. The Camag TLC scanner 3 was used to accomplish the densitometric scanning, with the help of winCATSsoftware (Version 1.3.0).

HPTLC profiling analysis

For this method, a 10x10 TLC plate with activation was employed. Camag Linomat V Automatic Sample Spotter was used to apply the following tracks:

Track 1- Standard Gallic Acid solution: 100 µg/ml, 1 µl)

Track 2- Standard Gallic Acid solution: 100 µg/ml, 2 µl)

Track 3- 10 mg/ml aqueous extract of *Bergenia ligulata* powder.

Track 4- 10 mg/ml ethanolic extract of *Bergenia ligulata* powder.

Track 5-10 mg/ml aqueous extract of marketed tablet (GBD)containing Gallic acid.

Track6- 10 mg/ml ethanolic extract of marketed tablet (GBD) containing Gallic acid.

Solvent System: Ethyl acetate: Toluene: Formic acid (6:6:1 v/v/v)

The plate was run in reported Mobile phase Ethylacetate: Toluene:Formic acid (6:6:1 v/v/v) in CAMAG glass twin trough chamber 10 × 10 cm.

The plate was placed under the Camag TLC Scanner 3 which was linked to win CATS software for track scanning and spectral scanning.

Linearity for standard gallic acid by HPTLC

Gallic acid linearity was performed on a 10x10 TLC plate that had been activated. Camag Linomat V Automatic sample spotters were utilized for sample application in the following way: Gallic acid solution (at increasing concentrations, from 100 ng to 600 ng) found on tracks 1 through 6. The plate was run in mobile phase Ethyl Acetate: Toluene: Formic acid (6:6:1 v/v/v) in CAMAG glass twin trough chamber (10-20 cm). After drying, the plate was scanned using Camag TLC Scanner 3, which was linked to the winning CATS program. Microsoft Excel was used to determine linearity.

Antimicrobial activity of Aqueous extract and herbal formulation

The Zone of Inhibition for various herbal compounds may be calculated using the agar well diffusion technique (Sarita *et al*., 2019).

Results and Discussion

Physicochemical analysis

Table 1: water and alcohol soluble extractive value of plant *Bergenia ligulata*

Water soluble extractive value % w/w		Alcohol soluble extractive value %w/w	
Reported	Experimentally	Reported	Experimentally
NLT 15	16 ± 0.5	NLT 9	20± 0.5

Table 2: Phytochemical analysis of plant *Bergenia ligulata*

Contents	**Presence or absence in aqueous extract**	**Presence or absence in Ethanolic extract**
Tannin	+	+
Alkaloid	-	-
Flavonoids	+	+
Steroids	-	-
Saponin	+	-
Terpenoids	-	-
Steroids	-	-
Phenols	+	+

Physiochemical parameters like extractive values (alcohol soluble and water-soluble extractive values) of plant were determined (Table 1) and were associated to the stated values specified in literature (Indian Herbal Pharmacopoeia, 2002).

Phytochemical analysis

Tannin, saponin, flavonoid, cardiac glycoside, and phenol content in the aqueous extract of *Bergenia ligulata* powder were analyzed phytochemically (Sarita *et al*., 2019). Table 2 illustrates phyto-chemical constituents of *Bergenia ligulata* extract (aqueous and ethanolic).

λ max of Gallic acid was found to be 261. It was verified by comparing the result to the gallic acid value that had been published earlier. Therefore, the 261 nm wavelength was used for all subsequent measurements (Fig. 1). For this method, a 10x10 HPTLC plate with activation was employed. The Camag Linomat V Automatic Sample Spotter was used during the sample application process (Table 3).

Since the Rf values of the standard and sample were so similar, we know that gallic acid is present in the extracts we tested. *Bergenia ligulata* and a commercially available formulation (GBD tablet) containing gallic acid have both been confirmed by track and spectrum scanning to contain gallic acid (Table 3, Figs. 2, 3).

Linearity for standard Gallic acid by HPTLC

The linearity was analyzed using an Activated 1010 TLC plate. CAMAG Linomat V automated sample spotter was used to apply the tracks. This led to the findings illustrated in Table 4.

