Advances in Pharmacology and Pharmacy 12(4): 338-350, 2024 http://www.hrpub.org
DOI: 10.13189/app.2024.120406

Review on Pharmacological Activities, Extraction and Analytical Techniques of Bergenin

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Received September 21, 2023; Revised December 20, 2023; Accepted February 17, 2024

Cite This Paper in the Following Citation Styles

(a): *[1] Keshavnam M. Chitte, Disha Prajapati, Tanvi Dodiya, Janvi Patel, Rishikesh Wagh , "Review on Pharmacological Activities, Extraction and Analytical Techniques of Bergenin," Advances in Pharmacology and Pharmacy, Vol. 12, No. 4, pp. 338 - 350, 2024. DOI: 10.13189/app.2024.120406.*

(b): *Keshavnam M. Chitte, Disha Prajapati, Tanvi Dodiya, Janvi Patel, Rishikesh Wagh (2024). Review on Pharmacological Activities, Extraction and Analytical Techniques of Bergenin. Advances in Pharmacology and Pharmacy, 12(4), 338 - 350. DOI: 10.13189/app.2024.120406.*

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Abstract The present review aims to compile various pharmacological activities, extraction techniques, and analytical techniques for Bergenin. Bergenin, a polyphenol compound that is a C-glycoside of 4-O-methyl gallic acid, has been discovered naturally via several different species, such as *Bergenia ciliata, Mallotus Philippines, Bergenia ligulata, Rivea ornata roxb, Bergenia Strachey, Flueggea virosa, Caesalpinia digyna Rottler, Actaea acuminata, Mallotus repandus Muell, etc.* Various online search engines like Google Scholar, PUBMED, the National Center for Biotechnology Information, MedlinePlus and Herbal Medicine were used for data mining of published research work. A comprehensive review was compiled, considering the various aspects related to Bergenin. Bergenin exhibits many pharmacological activities, including the ability to stop arrhythmia, HIV, anxiety, fungal infections, viruses, fungal infections, cancer, inflammation, and bacteria. Various methods are available for the extraction of bergenin from various plant species. The profoundly used extraction methods include soxhlet extraction and maceration using methanol as the solvent. The pharmacological actions, investigative methodologies, and extraction methods utilized for identifying and quantifying bergenin and its combinations are covered in detail in the current review. Bergenin can be identified and quantified using various analytical methods such as HPTLC, HPLC, HPLC-MS, LC-MS/MS, LC-MS, UPLC-PDA, and UHPLC-MS. The information compiled in the present review will guide the exploration and selection of analytical methods for evaluating bergenin in plant species, herbal, and ayurvedic formulations.

Keywords Bergenin, Pharmacology, Extraction, HPTLC, HPLC

1. Introduction

Bergenin, a C-glycoside of 4-O-methyl gallic acid, has been discovered spontaneously in a broad variety of species [1]. The Saxifragaceae family's *Bergenia ligulata*, a key medicinal plant used in traditional medicine, contains higher amounts of bergenin, a potent secondary metabolite, than any other identified species [2]. Bergenin is a colorless, crystal-like polyphenol obtained from medicinal plants such as *Bergenia ciliata, Mallotus Philippines, Bergenia ligulata, Rivea ornata roxb, Bergenia stracheyi, Flueggea virosa, Caesalpinia digyna Rottler, Actaea acuminata, Mallotus repandus Muell,* and others [3]. It is a perennial evergreen plant native to Central and East Asia. Additionally, it can be grown in the Khasi Hills at an elevation of 400 feet and in the upper elevations of the Himalayas' temperate climate, ranging from Kashmir up to Bhutan [4]. These phytoconstituents have several biological functions, including anti-diabetic, antioxidant, antimicrobial, anti-rheumatic, anti-urease, antiviral, antidiarrheal, antitussive, antianxiety, anti-tumor, anti-fungal, hepatoprotective, neuroprotective, immunomodulatory, anti-nociceptive, anti-H. pylori, and

anti-cancer activities [2,5,6,7,8,9,10,11]. Bergenin is derived from various plants, which are currently being identified and quantified using several methods, including HPTLC, RP-HPLC, HPLC-MS, LC-MS/MS, UHPLC-MS, and UPLC-PDA.

1.1. Physicochemical Profile [12-13]

The profile of bergenin is described in Table 1.

1.2. Sources of Bergenin

Various sources of bergenin, including the parts of plants and families to which it belongs, are described in Table 2, along with the extraction methods employed and the percentage yield obtained. Bergenin was isolated or extracted from 112 plant species that belong to 34 plant families [13].

Table 1. Physicochemical Profile of Bergenin

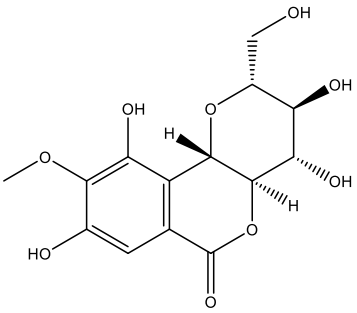
Biomarker	**Bergenin**
Structure:	
IUPAC Name:	(2R,3S,4S,4aR,10bS)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydro-2H-pyrano[3,2-c]isochromen-6-one
Molecular Weight:	328.27 gm/mol
Chemical formula:	$C_{14}H_{16}O_9$
Class:	Glycoside
Appearance:	White to Off-White Solid
Test:	Bitter-tasting
Odour:	Odourless
Solubility:	Methanol (Slightly), DMSO (Dimethyl sulfoxide) (Slightly)
Temperature:	37 ℃
Storage:	-20 ℃
Melting Point:	>231 ℃
Applications:	An herbal medicine with anti-inflammatory, antibacterial, and antioxidant effects.

