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Original Article

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF *BERGENIA LIGULATA (WALL.)* LEAVES EXTRACTS

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ABSTRACT

Objective: Evaluation of Antimicrobial and Antifungal potential of Phytoconstituents present in leaf extracts of *Bergenia ligulata (wall.)* plant.

Methods: The antimicrobial effects of leaves of *Bergenia ligulata* (wall.) were evaluated using both ethanol and aqueous extracts against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans,* and *Penicillium spp.* Agar well diffusion method was employed in determining the antimicrobial activity and the broth dilution method for determining the Minimum Inhibitory Concentration. All the isolates examined were susceptible to both the ethanol, aqueous, and the combined aqueous and ethanol extracts (synergetic effect) of the *Bergenia ligulata* leaves.

Results: The zone of inhibition ranged from 6.93±0.02[b] mm to 12.81±0.03[a] mm, with *Escherichia coli* being the most susceptible at 12.81±0.03[a] mm to the ethanol and 11.53±0.02[a] mm to the aqueous extracts at 250 mg/ml concentration while *P. aeruginosa* and *Penicillin* spp. were the slightest susceptible at 10.88±0.02[a] mm to the ethanol and 09.76±0.02[a] mm to the aqueous extract at 250 mg/ml concentration. The control/standard antimicrobial agent (*Gentamicin* and *Metronidazole*) exhibited higher inhibitory activity than the plant extracts. The least inhibitory value of 6.25 mg/ml was produced against *P. aeruginosa* by the ethanolic extract and against *C. albicans* by the combined (aqueous and ethanol) extracts of the plant. The qualitative and quantitative phytochemical screening of the leaves of *Bergenia ligulata* reveals the presence of flavonoids, tannins, saponins, alkaloids, and steroids. The most abundant percentage composition observed was flavonoids (7.72%), while tannins had the least component (4.29%).

Conclusion: The findings from this study show that the leaves extracts hold considerable antimicrobial activity against commonly encountered microorganisms in the environment. This, therefore, implies that it can be used as a chemotherapeutic agent which will contribute to the development of antibiotic drugs against the test organisms.

Keywords: Antimicrobial, *Bergenia ligulata,* Ethanolic extract, Phytochemical, Burgenin, Metronidazole

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INTRODUCTION

Plants are the basic sources of the acquaintance of modern medicine as several synthetic drugs are made from starting molecules extracted from plants and play an important role in drug development programs in the pharmaceutical industry based on their use in traditional medicine and natural products [1]. Medicinal plants are an abundant source of antimicrobial compounds, and an extensive range of these plant extracts are used to treat numerous infections caused by resistant microorganisms as they have potential antimicrobial activity [2]. They could have effects such as bacteriostatic, bactericidal, sterilizing disinfectant, antiseptic, and preservative.

Bergenia ligulata, commonly denoted as a "stone flower/stone breaker, a member of the *Saxifragaceae* family, is a well-known Ayurvedic medicine *Paashanbheda*. This plant is a highly regarded medicinal herb and one of the most well-known examples of controversial drugs in Indian medicine and is well-known for its ability to dissolve kidney stones. Bergenia comes in three varieties of *B. ligulata, B. ciliata,* and *B. stracheyi* [3]. It is mostly grown in cold and temperate climates and found in the Himalayas, from Kashmir to Bhutan [4]. The leaves are simple or compound, alternate, occasionally opposite, typically exstipulate, inflorescence cymose or racemose, rarely flowers solitary flowers, bisexual or sometimes unisexual are the main botanical characteristics of the Saxifragaceae family. For epochs, the plant's roots have been used in Ayurvedic medicine and we can also use Bergenia in over-the-counter weight-loss and kidney health supplements [5, 6].

Traditionally, the plants are also used to treat asthmatic disorders when taken orally, simply chewed if it's still fresh, to treat diarrhea and vomiting. The plant is also reported for the treatment of fever, cough, pulmonary affections, prolonged uterine hemorrhage, bladder disorders, dysentery, menorrhagia, splenic enlargement, and heart diseases in Ayurveda and Unani medicines [7]. The extract of the rhizome of *B. ciliata* is used as an anti-tussive for colds and cough by the local people of the Sikkim and West Bengal [8, 9].