The Area Under the Curve (AUC) was calculated via scanning the scanned tracks. The linearity was determined by making a scatter plot

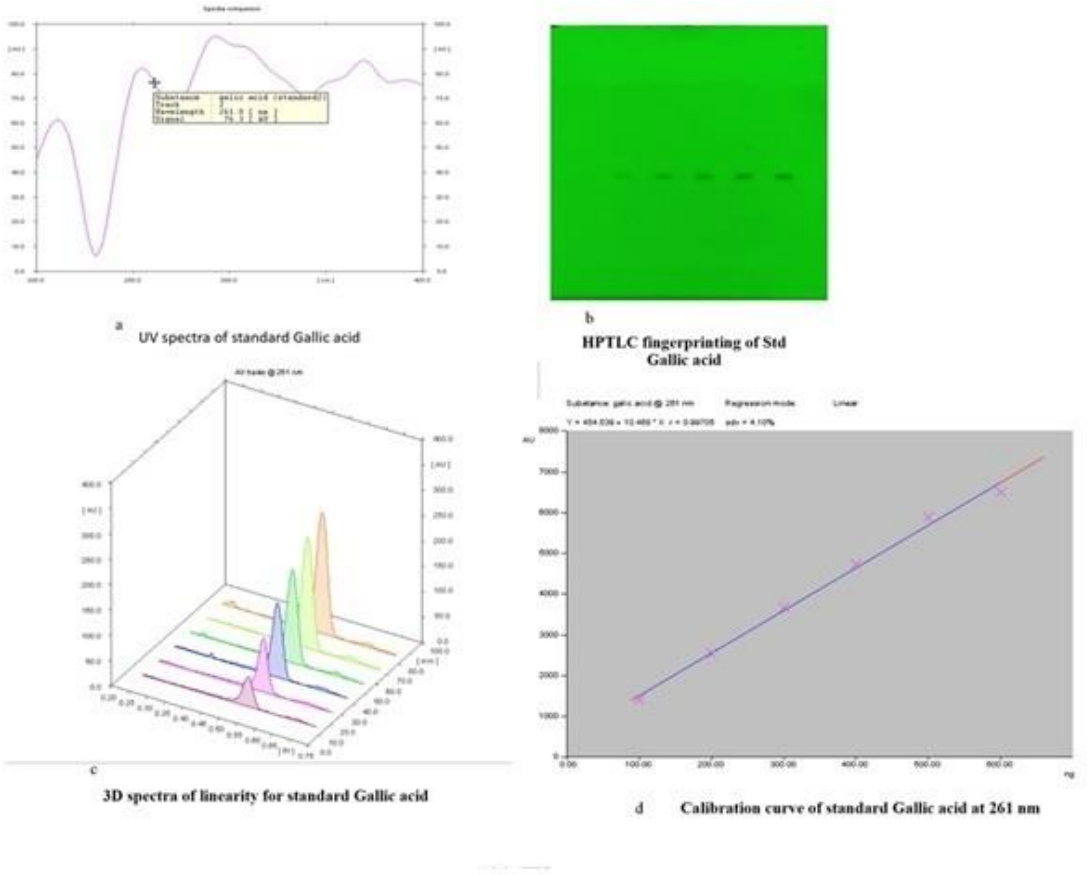

Fig. 1: (a) UV spectra of Gallic acid, (b) HPTLC fingerprinting of Gallic acid, (c) 3D spectra of linearity of Standard Gallic acid. (d) Calibration curve of Standard Gallic acid at 261 nm.

Table 3: Sample application on HPTLC plate

Track No.	**Name**	**Concentration**	**Application volume**	**Rf value**	**Area**
1	Standard Gallic Acid solution	100 µg/ml	1.0 µl	0.56	1721
2	Standard Gallic Acid solution	100 µg/ml	2.0 µl	0.59	3619.3
3	Aqueous extract of *Bergenia ligulata*	10000 µg/ml	2.0 µl	0.58	6294.6
4	ethanolic extract of *Bergenia ligulata*	10000 µg/ml	5.0 µl	0.57	3835
5	Aqueous extract of Marketed formulation(GBD Tablet)	10000 µg/ml	10.0 µl	0.59	586.6
6	Ethanolic extract of Marketed formulation(GBD Tablet)	10000 µg/ml	10.0 µl	0.60	498.8