Table 2. Sources of Bergenin

Species	**Parts**	**Family**	**Extract Methods**	**% yield found in Methods**	**Activity/ Action of Species**	Native Location of the Plants
Mallotus Philippines [6]	Roots, Stem-bark	Euphorbiaceae	1) Accelerated Solvent Extraction (ASE) 2) Cold extraction	1) Root 6.0% w/w and Stem-bark 4.4% w/w 2) Root 5.00% w/w and Stem-bark 2.9% w/w.	Antioxidant, Anti-HIV, Anti-arrhythmic, Hepatoprotective, Anti-inflammatory and Anti-microbial.	Himalayas, Sri Lanka, China and Australia
Bergenia ligulata [14]	Rhizome	Saxifragaceae	1) Cold Percolation 2) Solvent Fractions	1) CPM 20% 2) Chloroform 0.75%, Petroleum ether 6.25%, Sub-fraction 51.65%, and Ethyl acetate 36%.	Antioxidants, Anti-diarrheal, Anti-Inflammatory, Anti-bacterial, and Anti-tussive	Himalayan Region, South and East Asia, and European Countries
Bergenia ligulata [2]	Rhizome	Saxifragaceae	1) Methanol Extraction 2) Acetone Extraction	Methanolic 5.51 ±0.14, Acetone extracts 5.76 ±0.16	Diuretic, Anti-inflammatory, and Analgesic	East and South Asia, and European countries
Rivea ornata roxb [7]	Aerial	Convolvulaceae	1) Solvent Extraction 2) Methanol Extraction	1) Petroleum ether extract 2.50% w/w, chloroform extract 2.13% w/w, Ethyl acetate extract 3.21% w/w and water extract 9.61 % w/w. 2) Methanol extract 6.21% w/w	Antioxidant, Anti- Diabetic and Anti-anxiety Activity	South India in Tripura
Bergenia stracheyi [15]	Rhizome	Saxifragaceae	Soxhlet extraction	Crude methanol extract 215 g (17.9% (w/w))	Analgesic, anti-inflammatory, diuretic, and antibacterial activity	Himalaya
Flueggea virosa [16]	Aerial	Euphorbiacea	Methanol Extraction	Bergenin 15.25±0.03 w/w, and Menisdaurin 4.22±0.05 w/w	Antimicrobial, Anti-Inflammatory and Anti-hepatitis-B	Saudi Arabia, Africa
Bergenia crassifolia [17]	Roots, Leaves	Saxifragaceae	Methanol extraction (Ultrasound bath)	The presence of leaves Bergenin 5.05 ±0.23 to 6.90 ±0.66 mg/g, Ellagic acid 0.47 ±0.10 to 2.00 ±0.09 mg/g, and Gallic acid 0.74 ±0.07 to 6.47 ±0.22 mg/g.	Antimicrobial, Anti-oxidative, Anti-inflammatory and Antiviral.	Russia
Caesalpinia digyna Rottler [18]	Roots	Caesalpiniaceae	Ethanol extraction	0.56%.	Anti-anxiety	Western Countries
Endopleura uchi [8,9]	Bark	Humiriaceae	1) Methanol extraction 2) Dynamic maceration Extraction	1) Hexanes 1.1gm, Chloroform 6.5g, Ethyl acetate 39.6gm, and Hydro alcoholic fractions 62.0 gm. 2) Bergenin 35.58%.	Anti-inflammatory	Brazilian Amazon.
*Actaea acuminat*a [19]	Roots	Ranunculaceae	Soxhlet extraction	Bergenin 0.8010% w/w, and gallic acid 0.1242% w/w	Anti-inflammatory	Himalayas, India, and Afghanistan
Mallotus repandus (Willd.) Muel l [20]	Stem	Euphobiaceae	1) Soxhlet extraction 2) Maceration extraction	1) Bergenin- 12.67% 2) Bergenin- 19.38%	Anti-inflammatory and Anti-oxidant	Thailand, Tropical and Sub-tropical Asia and New Caledonia
Bergenia ciliata [11]	Rhizome	Saxifragaceae	Maceration extraction	Crude extract 130 gm.	Anti-oxidant, Anti-diabetic, Anti-arrhythmic, Anti-hepatotoxic, and Anti-plasmodial	Himalaya, India
Bergenia ciliata [5]	Rhizome, Root and Leaves	Saxifragaceae	Methanol Extraction	Bergenin yield found in Rhizome part 19.4%, Root 9.2% and Leaves 6.9%	Anti-oxidant, Anti-diabetic, Anti-fungal, Anti-malarial And Anti-Cancer	Himalaya, India, and Pakistan