The phytochemical screening of *Bergenia ligulata* has revealed the leaf extracts of the plant to possess numerous active compounds with Bergenin, afzelechin, and gallic acid as the most active compounds [10]. The methanolic extract of *B. ciliata* rhizomes holds antibacterial activity and inhibited influenza virus replication in a dose-dependent manner but lacked virucidal activity at an appropriate concentration due to the presence of condensed tannins in the extract [8, 11]. Therefore, this study was intended to consider the antimicrobial properties and phytochemical investigation of the leaves extract of *Bergenia ligulata.*

MATERIALS AND METHODS

Collection and identification of plant material

The leaves of *B. ligulata* were purchased from an online source *nursery live* and were identified and authenticated by the Central Institute of Medicinal and Aromatic Plants (CIMAP) Lucknow, India by Dr. Rakesh Kumar Shukla, Senior scientist, Department of Botany and a voucher specimen (CIMAP/2020/3152) was deposited at the research Centre CIMAP Lucknow, India.

Preparation of extract

The leaves were washed with sterilized water, dried in shade and ground to a fine powder using a mechanical blender. The ethanol and aqueous extracts were prepared by soaking 200 grams of the powdered leaves in 650 ml of 95% ethanol and 600 ml of distilled

water individually at room temperature for 24 h. The extracts were filtered separately through Whatman's filter paper and concentrated using a rotary evaporator, warmed in a water bath at 75 °C for the aqueous extract and temperature of 50 °C for ethanol extracts, to obtain crude extracts [12]. The extracts were stored at 4 °C in a refrigerator till further use. A mixture of both extracts (ethanol and aqueous) was used in the synergetic valuation.

Collection and maintenance of test organisms

Stock cultures of bacterial isolates of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and fungal isolates of *Candida albicans* and *Penicillium* spp., were obtained from the laboratory stock of the Department of Microbiology, Ganesh Shankar Vidyarthi Memorial Medical College Kanpur, Uttar Pradesh, India. They were resuscitated and their identities were confirmed using standard procedures and methodology [13, 14]. Pure culture of the bacterial species was maintained on nutrient agar slant and the fungi species on potato dextrose agar slant at 4 °C before use.

Antimicrobial assay

The agar well diffusion method was executed to influence the antimicrobial activities of the leaves extract. Sterile Mueller-Hinton agar plates and potato dextrose agar plates were inoculated with standardized 50 µl inoculum of each selected test organism using the spread plate technique. Wells of 6 mm in diameter were made and 0.1 ml of the various concentrations (50 mg/ml, 100 mg/ml and 250 mg/ml) of the leaf extracts were distributed into the wells. *Gentamicin* and *Metronidazole* 250 mg/ml were used as a positive control. The plates were incubated at 37±2 °C for 24 h. for bacteria and room temperature (25±2 °C) for 72 h for fungi. The antimicrobial potential was estimated by measuring the diameter of the resultant zones of inhibition in millimeters (mm) around the wells [15].

Determination of minimum inhibitory concentration

For the determination of the Minimum inhibitory concentration (MIC) of the extracts, the broth dilution method was employed. Various concentrations of the extracts were prepared by serial dilution method to acquire concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml 1.5625 mg/ml. 2 ml of nutrient/potato dextrose broth holding the test organisms was each distributed into sterilized test tubes and a 1 ml extract of various concentrations added to the test tubes containing the test organisms. The test tubes were corked and incubated aerobically at 37±2 °C for 24 h. for bacteria and 25±2 °C for 72 h for fungi. The turbidity in the test tubes was determined and compared after incubation to determine the MIC. The MICs for each organism were articulated as the lowest concentrations, which inhibit the growth of the microbial test isolates [16].

