CHAPTER 7

PHARMACOLOGICAL STUDIES OF *BERGENIA CILIATA*

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Abstract

Bergenia ciliata is distributed in cold and temperate regions throughout Himalayas from Kashmir to Bhutan at an altitude of 800-3000m. In the Ayurvedic system of medicine, plant rhizome is known as 'Pashaanabheda'. *Bergenia ciliata* is well known for the treatment of kidney disorders, especially kidney stones. The plant is used in various herbal preparations for the treatment of several diseases in Nepal, India, Pakistan, Bhutan and some other countries. Also, different parts of the plant are used traditionally for the treatment of various health-related ailments including skeletomuscular, gastrointestinal, gynecological, respiratory problems, rheumatism, constipation, ENT problems, skin problems, *etc.* Bergenin is the main constituent of the plant which may be responsible for different pharmacological properties. The plant has been studied for its antioxidant, anti-inflammatory, anti-tussive, anti-ulcer and anti-neoplastic activities. Phenolic compounds helps in dissolution of calcium phosphate and oxalate stones. (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin isolated from *B. ciliata* may be responsible for its antidiabetic and antiplasmodial effect. Further, detailed clinical studies of various properties and toxicity of this plant may help in its commercial use in food, pharmaceutical and cosmetic industries.

Keywords: Ailments, Ayurvedic system, *Bergenia ciliata,* Himalayas, Pharmacological properties.

Introduction

Bergenia ciliata is a small perennial herb that belongs to the family Saxifragaceae. It grows well in moist shady areas and is found in the temperate Himalayas between elevations

of 800- 3000m. It is commonly known as *Pashaanabheda*, *Zakhhm-e-Hayat* and *Rock foil*. The rhizome of the plant is solid, hard, dark brown, barrel-shaped, cylindrical with ridges and furrows having rootlets and aromatic. Leaves are less in number, spreading, glabrous or hirsute, suborbicular to orbicular, broadly obovate, base cordate or sometimes rounded, apex round or sometimes abruptly acuminate, margin entire to occasionally denticulate at top. Petiole is 1-5 cm long, glabrous or hirsute. The inflorescence is raceme or corymbose type, pink-tinged, often subtended by an ovate leafy bract. Bract is glabrous or sparsely ciliate and scape. Flowers are pink to purplish, pedicellate and the peduncle is up to 10 cm long. The sepals are pink to red, stamens are inserted with the petals. Carpels and styles are green or pinkish and 7mm long. The ovary is bicarpellary with axile placentation, occasionally 1-celled with parietal placentas, ovules numerous, anatropous. Capsule 13×6 mm in size, including styles. Seeds are 1 mm long, numerous and minutely tuberculate (Srivastava *et al*., 2008; Ruby *et al*., 2012; Singh *et al*., 2018).

Ethno-botanical uses

From the previous literature, it has been found that this species of *Bergenia* is used to treat 104 different disorders while its highest potential was observed in the treatment of gastrointestinal problems (Ahmad *et al*., 2018). The plant is used for the treatment of different ailments in different parts of the world. In Pakistan, leaves and rhizomes of the plant are used in muscular pain, pus discharge and as a tonic (Hamayun *et al*., 2006). In Nepal, a vital tonic is prepared from the rhizome of the plant which is prescribed with honey to post-partum women. It is also used in the treatment of diarrhea, dysentery and stomachache (Bhattarai, 1994; Rajbhandari *et al*., 2009). Dhunkharka community of Nepal uses the rhizome of the plant as carminative, to treat diarrhea and roundworm infestation (Adhikary *et al*., 2011). In Uttarakhand, the sap of the root is used as a hair tonic and in liver problems (Rana *et al*., 2013). In the Kangra and Chamba districts of Himachal Pradesh, the rhizome of the plant is used in kidney stones, menstrual pain, fever, diarrhea, pulmonary infections, bruises and boils (Thakur *et al*., 2016). Different parts of the plant are used in various ailments by traditional healers of two districts of Kashmir valley. Rhizome, roots, leaves, flowers, latex is used for the treatment of urinary disorders, skeletomuscular, gastrointestinal, gynecological, respiratory problems, rheumatism, constipation, ENT problems and skin problems (Rafiq *et al*., 2019). The rhizomes are applied to the gums with honey in children during teething to relieve irritation (Nadkarni 1976). In Sikkim, the rhizome is used for the treatment of diarrhea, oral inflammations and vomiting. It is also used to treat fever, cough and respiratory infections, boils and as an antiscorbutic (Rai *et al*., 2000). Also, the crushed rhizome of the plant is tied around the fractured bone and paste is applied to treat cuts and wounds by the Lepcha tribe in North Sikkim (Pradhan and Badola, 2008). Fresh or dried rhizome powder or paste is used to cure diarrhea and vomiting by tribal communities in East Sikkim. The rhizome is also simply chewed for the same action and its paste is used to cure cuts and wounds. Inflorescence of the plant is used in veterinary problems by mixing with barley flour and given to cattle to cure bleeding in the urine (Tamang *et al*., 2021). In another study, the roots are crushed and the extract is given orally to cattle three times a day to treat diarrhea, dysentery (Bharati and Sharma, 2012) and mastitis (Tiwari and Pande, 2004). In Pakistan, fresh leaves are crushed and applied to the bleeding wounds of cattle. On the other side, dried rhizome powder is sprinkled onto wounds for 8–10 days to heal the wounds of livestock. *B. ciliata* is one of the important species that supports a diverse group of pollinators by providing the necessary forage. Therefore, it is important to protect and conserve this species wisely so that they may provide forage to pollinator insects during the winter season (Pandey *et al*., 2019).