Table 3. Summary of Analytical Methods used for estimation of Bergenin

Method	Biomarker	Stationary phase	Mobile phase	Retention factor (R_f)	Reference
HPTLC	Bergenin	Silica gel 60 F_{254} plates	Chloroform: methanol: acetic acid (8:1:1 v/v/v)	0.28	Khan et al., 2015 [2]
		Precoated silica gel 60 F_{254}	Toluene: ethyl acetate: acetone (2:4:4, v/v/v)	0.36	Sharma & Patel, 2017 [7]
		Silica gel 60 F_{254}	Ethyl acetate: methanol: acetic acid: formic acid (8:1:0.5:0.5, v/v)	0.59 ±0.02	Haribabu et al., 2012 [6]
		Precoated silica gel 60 F_{254} 0.2 mm thickness of plate	Toluene: Ethyl acetate: Formic acid (6:6:1, v/v)	0.13	Pushpalatha et al., 2015 [25]
		(Merck) Glass plates Kieselgel 60 F_{254} (10×610 cm)	Toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2, v/v)	0.2	Pozharitskaya et al., 2007 [17]
	Gallic acid, Bergenin, and Catechin	Precoated silica gel 60 F_{254}	Toluene: ethyl acetate: formic acid (4:6:1, v/v)	Gallic acid- 0.60 Bergenin-0.29, Catechin- 0.54	Dhalwal et al., 2008 [24]
	Bergenin, Gallicin, Catechin and Gallic acid	Silica gel 60 F_{254}	Toluene: Ethyl acetate: Formic acid (6:6:1, v/v/v)	Bergenin-0.13, Gallicin-0.56, Catechin-0.49 and Gallic acid-0.64	Dharmender et al., 2010 [23]
	Bergenin and Menisdaurin	Glass-backed silica gel 60 F_{254}	Dichloromethane: methanol (8.5:1.5, v/v)	Bergenin- 0.29+0.01 and menisdaurin- 0.16+0.01	Siddiqui et al., 2015 [16]
	Gallic acid and bergenin	Pre-coated plates from E. Merck HPTLC with an aluminum base and a 0.2 mm thickness	Chloroform: methanol (17:3)	Gallic acid-0.1242% and bergenin-0.8010% w/w	Kumar et al., 2020 [19]
	Bergenin and Gallic Acid	(Merck) TLC silica gel 60F_{257}	Toluene: ethyl acetate: formic acid (3.5:5.5:1, v/v/v).	Bergenin- 0.41, Gallic Acid- 0.81	Srivastava et al., 2013 [22]

Table 3 continued

Method	Biomarker	Column	Solvent system	Retention Time (R_t)	Reference
HPLC	Bergenin	Phenomenex C18 (250mm×4.6 mm ID, 5µm)	Methanol: water (30:70, v/v)	4.012 min.	Shailajan et al., 2021 [27]
		Waters bondapak C18 (3.9×300 mm, 5µm)	Methanol:0.05%v/v trifluoroacetic acid in water	14.20 ±0.01 min.	Dziedzom Amenu et al., 2019 [29]
		C-18 Shim-PACK CLS (ODS 280×4.0 mm and particle size 5µm)	Methanol: water (20:80)	17±1	Tacon & Freitas, 2011 [9]
	Bergenin, Arbutin, and Gallic Acid	RP-Luna C18 (2)-HST [100×3.0 mm, DP = 2.5 m (Phenomenex, USA)]	Methanol: water (1:1, v/v)	Bergenin -10.7 min, gallic acid -2.6 min and arbutin- 3.3 min.	Boros et al., 2014 [31]
	Bergenin, Epicatechin, (+)-Catechin, and Gallicin	Reodein; RP-C18 (4.5×250mm, 5.0 µm)	Water: phosphoric acid (99.6:0.3, v/v) for bergenin and Acetonitrile: water: phosphoric acid (79.6:20:0.3, v/v/v) for epicatechin	Bergenin-8.8±0.3, epicatechin-10.3±0.2, (+)-catechin-11.01±0.3 and gallicin- 0.54±0.2	Srivastava, Srivastava, et al., 2015 [28]
	Bergenin Gallic acid	Reodein; RP-C18 (4.5×250 mm, 5.0 µm)	Water: phosphoric acid (99.7:0.3, v/v) for Gallic acid and Acetonitrile: water: phosphoric acid (79.7:20:0.3, v/v) for Bergenin	Bergenin-11.06±0.23, and gallic acid-5.91 ±0.12	Singh et al., 2007(32) (Srivastava, Singh, et al., 2015 [4]
	Bergenin (+)-afzelechin	RP-C18 (250×4.6 µm)	Water: acetonitrile	Bergenin- 10.1 min. and (+)-afzelechin- 14.8 min.	Chandra Reddy et al., 1999 [30]
LC-MS/MS	Apigenin, Bergenin, Scutellarin, Baicalin, Wogonin, Wogonosideand and Chlorogenic Acid	RP-Agilent Poroshell 120 EC-C18	Methanol: 0.1% formic acid added to water (v/v)	Apigenin-8.6min, bergenin-3.8min, scutellarin-6.2min, baicalin-7.3 min, wogonin-9.6 min, wogonoside-8.1 min and chlorogenicacid-4.2min.	Zhao et al., 2014 [34]
	Bergenin	Agilent zorbaxSB-C18	Acetonitrile:10 mm ammonium acetate (20:80 v/v)	2.01	W. Yu et al., 2009 [10]
	Bergenin	Diamonsil® C18 (150×4.6 mm,5µm)	Water: methanol (30:70, v/v)	2.62 min.	Li et al., 2013 [36]
	Bergenin, Epicatechin, Isoquercitrin, Epicatechin gallate, Quercetin-3-Rhamnoside, Gallic Acid	Eclipse plus C18 (4.6mm×100 mm, 1.8 µm)	Acetonitrile: formic acid aqueous solution at (0.05%)	Bergenin-4.6 epicatechin-4.9, isoquercitrin-8.4, epicatechin gallate-7.2, quercetin-3-rhamnoside-9.5 and gallic acid-4.2 min.	X. A. Yu et al., 2018 [37]
UHPLC-MS/MS	Bergenin	Acquity UPLC™ BEH C18 (50 ×2.1 mm id, 1.7 µm)	Acetonitrile: aqueous formic acid solution at 0.1% (v/v)	2.49 min.	Pandey et al., 2017 [39]
UPLC-PDA	Bergenin Menisdaurin	Eclipse C_{18} (4.6×100 mm, 3.5 µm)	Acetonitrile: water	Bergenin-2.723 min. menisdaurin 3.068 min.	Hussain et al., 2018 [42]