Determination of minimum bactericidal/ fungicidal concentration

The minimum bactericidal and fungicidal concentrations (MBCs and MFCs) were determined by subculturing 2µl from each of the tubes showing no growth (MIC culture tubes) onto nutrient and potato dextrose agar plates and incubating for 24 h. at 37±2 °C and 72 h. at 28±2 °C correspondingly. The lowest concentration with no observable growth was defined as MBC and MFC correspondingly, indicating 99.90% killing of the original inoculums [12].

Qualitative and quantitative phytochemical analysis

Phytochemical screening of the plant extracts was carried out using standard methods to ascertain the presence or absence of the different metabolites (Glycosides, flavonoids, tannins, saponins, steroids and alkaloids) [17, 18].

Statistical analysis

Data was collected and analyzed using One-way analysis of variance (one-way ANOVA). Values were reported as means of triplicate determination±standard deviation.

RESULTS

The diameter of the zone of inhibition of the ethanol and aqueous extracts of *Bergenia ligulata* against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Penicillin* species illustrates that both extracts had inhibitory activity against the isolates at higher concentrations of between 100 and 250 mg/ml, with the ethanolic extracts represents more activity (table 1) (fig. 1).

Table 1: Antimicrobial screening of ethanolic and aqueous extracts of *Bergenia ligulata* against selected test organisms

Test organisms	Ethanolic extract concentration (mg/ml)			Aqueous extract concentration (mg/ml)			Control (Gentamicin and metronidazole 250 mg/ml)
	250	100	50	250	100	50	
E. coli	12.81±0.03^{a}	6.93±0.02^{b}	0.04±0.01^{c}	11.53±0.02^{a}	2.01+0.00^{b}	0.03±0.00^{c}	21.91±0.04
S. aureus	11.78±0.02^{a}	7.11±0.02^{b}	1.30+0.01^{c}	10.21±0.01^{a}	1.02±0.00^{b}	1.27±0.01^{c}	19.08±0.02
P. aeruginosa	10.88±0.02^{a}	6.87±0.01^{b}	0.91±0.01^{c}	10.76±0.02^{a}	0.91±0.02^{b}	0.86±0.00^{c}	23.89±0.02
C. albicans	12.23±0.01^{a}	7.98±0.01^{b}	1.21±0.01^{c}	10.13±0.02^{a}	2.78±0.02^{b}	1.15±0.01^{c}	26.17±0.06
Penicillin spp.	10.97±0.02^{a}	7.35±0.02^{b}	0.88±0.01^{c}	9.76±0.02^{a}	6.44±0.02^{b}	0.74±0.00^{c}	28.37±0.06

Values with different superscripts are significantly dissimilar from each other (P<0.05).

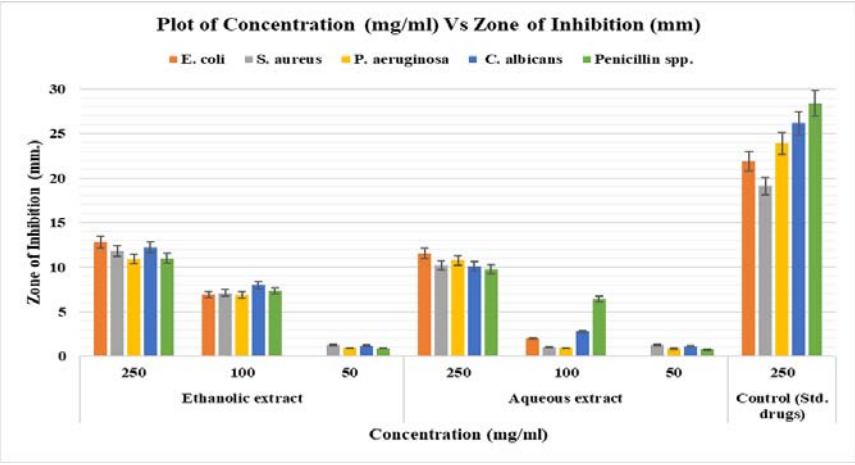

Fig. 1: Plot of antimicrobial screening of ethanolic and aqueous extracts of *Bergenia ligulata* against selected test organisms

Comparison made between standard drugs (*Gentamicin* and *Metronidazole* 250 mg/ml) Vs various concentrations of the ethanolic and aqueous extract of *B. ligulata* (50,100 and 250 mg/ml). P<0.05 considered significant data.