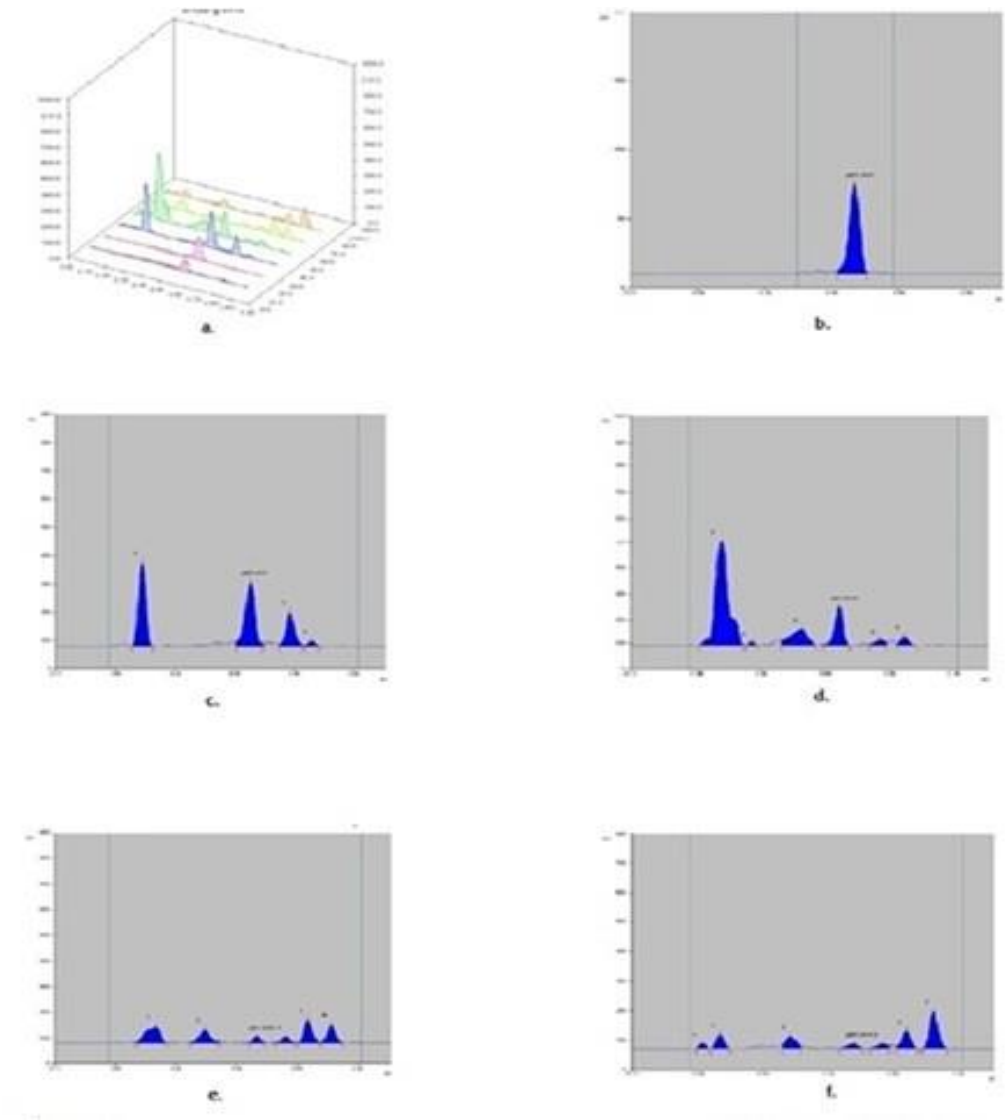

Fig. 2: HTPLC Fingerprinting profile, (a) 3D spectra of standard, aqueous and ethanolic extract of *Bergenia ligulata* along with aqueous and ethanolic extract of marketed formulation (GBD tablet), (b) standard gallic acid, (c) aqueous extract of *Bergenia ligulata*, (d) ethanolic extract of *Bergenia ligulata*, (e) aqueous extract of marketed formulation (GBD tablet), (f) ethanolic extract of marketed formulation (GBD tablet),

Table 4: Standard Gallic acid solution (in escalating volumes) found on Tracks 1 - 6

Track No.	Name	Concentration	Volume applied	Final volume on TLC Plate	Rf	Area
1	Std. Gallic acid	100 µg/ml	1 µl	100 ng/band	0.53	1396
2	Std. Gallic acid	100 µg/ml	2 µl	200 ng/band	0.53	2534
3	Std. Gallic acid	100 µg/ml	3 µl	300 ng/band	0.53	3665
4	Std. Gallic acid	100 µg/ml	4 µl	400ng/band	0.53	4729
5	Std. Gallic acid	100 µg/ml	5 µl	500 ng/band	0.52	5879
6	Std. Gallic acid	100 µg/ml	6 µl	600 ng/band	0.52	6504

Table 5: Zone of inhibition of aqueous extract of *Bergenia ligulata* and formulation (GBD tablet)