2. Analytical Methods for Determination of Bergenin and their Combinations

This study emphasizes the role that analytical techniques and procedures have in determining the quality of pharmaceuticals and biomarkers. This review details all of the analytical methods used for the medications described, including HPTLC, HPLC, HPLC-MS, LC-MS/MS, LC-MS, UPLC-PDA, and UHPLC-MS. A summarized overview of the analytical techniques for bergenin estimation is described in Table 3, along with their chromatographic conditions.

2.1. HPTLC Methods

A sophisticated technique known as HPTLC based on TLC, HPTLC is an analytical method that has been improved to allow for quantitative analysis of the compounds and to improve the resolution of the compounds that need to be separated [21].

Actaea acuminata roots were analyzed for gallic acid and bergenin contents using an HPTLC technique developed by Kumar et al. [19]. They used the solvent systems chloroform and methanol (17:3 v/v) for the mobile phase, and on pre-coated plates from E. Merck HPTLC with an aluminum base and a 0.2 mm thickness, bergenin, and gallic acid were calculated to have respective weight-by-weight percentages of 0.8010% and 0.1242%, by using densitometric scanning at an absorbance wavelength of 280 nm.

A technique aimed at quantifying bergenin using HPTLC has been developed by Sharma and Patel et al. [7] using precoated silica gel 60 F_{254} as the stationary phase and a mobile phase mixture of toluene, ethyl acetate, and acetone in a ratio of 2:4:4 v/v. The R_f value for bergenin is 0.36, respectively. UV light is absorbed at a wavelength of 254 nm. Bergenin was discovered to be 0.331 mcg/ml.

Siddiqui N. et al. [16] have quantified the simultaneous measurement of the markers bergenin and menisdaurin in the aerial portions of *Flueggea virosa* using an HPTLC densitometric approach. Glass-backed silica gel 60 F_{254} HPTLC plates and a mixture of dichloromethane and methanol in a ratio of 8.5:1.5, v/v, as the mobile phase system. Retention factor values of 0.16+0.01 and 0.29+0.01 were found by Menisdaurin and Bergenin, respectively. Scanning and quantification were carried out at 260 nm UV absorption maxima. Menisdaurin and bergenin were discovered to be present in FVME in amounts of 4.22 and 15.25% (w/w), respectively.

Bergenin and gallic acid were simultaneously estimated by Srivastava et al. [22]. Toluene, ethyl acetate, and formic acid were used as the solvents at a ratio of 3.5:5.5:1, v/v/v, and the stationary phase used a TLC silica gel (Merck) 60F_{257} plate. At scanning wavelengths of 250 and 280 nm, gallic acid and bergenin were discovered to have R_f values of 0.81 and 0.41, respectively.

Simultaneous estimation of bergenin, gallic acid, (+)-catechin, and gallicin by HPTLC was developed and validated by Dharmender et al. [23] using silica gel 60 F_{254} TLC plates and the solvent system of toluene: ethyl acetate: formic acid in a ratio of 6:6:1% v/v/v. With R_f values of Bergenin, 0.13; (+)-Catechin, 0.49; Gallicin, 0.56; and Gallic acid, 0.64. The 254 nm and 366 nm ultraviolet wavelengths were used for detection and quantification. The contents of bergenin, gallic acid, and (+)-catechin were 0.22, 0.25, and 0.63 percent, respectively.

Khan et al. [2] have reported that bergenin was found in different extracts using the developed HPTLC method. Using the stationary phase as a silica gel on 60 F_{254} plates, the mobile phases for the detection and quantification were chloroform: methanol: acetic acid in a ratio of 8:1:1 v/v/v, respectively. Densitometry at a wavelength of 276 nm revealed that an R_f value of 0.28 created a sharp and identifiable peak. Bergenin contents of 5.76±0.16 and 5.51±0.14, respectively, were found in the acetone and methanolic extracts.