The collective (Synergetic) effects of the ethanol and aqueous extracts of *B. ligulata* against the test isolates illustrate that the highest *in vitro* antimicrobial activity (14.17±0.02 mm) was revealed at the concentration of 250 mg/ml against *E. coli*, while the least antimicrobial activity (11.96±0.01 mm) against *P. aeruginosa* and *Penicillin* spp. was also revealed at the concentration of 250 mg/ml. The zone of inhibition produced by the standard antimicrobial agents (*Gentamicin* and *Metronidazole*) against the test microorganisms exhibited higher inhibitory activities than the extracts of *Bergenia ligulata* (table 2) (fig. 2).

Table 2: Antimicrobial screening for the synergetic effect of aqueous and ethanol extracts of *Bergenia ligulata* against selected test organisms

Test organisms	Ethanol+Aqueous extract concentration (mg/ml)			Control
	200	100	50	(Gentamicin and metronidazole 250 mg/ml)
E. coli	14.17±0.02[a]	11.07±0.01[b]	1.51±0.01[c]	21.91±0.04
S. aureus	13.19±0.03[a]	9.21±0.02[b]	1.41±0.01[c]	19.08±0.02
P. aeruginosa	11.96±0.02[a]	7.21±0.01[b]	1.92±0.01[c]	23.89±0.02
C. albicans	13.23±0.02[a]	8.16±0.01[b]	1.76±0.01[c]	26.17±0.06
Penicillin spp.	11.97±0.02[a]	7.34±0.01[b]	1.24±0.01[c]	28.37±0.06

Values with different superscripts are significantly dissimilar from each other (P<0.05).

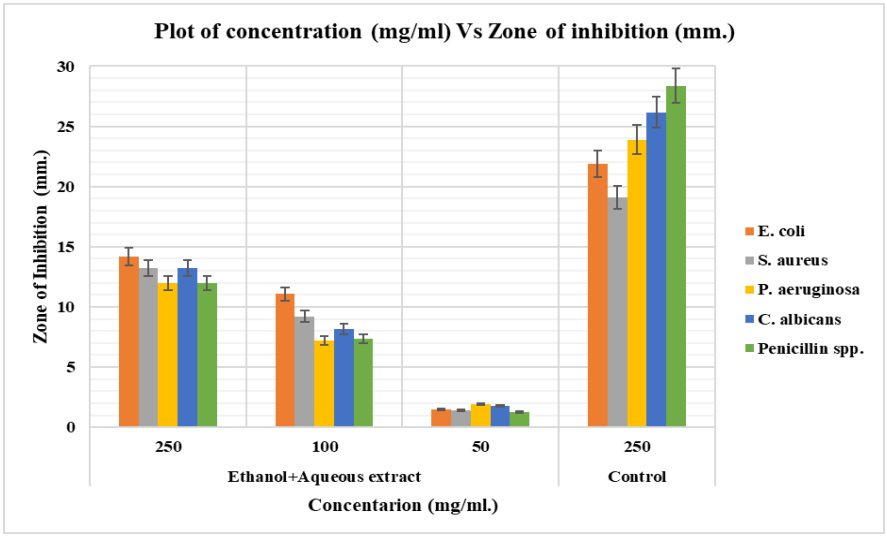

Fig. 2: Antimicrobial screening for the synergetic effect of aqueous and ethanol extracts of *bergenia ligulata* against selected test organisms

Comparison made between standard drugs (*Gentamicin* and *Metronidazole* 250 mg/ml) Vs various concentrations of the combination of ethanolic and aqueous extract of *B. ligulata* (50,100 and 250 mg/ml). P<0.05 considered significant data.