Bacteria		Zone of inhibition(in mm)		
	Concentration (%)	Aqueous Extract	Marketed Formulation (GBD tablet)	Standard
Escherichia coli	5	19	13	39
	10	20	15	41
	15	21	16	41
	20	22	19	42
Pseudomonas aeruginosa	5	0	10.6	33
	10	21	12	36
	20	21	13	37
	40	23	13	37
Staphylococcus aureus	5	15	12	31
	10	14	13	34
	20	17	13	34
	40	19	14	36

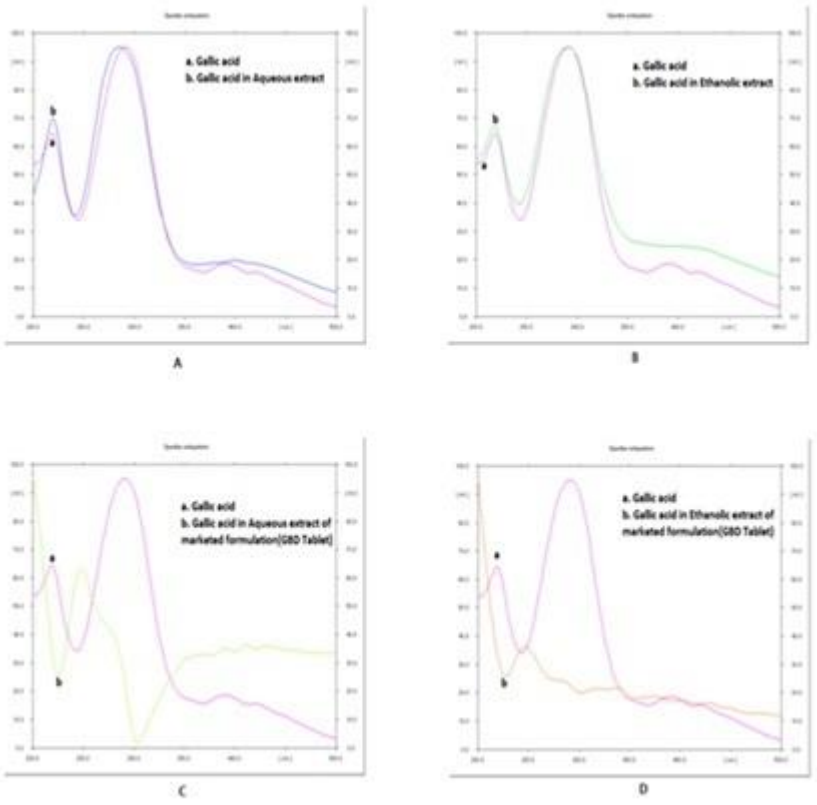

Fig. 3: Overlay UV absorption spectra of Gallic acid in peaks of (A) standard with aqueous extract, (B) ethanolic extract, (C) *Bergenia ligulata* Gallic acid in peaks of standard and aqueous extract, (D) ethanolic extract of marketed formulation (GBD tablet).

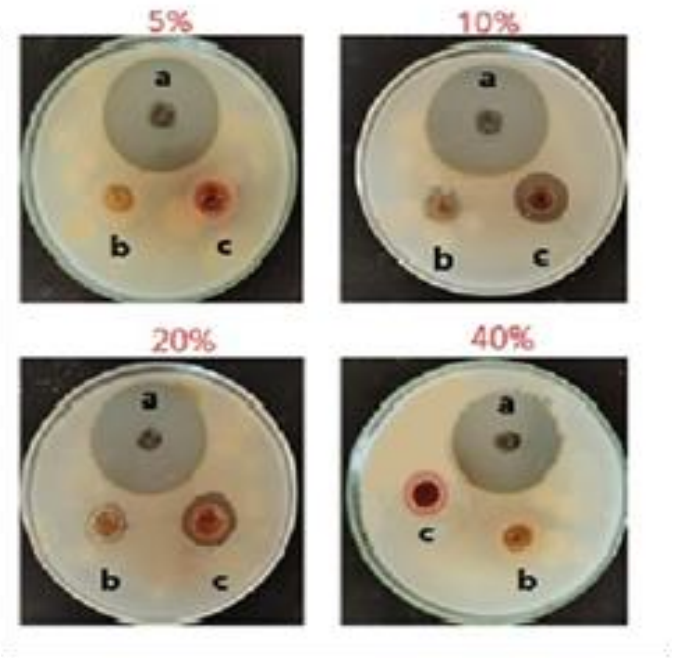

Fig. 4: Zone of inhibition against *E. coli* (a) standard (Ciprofloxacin), (b) marketed formulation and (c) aqueous extract.