According to Haribabu et al. [6], several extraction methods have been studied, and the bergenin in *Mallotus philippinensis* was quantified using HPTLC-MS. Combining ethyl acetate, methanol, acetic acid, and formic acid in the ratio of 8:1:0.5:0.5, v/v, as the mobile phase and utilizing the stationary phase, 20×10 silica gel 60 F_{254}, scanning with densitometry at 284 nm. The R_f value was found to be 0.59 ± 0.02. Its [M + Na] + ions for bergenin were discovered at m/z value 351.

A high-performance thin-layer chromatography technique was developed by Dhalwal et al. [24] to simultaneously quantify biomarkers such as bergenin, catechin, and gallic acid. From the species *Bergenia ciliata* and *Bergenia ligulata*, each biomarker has been examined at a wavelength of 280 nm utilizing the mobile phase comprising toluene, ethyl acetate, and formic acid in a ratio of 4:6:1, v/v, on an HPTLC plate precoated with silica gel 60 F_{254} as the stationary phase. At 0.60, 0.54, and 0.29, respectively, gallic acid, catechin, and bergenin retention were discovered.

Pushpalatha et al. [25] have developed novel quantitative methods for estimating bergenin using HPTLC. Using a 0.2 mm thick precoated silica gel 60 F_{254} plate and toluene, ethyl acetate, and formic acid at a 6:6:1 ratio as a mobile phase, detection occurred at 275, and the R_f value was found to be 0.13.

In 2007, Olga N. Pozharitskaya et al. [17] described their use of the HPTLC-DPPH method to separate the phenol components of *Bergenia crassifolia's* green, brown, and black leaves. They assessed the leaves' ability to neutralize free radicals. Methanol, toluene, formic acid, and ethyl acetate in a ratio of 0.2:3:0.8:3 % v/v are utilized on a mobile phase. With a glass plate measuring 60 F_{254} (106×10 cm), the results were found at 517 nm.

2.2. HPLC Methods

To separate, recognize, and measure active compounds, high-pressure liquid chromatography, a form of column-based chromatography, is frequently employed in biological chemistry as well as analysis. It is a well-liked analytical approach for disentangling, determining, and characterizing every component of a mixture [26].

Shailajan et al. [27] have developed the HPLC method to estimate bergenin using a Phenomenex C18 (250 mm ×4.6 mm ID, 5 µm) column as the stationary phase and a mixture of methanol and water (30:70) in volume by volume as the mobile phase. At the detection wavelength of 275 nm, the Bergenin retention period was found to be 4.012 minutes. Bergenin content in the formulation was found to be 0.27 mg/gm in the investigation.

Srivastava et al. [28] have reported a combined estimation of (+)-catechin, bergenin, gallicin, and epicatechin within Bergenia ciliata. Applying high performance liquid chromatography on the RP-C_{18} 4.5 ×250 mm, 5.0 µm, the 1 ml/min flow rate, the following ratios were used: 99.6:0.3 for Bergenin and 79.6:20:0.3 for epicatechin, (+)-catechin, and gallicin. Bergenin and catechin were detected and quantified at a wavelength below 280 nm with R_f values of 8.8 ±0.3 and 11.01 ± 0.3, respectively.

Bergenin in *Securinega virosa* root has been characterized and developed by Dziedzom Amenu et al. [29]. The Waters Bondapak C18 (3.9 ×300 mm, 5 µ) column was used in a gradient elution of methanol and 0.05% v/v trifluoroacetic acid in the water, with a flow rate of 1.0 ml/min and an absorbance of 220 nm. The bergenin retention time was discovered to be 14.20 ±0.01 minutes.

Chandra Reddy et al. [30] have determined the HPLC method of evaluation of afzelechin and bergenin from various parts of *Bergenia ligulata*. Using water: acetonitrile as the mobile phase, with a 250 × 4.6 mm C18 column and maximum absorbance measured at 200 nm. The retention times were 14.8 min and 10.1 min, respectively, for the amounts of afzelechin (0.168% w/w) and bergenin (0.907% w/w).

Boros B. et al. [31] have published a validated HPLC technique for simultaneously quantifying bergenin, gallic acid, and arbutin in leaves from various Bergenia species. Develop a mobile phase with a methanol: water ratio of 1:1 (v/v). The Rp-Luna C_{18} (2)-HST column was used for the analysis. Absorbance at 280 nm and retention time peaks of gallic acid (2.6 min), arbutin (3.3 min), and bergenin (10.7 min) were seen.

The Box-Behnken design was used in this work by Tacon & Freitas et al. [9] to analyze the antioxidant activity and bergenin content of *Endopleura uchi* bark extract produced using dynamic maceration. A C18 Shim-PACK CLS (ODS 280 4.0 mm; 5 m) column was used as the stationary phase, and methanol: water (20:80) was used as the mobile phase with a flow rate of 0.8 mL/min. The wavelength of the HPLC detector has been fixed at 272 nm.