The minimum inhibitory concentration (MIC) of the extracts of *B. ligulata* in contradiction to the test isolates are epitomized in table 3 and table 4. Both extracts (aqueous and ethanol) exhibited active inhibitory effects, but the ethanolic extracts showed more activity. The least inhibitory value of 6.25 mg/ml was produced against *P. aeruginosa* and *C. albicans* by the ethanolic extract and by the combined extract of the aqueous and ethanolic extracts of *B. ligulata* correspondingly.

Table 3: MIC values of aqueous and ethanol extracts of *B. ligulata* against test isolates

Extracts	Test organisms	Concentrations (mg/ml)						
		50	25	12.5	6.25	3.12	1.56	MIC
Aqueous	*E. coli*	+	+	+	+	+	+	50
	S. aureus	+	+	+	+	+	+	50
	P. aeruginosa	+	+	+	+	+	+	50
	Penicillin spp.	-	+	+	+	+	+	25
	C. albicans	-	-	+	+	+	+	12.5
Ethanolic	*E. coli*	-	+	+	+	+	+	25
	S. aureus	-	+	+	+	+	+	25
	P. aeruginosa	-	-	-	+	+	+	6.25
	Penicillin spp.	-	-	+	+	+	+	12.5
	C. albicans	-	-	+	+	+	+	12.5

Key: += Positive,-= Negative, MIC–Minimum Inhibitory Concentration.

Table 4: MIC values of collective extract (Aqueous+Ethanol) of *B. ligulata* against test isolates

Combined extract (Aqueous and ethanolic	Test organisms	Concentrations (mg/ml)						
		50	25	12.5	6.25	3.12	1.56	MIC
	E. coli	-	+	+	+	+	+	25
	S. aureus	-	+	+	+	+	+	25
	P. aeruginosa	-	-	+	+	+	+	12.5
	Penicillin spp.	-	+	+	+	+	+	25
	C. albicans	-	-	-	+	+	+	6.25

Key: += Positive,-= Negative, MIC–Minimum Inhibitory Concentration.

The minimum bactericidal/fungicidal concentration showed more activity by the ethanolic extracts of *B. ligulata* against *P. aeruginosa* at 12.5 mg/ml (table 5), and the combined (ethanolic+aqueous) extracts against *C. albicans* at 12.5 mg/ml (table 6), while the aqueous plant extract had cidal action at the concentration of 50 mg/ml for all the isolates tested.

Table 5: Minimum bactericidal/fungicidal concentration (MBC/MFC) values of aqueous and ethanolic extracts of *B. ligulata* against test isolates

Extracts	Test organisms	Concentrations (mg/ml)						
		50	25	12.5	6.25	3.12	1.56	MBC/MFC
Aqueous	*E. coli*	+	+	+	+	+	+	50
	S. aureus	+	+	+	+	+	+	50
	P. aeruginosa	+	+	+	+	+	+	50
	Penicillin spp.	-	+	+	+	+	+	50
	C. albicans	+	+	+	+	+	+	50
Ethanolic	*E. coli*	-	+	+	+	+	+	50
	S. aureus	-	+	+	+	+	+	50
	P. aeruginosa	-	-	-	-	+	+	12.5
	Penicillin spp.	-	-	+	+	+	+	25
	C. albicans	-	-	+	+	+	+	25

Key: += Positive,-= Negative, MBC–Minimum Bactericidal Concentration, MFC–Minimum Fungicidal Concentration.

Table 6: Minimum bactericidal/fungicidal concentration (MBC/MFC) values of combined extracts (Ethanolic+Aqueous) of *B. ligulata* against test isolates

Combined extract (Aqueous and ethanolic)	Test organisms	Concentrations (mg/ml)						
		50	25	12.5	6.25	3.12	1.56	MIC
	E. coli	-	+	+	+	+	+	50
	S. aureus	-	+	+	+	+	+	50
	P. aeruginosa	-	-	+	+	+	+	50
	Penicillin spp.	-	+	+	+	+	+	50
	C. albicans	-	-	-	-	+	+	12.5

Key: += Positive,-= Negative, MBC–Minimum Bactericidal Concentration, MFC–Minimum Fungicidal Concentration.