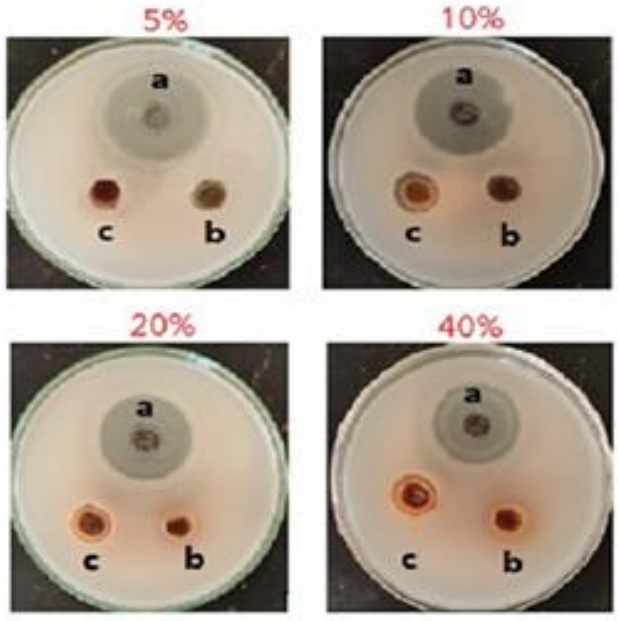

Fig. 5: Zone of inhibition against *P. aerusinosa* (a) standard (Ciprofloxacin), (b) marketed formulation and (c) aqueous extract.

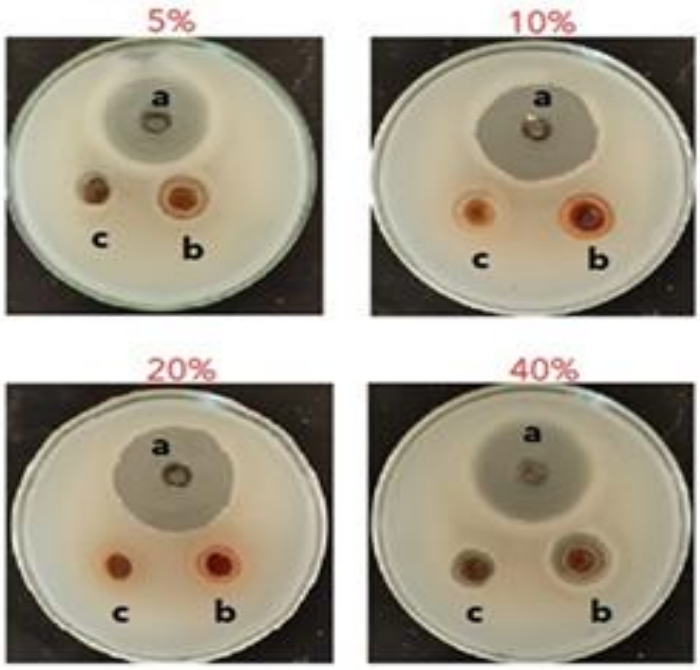

Fig. 6: Zone of inhibition against *S. aureus* (a) standard (Ciprofloxacin), (b) marketed formulation and (c) aqueous extract.

of gallic acid concentrations in Microsoft Excel. When area measurements were compared to concentration values, the R^2 value that was calculated was 0.99705. (Table 4, Fig. 1)

Antimicrobial activity:

Aqueous extract of *Bergenia ligulata* at various concentration (5, 10. 15, 20%) were tested for antibacterial activity. Inhibition zones were determined and noted between 10 and 25 mm (Table 5). In this investigation, we found that aqueous extract had potent antimicrobial properties against *Escherichia coli*, *Staphylococcus aureus*, *and Pseudomonas aeruginosa* (Table 5, Figs. 4, 5, 6)

Conclusion

In present study phytochemical analysis of *Bergenia ligulata* reveals that tannins, phenols, lactone, flavonoids, were present in the aqueous extract. High Performance thin layer chromatography was performed for the determination of Gallic acid in *Bergenia ligulata* and in its polyherbal formulation known as GBD tablet. The optimized solvent system was accurate for the analysis of Gallic acid in crude drug sample and polyherbal sample. Gallic acid has several pharmacological functions including antiviral, free radical scavenging, antidiabetic, hepatoprotective, diuretic, anti-inflammatory, cardioprotective, and antipyretic. The present antimicrobial study of *Bergenia ligulata* and its polyherbal formulation was found to give satisfactory results.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Pharmacognosy, P. E. Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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