Bergenin was discovered in many Bergenia species, including *Bergenia stracheyi, B. ligulata, and B. ciliata*, using high-performance liquid chromatography in the study by D. Singh et al. [32]. Solvent systems: acetonitrile, water, and phosphoric acid in a ratio of 79.7:20:0.3 (Bergenin) and water and phosphoric acid 99.7:0.3 (v/v) (Gallic acid). Stationary phase waters symmetry column (150 mm ×3.6 mm) pro-detecting at 272 nm, retention time was 5.91 ± 0.12 for gallic acid while bergenin was 11.06 ± 0.23. Bergenin content (%) in B. stracheyi was 3.277, B. ligulata was 2.419, and Bergenia ciliata was 3.275.

2.3. LC/MS Method

Pharmaceutical analysis can take advantage of the special features offered by LC/MS-based methods. Continuous advancements in LC/MS interface technology, along with sophisticated capabilities for structural analysis both qualitative and quantitative, have led to a broad scope of applications [33].

Zhao et al. [34] investigated the use of the LCMS method to simultaneously measure and carry out pharmacokinetic analyses on bergenin, chlorogenic acid, and the 4 flavonoids in plasma from rats after taking a decoction extract of QingGanSanJie orally. They used an Rp Agilent Poroshell 120 EC-C18 column with a gradient mobile phase of methanol and water containing 0.1% formic acid (v/v). 20 ml were injected, and the flow rate was 0.2 ml/min. Bergenin obtained 3.8 minutes, chlorogenic acid obtained 4.2, puerarin obtained 4.7, scutellarin obtained 6.2, baicalin obtained 7.3, wogonoside obtained 8.1, apigenin obtained 8.6, and wogonin obtained 9.6. Bergenin, wogonin, chlorogenic acid, apigenin, scutellarin, baicalin, and wogonoside were all present in QGSJD extract in amounts of 0.79, 0.58, 2.34, 0.10, 0.78, 10.69, 0.05, and 2.02 mg/ml, respectively.

2.4. LC-MS/MS Methods

The powerful analytic method known as liquid chromatography with tandem mass spectrometry (LC-MS-MS) mixes the ability to separate substances with the very precise and targeted mass analysis capability of triple quadrupole mass spectrometry. The combined operation of two series-operated mass analyzers can further enhance specimen identification and precise quantification. The two tandem mass spectrometers that are most frequently used are quadrupole time-of-flight and triple quadrupole mass spectrometers [35].

According to Li BH et al. [36], the LC-MS/MS technique has been created and evaluated for the detection of bergenin in rat plasma. Using an isocratic elution method and a mobile phase made up of water and methanol in a ratio of 30:70, v/v, with a flow rate of 0.6 ml/min, chromatographic separation was carried out on a Diamonsil C18 (150 mm ×4.6 mm, 5 µm). The bergenin quantity was determined to be 16.3 mg/ml in the extract. Bergenin had the best m/z

value for quantifying at 327.3/192.0, and the interval after injections was limited to 3.5 minutes for every analytical run.

W. Yu et al. [10] have developed the LC-MS-MS method with an Agilent zorbax SB-C18 column, comprising a mobile phase composed of acetonitrile and 10 mm ammonium acetate (1% formic acid) in a ratio of 20:80 v/v, the concentration of bergenin in the plasma of humans, and the rate of flow was 1 ml per minute. The transition at m/z 188.9→42.2was utilized to monitor 5-Br, while the most intense [MH] MRM (multiple reaction monitoring) transitions of bergenin at m/z 326.9→312.3 were employed for quantification. As a qualification, the transition at m/z 326.9→234.1 was employed. Retention durations of 1.92 and 2.01 minutes were found by the initial standard and bergenin.

X. A. Yu et al. [37] published measurements simultaneously of epicatechin gallate, quercetin-3-rhamnoside, gallic acid, isoquercitrin, and bergenin, in rat blood plasma. Post-administration of an oral dose of an *Ardisia japonica* extract with the LC-MS/MS method, all six compounds and IS were separated using the 300 L per minute flow rate, C18 column with total injection volume is 10 µl, and a 16-minute elution time using acetonitrile and formic acid aq. solution 0.05% in the mobile phase at m/z 168.8, 327.0, 288.9, 441.1, 462.9, 447.0, 192.8, 415.0, and 307.0, respectively, epicatechin gallate, gallic acid,quercetin-3-rhamnoside bergenin, isoquercitrin, and epicatechin all observed retention times of 4.9 min, 4.2 min, 9.5 min, 4.6 min, 8.4 min, and 4.9 min, respectively.

2.5. UHPLC-MS/MS Method

The methodology is UHPLC-MS/MS in a variety of study institutions to conduct qualitative and quantitative analyses on pharmaceutical substances, pharmaceutical goods, and biological specimens. Mass spectrometers are successively used to analyze the substance. This gives more detailed information and is particularly helpful for breaking down big, complicated macromolecules into smaller, more manageable pieces for analysis [38].