The qualitative and quantitative phytochemical screening of the leaves of *Bergenia ligulata* showed the presence of flavonoids, tannins, saponins, alkaloids and steroids. The most copious percentage composition detected was flavonoid (7.72 %), followed by and Tannins (4.29 %), Alkaloid (3.81 %) and saponins having the least component (1.26 %). Some tests also represent the presence of steroids in the least quantity (0.90 %).

Table 7: Qualitative phytochemical analysis of the leaves extract of *Bergenia ligulata*

Group of phytochemicals	Observations	% Composition
Flavonoids	+++	7.72±0.17
Tannins	+++	4.29±0.12
Alkaloids	++	3.81±0.04
Saponins	+	1.26±0.02

Values are results of triplicate analysis.

DISCUSSION

Antibiotics have persisted as the pillar of drug therapy for infectious diseases worldwide. Investigations of natural components from plants that may oblige as valuable sources of antimicrobial agents for therapeutic uses seem to be a practicable alternative to conventional antibiotics in the face of enhancing antibiotic resistance. In this study, antimicrobial influence and synergistic effect of crude aqueous and ethanolic extracts of leaves of *Bergenia ligulata* against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Penicillium* species were examined (table 1 and 2).

The results attained from the antimicrobial potential of the extract of the leaves of *Bergenia ligulata,* independently and in combination (synergy) possess considerable antimicrobial activity against commonly encountered microorganisms considered.

The antimicrobial potential of the extracts was detected to be concentration-dependent, and the activity varied with concentration against the tested pathogens. All the organisms tested were sensitive to the extracts of *Bergenia ligulata* between the concentrations of 100 mg/ml to 250 mg/ml. The inhibition zone ranged from 6.97±0.00 mm to 14.17±0.02 mm, with *Escherichia coli* being the most vulnerable (at 14.17±0.02 mm, 12.81±0.03 mm and 11.53±0.02 mm) to the synergy, ethanol and aqueous extracts at 250 mg/ml concentration correspondingly while *P. aeruginosa* and *Penicillin* spp. were the least susceptible (at 11.97±0.02[a] mm, 10.88±0.02 mm and 10.76±0.02 mm) to the synergy, ethanol and aqueous extract at 250 mg/ml concentration correspondingly.

The circumstance that the various microbial isolates subjected to the extract of *Bergenia ligulata* were susceptible shows that the leaves of this plant have antimicrobial potency. The results were approved with the work of [19], who reported that the leave extract of *Bergenia ligulata* has antimicrobial and antioxidant potential against some selected range pathogens and infectious diseases. The results obtained show that the ethanolic extracts possess maximum potential against the extract on all microorganisms tested than the aqueous extracts. This probably indicates that ethanol is a better solvent than water in the extraction of the active components of the plant. This. Additionally, ethanol extract produced the maximum zones of inhibition, agreeing with the previous studies [20]. The result attained in this study is comparable with similar studies on *Moringa oleifera* extracts that have been reported to validate antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans*, and *Mucor* species [21-23].

The minimum inhibitory concentration (MIC) of the various extracts was detected to be between the concentrations of 6.25 mg/ml and 12.5 mg/ml for the ethanolic and combined (synergy) extracts on both bacterial and fungal isolates and 50 mg/ml for the aqueous extracts on both bacterial and fungal isolates (table 3 and 4). These findings are in favor of similar reports that documented remarkable activity against the growth of *E. coli, S. aureus* and *K. pneumonia* with zones ranging from 13 mm to 26 mm and MIC values of 25 mg/ml, 50 mg/ml and 100 mg/ml for the test organisms correspondingly [24, 25].