According to Pandey et al. [39], major bioactive phenolic found in Indian Himalayan Bergenia species: developing a UHPLC-MS/MS method under UHPLC conditions using a (50 mm×2.1 mm id, 1.7µm Acquity UPLC™ BEH C_{18} column kept at 25℃, the chromatographic separation was completed. Acetonitrile and 0.1% (v/v) formic acid in an aqueous solution made up the mobile phase. 0.3 mL/min was the constant flow rate. The amount of injection for the specimen was 2 µl. MS-related conditions. To identify the target analytes quantitatively, the MS instrumentation was operated in a negative electrospray ionizing mode with MRM acquisition set as the unit resolution for Q1 as well as Q3. The source of electrospray was operated under the following ideal conditions: -4200 V for the ion spray voltage; 20 psi for the curtain gas (CUR); 50 psi for the nebulizer gas (GS1) as well as heater gas (GS2); 400 ℃ for the ion source temperature; medium for the collision-activated dissociation gas; and on for the interface heater. Bergenin has a retention time of 2.49 minutes.

2.6. UPLC Method

Modern techniques like UPLC, which primarily improve in the areas of "sensitivity, resolution, and speed," provide liquid chromatography with a new path. When compared to high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography is appropriate for particles with a diameter of less than 2µm and can achieve superior sensitivity, resolution, and speed [40-41].

Hussain et al. [42] have reported that the menisdaurin and bergenin aerial portions of Flueggea virosa were simultaneously analyzed using the development and validation of the UPLC-PDA technique, employing a gradient system with acetonitrile and water as the mobile phase and an Eclipse C18 (4.6 ×100 mm, 3.5 mm) UPLC column with a 0.16 ml/min flow rate and absorbance at 235 nm. Menisdaurin and bergenin retention times were found to be 2.72 and 3.068 minutes, respectively. Menisdaurin and bergenin were present in the FVME samples at 15.16% and 3.28% w/w, respectively.

3. Pharmacology of Bergenin

Traditional Chinese medicine and other medicinal systems frequently use bergenin because of the wide range of pharmacological activities it exhibits.

3.1. Effects on the Urinary System

Bergenin effects on urolithiasis in albino rats brought on by 3% glycolic acid were examined. Glycolic acid therapy raised the levels of oxalate and phosphorus in urine, as well as the excretion of and activity of lactate dehydrogenase (LDH) in the kidneys. Bergenin action in the current model system was less substantial [3].

3.2. Antidiabetic Activity

Yang et al. [43] investigated the bergenin impact on diabetic nephropathy. Bergenin therapy restores normal levels of elevated serum creatinine, urea nitrogen, and urine albumin. Additionally, it enhances kidney histopathology and prevents mesangial cell growth. BEG both in vivo and in vitro significantly reduced the formation of TGF-1 and Transforming Growth Factor-1, downregulated the expression of p-Smad2/3, and increased the expression of suppressors in mothers against decapentaplegic homolog 7. Bergenin functioned via the mTOR/-TrcP/Nrf2 pathway to prevent damage from oxidative stress, which in turn prevented the formation of an extracellular matrix in the glomerular mesangial cell's response to glucose [44].

Bergenin was extracted by Sanjeev et al. [45] using the methanolic extract from *Ardisia colorata* leaves, and its effectiveness against diabetic testicular problems in rats was examined. Bergenin supplementation improves the histopathological and histomorphometric manifestations of diabetic testicular dysfunction by increasing proliferating cell nuclear antigen patterns of expression and serum testosterone levels, modulating the levels of antioxidant enzymes, enhancing the quality of sperm, reducing sperm-damaging DNA, controlling spermatogenic activity and sperm cell proliferation, and boosting sperm quality.

3.3. Anti-Microbial Activity

Raj et al. [46] have reported that Indian medicinal herbs have antimicrobial characteristics, and several have been examined to see whether they may minimize the spread of specific harmful bacteria and fungi. *Peltophorum spp.* bark has been shown to have antibacterial properties, and both its aqueous and ethanolic extracts have been used to treat ulcers, painful muscles, and dysentery.

3.4. Anti-Fungal Activity

Bajracharya et al. [47] have reported antifungal activity. With MIC values of 14.9, 29.8, and 14.9 μm for *Candida albicans*, *Candida guilliermondii*, and *Candida tropicalis*, bergenin showed antifungal activity. *Aspergillus flavus*, however, showed less activity.

3.5. Anti-Bacterial Activity

Bacillus subtilis, Salmonella paratyphi, Salmonella typhi, Shigella flexneri, Staphylococcus aureus, Salmonella typhimurium, Erwinia sp, Proteus vulgaris, and Enterococcus faecalis were among the bacteria that bergenin was found to be ineffective against. Bergenin was found to have an inhibitory impact on Staphylococcus aureus, Klebsiella pneumoniae, beta-streptococci, and A. aeruginosus bacillus. Bergenin's antitussive action has been documented elsewhere [47].

3.6. Anti-Arrhythmic Activity

There aren't many studies on bergenin antiarrhythmic properties, but one of them did this evaluation. Bergenin, which was isolated using an aerial portion of *Flueggea virosa*, was examined for its antiarrhythmic properties at doses of 0.8 mg/kg, 0.4 mg/kg, and 0.2 mg/kg. It demonstrated different therapeutic benefits for arrhythmias in rats caused by barium chloride [13]. At doses of 0.8 mg/kg and 0.4 mg/kg, bergenin significantly reduced arrhythmias induced by coronary artery channel and reperfusion. Bergenin has the potential to treat cardiac arrhythmias as a result of at a dosage of 0.8 mg/kg, it increased the atrial tachycardia thresholds in rabbits from 1.34 to 1.92 mV. The electric current can flow with enough force to contract the ventricular muscles and induce involuntary systole thanks to the raising of the atrial fibrillation threshold [1].