The minimum bactericidal/fungicidal concentration (MBC/MFC) values imitate the superior activity of the ethanol extracts against *P. aeruginosa* at 12.5 mg/ml, *Penicillin spp.* and *C. albicans* at 25 mg/ml when associated with the aqueous extracts at concentrations of 50 mg/ml against all tested isolates (table 5). The combined (synergy) extracts had values of 12.5 mg/ml against *C. albicans* and 50 mg/ml against all bacterial isolates tested (*E. coli, S. aureus, P. aeruginosa)* and *Penicillin* spp. (table 6). The study concluded that the ethanolic extracts have more inhibitory effects as an antimicrobial agent in comparison to aqueous extracts and when compared with the control (standard antibiotics) that produced maximum inhibition than the various extracts against all the test organisms.

Phytochemical screening of leaves of *Bergenia ligulata* shows the presence of flavonoids, tannins, saponin, alkaloids and traces of steroids. Quantitatively, the percentage yields of phytochemical content of the leaves of *Bergenia ligulata* resulted from Flavonoids (7.72%), Tannins (4.29%), Alkaloids (3.81%) and Saponins (1.26%) which shows that the leaves of the plant *B. ligulata* having considerable amount of the phytochemicals responsible for its therapeutic importance.

CONCLUSION

The *in vitro* antimicrobial potential established by *Bergenia ligulata* leaves extracts against the microbial test isolates designates that the plant has potential antimicrobial strength. Thus, validating the local use of *Bergenia ligulata* for medicinal purposes in treating infectious diseases such as gastrointestinal disorders, gonorrhea, diarrhea, typhoid and other infections in which the tested pathogens may be concerned. The presence of phytochemicals and the ability of the leaves extracts to inhibit the growth of numerous microbial species is an indication of the broad-spectrum antimicrobial potential of *Bergenia ligulata,* which makes it a potential candidate for a prospective antimicrobial drug. Thus, this plant is effective and comprises natural compounds that could be used in the treatment of infections and helps promote alternative medicine as better substitutes to synthetic antimicrobials.

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Nil

AUTHORS CONTRIBUTIONS

Dr. Pranay Wal gave a substantial contribution by drafting the manuscript and extensively revised to improve its quality of the manuscript. Dr. Awani Kumar Rai suggest the Conception, the design of the study and supervision of the work were done. Mr. Yatendra Singh performed bench work, experiments, and statistical analysis. Mr. Shashi Pratap Singh executed the experimental work in the laboratories.