3.7. Antioxidant Activity

Mehta et al. [48] have reported that antioxidant actions are the ability of an active molecule to reduce the creation of free radicals and scavenger reactive oxygen species, repairing and preventing damage brought on by the oxidation and degradation of biomolecules and other molecules. By scavenging the different ROS produced and lowering the production of free radicals, bergenin demonstrates its antioxidant function. According to published research, Bergenin effectively neutralizes the free radical DPPH (2, 2-diphenyl-2-picryl hydroxyl), which it does by acting as a scavenger. Bergenin has also shown antioxidant efficacy in DPPH radical tests, lipid peroxidation, and superoxide in another research [3], [13].

3.8. Anti-Inflammatory Activity

Amazon has long employed *Endopleura uchi* bark as an anti-inflammatory. Three significant enzymes that play key roles in an inflammatory response and accelerate the production of prostaglandins were tested for inhibitory activity against the chemicals isolated through *Endopleura uchi*, Bergenin-designated phospholipase A2, COX-1, and COX2. Bergenin was around 70 times less active than the PLA2 inhibitor thioetheramide-PC. Bergenin was 50 times less active than the positive control, resveratrol, in the COX-1 experiment, the inhibitory activity is reported in Table 4. Niflumic acid showed a difference from the control group that was positive, and bergenin demonstrated strong COX-2 inhibitory efficacy with a selectivity score of 89.3. Bergenin showed very strong COX-2 inhibition, in accordance with the COX-1/COX-2 IC50 ratio values. The primary COX isoform involved in inflammation is COX-2, and the stimulation of COX-2 is what causes the formation of PGs at the site of inflammation. Given that human colonic nonmalignant tumors and cancer excessively express COX-2, the growth of cancer is significantly aided by COX-2. However, other studies showed that COX-1 inhibitors commonly have negative effects on the gastrointestinal system [8], [3].

Table 4. Bergenin has phospholipase (PLA2) and cyclooxygenase (COX-1/COX-2) inhibitory activity [8]

IC50 / ($\mu mol\ l^{-1}$)			
	COX-1	COX-2	PLA2
Niflumic acid	-	0.2	-
Thioeteramide-PC	-	-	2.1
Bergenin	107.2	1.2	156.6
Resveratrol	3.1	-	-

3.9. Anti-HIV Activity

Piacente et al. [49] reported that all of the identified compounds from *A. japonica* were examined for their anti-HIV potential. Norbergenin as well as bergenin had considerable action against HIV, despite triterpene saponins not preventing HIV replication. Through the use of 2D shifting correlation spectroscopy for 1H-13C and 1H-1H, we describe the discovery of the novel triterpene glycoside 4 and its isolation and structural analysis, as well as the in vitro HIV inhibition of norbergenin and bergenin in infected C8166 cells.

3.10. Antianxiety Activity

J. Singh et al. [18] have reported that the common anxiolytic medication diazepam served as the positive control throughout all of the studies. 40 mg/kg of F4 administered orally showed anti-anxiety effects. According to Dawson & Tricklebank et al. [50], it is recognized as an etiologically reliable model for animals. The three fractions elevated plus maze (EPM), F4.1, F4.2, and F4.3 were employed to test the antianxiety effect of the three fractions because they employ natural stimuli, such as the dread of balance on an extremely narrow rising platform.

3.11. Anticancer Activity

Bergenin has substantial cytotoxicity against HePG2 human liver cancer cells, according to a study by Newell et al. [51]. Cells from the G1 phase build up as a result of the compound and decrease in the G2/M phase, which causes death.

3.12. Antiviral Activity

The aerial portions of *Ardisia japonica* were isolated for nor-bergenin and bergenin, and their in vitro anti-HIV efficacy was insufficient with 6.25 g/ml as the IC50 value. The *Astilbe rivularis* plant's bergenin, which was extracted from its rhizomes part, exhibited antiviral properties against the virus that caused herpes simplex [49] [52].

4. Conclusions

A wide range of medicinal plants contain the active secondary metabolite bergenin, which has many different kinds of pharmacological actions. There are many extraction methods for Bergenin, including soxhlet extraction, maceration extraction, methanol extraction, accelerated solvent extraction, cold percolation method, etc. Methanol and petroleum ether are the most commonly used solvents in bergenin extraction. This review reports several analytical methods for identifying and quantifying Bergenin and its combination with other phytoconstituents like gallic acid, catechin, menisdaurin, arbutin, and epicatechin in herbal formulation, ayurvedic formulation, and different plant species. The review reports 10 HPTLC methods, 7 HPLC methods, 1 LC/MS method, 3 LC-MS/MS methods, 1 UHPLC-MS/MS method, and 1 UPLC method for estimation of bergenin. These approaches can be used to assist the estimation of bergenin with enhanced accuracy, precision, specificity, linearity, and range. As a result, it can be concluded that the available analytical methodologies and recent trends demonstrate that the available data is valuable in the process of analytical method development and its validation.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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