CONFLICT OF INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

REFERENCES

1. Ugboko HU, Nwinyi OC, Oranusi SU, Fatoki TH, Omonhinmin CA. Antimicrobial importance of medicinal plants in Nigeria. Scientific World Journal. 2020;2020:7059323. doi: 10.1155/2020/7059323. PMID 33029108.
2. Renisheya JJMT, Johnson M, Mary UM, Arthy A. Antibacterial activity of ethanolic extracts of selected medicinal plants against human pathogens. Asian Pac J Trop Biomed. 2011;1(1):S76-8. doi: 10.1016/S2221-1691(11)60128-7.
3. Haritha C, Ramya D, Naveen R, Prasanna SV, Salomi P. A comprehensive review on Bergenia ligulata (Paashanbheda) and its role in the treatment of kidney stone formation. Int J Res Ayurveda Pharm. 2021;12(4):94-9. doi: 10.7897/2277-4343.1204113.
4. Handa SS. Indian herbal pharmacopoeia. Vol 1. Mumbai: A joint publication of RRL Jammu and IDMA; 1997. p. 17-24.
5. Kirtikar K, Basu B. Indian medicinal plants. Lalit Mohan Basu publication. 2nd ed. Vol. III; 1983. p. 993-4.
6. Pandey G, Vijnana DG. Varanasi. Krishna Acad. 1988;III:69-71.
7. Kirtikar K, Basu B. Textbook of Indian medicinal plants. Volume II. 2nd ed. Dehradun. India. International Book Distributors; 2005. p. 993-4.
8. Sinha S, Murugesan T, Maiti K, Gayen JR, Pal B, Pal M. Antibacterial activity of Bergenia ciliata rhizome. Fitoterapia. 2001;72(5):550-2. doi: 10.1016/s0367-326x(00)00322-1, PMID 11429252.
9. Srivastava S, Rawat AKS. Botanical and phytochemical comparison of three Bergenia species. J Sci Ind Res. 2008;67:1:65-72.
10. Umashanka DC, Chawla AS, Deepak M. High-pressure liquid chromatographic determination of bergenin and afzelechin from different parts of Paashaanbhed (Bergenia ligulata yeo). Phytochem Anal. 1999;10(2):44-7.
11. Rajbhandari M, Wegner U, Jülich M, Schöpke T, Mentel R. Screening of Nepalese medicinal plants for antiviral activity. J Ethnopharmacol. 2001;74(3):251-5. doi: 10.1016/s0378-8741(00)00374-3, PMID 11274826.
12. Addai MD, Richard M, Suurbar J. *In vitro* bacteriostatic and bactericidal activities of Senna alata, Ricinus communis and Lannea barteri extracts against wound and skin disease-causing bacteria. J Anal Pharm Res. 2016;3:1-6.
13. Cheesbrough M. District laboratory practice in tropical countries. Cambridge University Press; 2006. p. 434.
14. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. Minneapolis: Burgress Publishing Company; 1972. p. 241.
15. Long HN, Thi HYN, Vu KT. Screening antibacterial activity of Vietnamese plant extracts against human pathogenic bacteria. Asian J Pharm Clin Res. 2021;14(6):62-5.
16. Nweze EI, Onyisi MC. *In vitro* antimicrobial activity of ethanolic and methanolic fruit extracts of Xylopia aethiopica and its combination with disc antibiotics against clinical isolates of bacteria and fungi. J Rural Trop Publ Hlth. 2010;9:1-6.
17. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. London: Chapman & Hall Ltd.; 1973. p. 279.
18. Mutia SW, Mahdi J, Abdul M. Physicochemical properties and nutrition of Moringa oleifera lam. Leaf extract: A preliminary study on preparation of Phytosomes an herbal supplement for children. Int J Appl Pharm. 2022;14(1):281-7.
19. Agnihotri V, Sati P, Jantwal A. Antimicrobial and antioxidant phytochemicals in leaf extracts of Bergenia ligulata: a

Himalayan herb of medicinal value. Nat Prod Res. 2015;29(11):1074-7. doi: 10.1080/14786419.2014.980244, PMID 25420426.

20. Zbikowska B, Franiczek R, Sowa A, Połukord G, Krzyżanowska B, Sroka Z. Antimicrobial and antiradical activity of extracts obtained from leaves of five species of the genus Bergenia: identification of antimicrobial compounds. Microb Drug Resist. 2017;23(6):771-80. doi: 10.1089/mdr.2016.0251, PMID 28118088.
21. Chiranjeevi CH, Vijayalakshmi M. Taxonomic characterization, antimicrobial activity and antioxidant potential of rare actinobacterium isolated from mangrove ecosystem of machilipatnam, Andhra Pradesh, India. Asian J Pharm Clin Res. 2022;15(3):113-7.
22. MA, OAO, ARK. Antibacterial activities of azadirachta Indica against some bacterial pathogens. Adv Life Sci. 2012;2(2):5-8. doi: 10.5923/j.als.20120202.02.
23. Shadia SA. A methodology to develop and validate PCR tests to detect of UTI 89 by comparative genomics of bacterial pathogens. Crit Rev. 2020;7(3):1884-90.
24. Vinoth B, Manivasagaperumal R, Balamurugan S. Phytochemical analysis and antibacterial activity of Moringa oleifera. Int J Res Biological Sciences. 2012;2:98-102.
25. Gatsing D, FN Nkeug C, F Nji-Nka B, Kuiate J, M Tchouan F. Antibacterial activity, bioavailability and acute toxicity evaluation of the leaf extract of Alchornea cordifolia (Euphorbiaceae). Int J Pharmacol. 2010;6(3):173-82. doi: 10.3923/ijp.2010.173.182.