Chemical composition

Alkaloids, tannins, flavonoids, coumarins and glycosides were reported in a methanolic crude extract of rhizome of *B. ciliata* collected from the Dhunkharka community of Nepal (Adhikary *et al*., 2011). Uddin *et al*., (2012) observed anthraquinone, flavonoids, steroids, terpenoids, saponins and tannins in hexane, chloroform, aqueous and methanol extracts of the plant. Approximately 58 phytochemicals were found in *B. ciliata* plant and these phytochemicals are known for different biological activities (Singh *et al*., 2018). Methanol, petroleum ether and ethyl acetate extract of *Bergenia ciliata* rhizome showed the presence of carbohydrates, glycosides, alkaloids, saponins, flavonoids, terpenoids, steroids, tannins and phenolics (Kanth *et al*., 2019). The root of *B. ciliata* contains bergenin, gallic acid, tannic acid, glucose, mucilage and wax. Some preclinical and clinical studies revealed that highly purified tannic acids can be used as an importable tool in wound healing and also reduce scar tissue formation (Bhattarai, 1994; Halkes *et al*., 2001). Phenolic compounds were found maximum in ethyl acetate extract (Ahmed *et al*., 2016; Zafar *et al*., 2019) whereas flavonoids were found highest in crude extract. The phenolic compounds namely rutin, morin and pyrogallol were also isolated from the rhizome of *B. ciliata* (Zafar *et al*., 2019). Phenolic compound, isolated from the ethyl acetate fraction of the leaves, revealed the highest dissolution of calcium phosphate stones and oxalate stones. However, it was found that the ethyl acetate fraction effectively dissolves calcium phosphate stones as compared to oxalate (Byahatti *et al*., 2010).

It has been found that methanolic extract of *Bergenia ciliata* showed the highest content of total phenolic compound as compared to ethyl acetate and hexane extract which indicate that the content of phenolic compounds depends upon the polarity of the solvent. In contrast, flavonoid content was more in ethyl acetate extract followed by methanol and hexane extract (Singh *et al*., 2017). Recently, it was reported that among the different solvents, methanol was found to be the best extractive solvent which showed the presence of the highest total phenolic content (Dulta *et al*., 2021).

According to Gorki *et al*., (2021) HPTLC analysis of ethanol extract of *B. ciliata* indicated the presence of bergenin, epicatechin and gallic acid. Gallic acid is an important constituent that is attributed with NF-KappaB inactivation activity (Ho *et al*., 2010). The ethanol rhizome extract of the plant confirmed the presence of various phytochemicals including alkaloids, saponins, tannins, flavonoids and cardiac glycosides. Gas Chromatography/Mass Spectrometry of ethanolic rhizome extract showed the presence of beta-sitosterol, ethyl iso-allocholate, hexadecanoic acid, cetene, monolinolein TMS, bergenin, gallic acid, linolool, β-caryophyllene, calcitriol, carotene, oleic acid and astaxanthin (Verma *et al*., 2019). Beta-sitosterol has confirmed anti-inflammatory, anti-cancerous, hypocholesterolemic, angiogenic, analgesic, anthelmintic, immunomodulatory, antioxidant, neuroprotective, anti-diabetic effects in various studies (Saeidnia *et al*., 2014). HPTLC analysis of plant extract showed that *B. ciliata* can be a good source of bergenin (Srivastava *et al*., 2008). Previously, bergenin has been known for hepatoprotective effects against GalN-induced hepatotoxicity in rats (Lim *et al*., 2001). Bergenin has been evaluated for different pharmacological activities and observed for showing anti-inflammatory, antimicrobial, antioxidant, anti-hepatotoxic, antidiabetic, antilithiac, anti-nociceptive, anti-HIV, cytotoxic and immunomodulatory properties (Patel *et al*., 2012; Oliveira *et al*., 2011; Oliveira *et al*., 2019).

Antibacterial

The methanol extract of *B. ciliata* rhizomes revealed antibacterial activity at concentrations 200, 400, 800 and 1000µg/disc against *Bacillus pumilis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Vibrio cholera* (Sinha *et al*., 2001). The root and leaves extract were effective against *Microsporum canis*, *Pleuroetus oustreatus* and *Candida albicans* (Islam *et al*., 2002a). Different extracts of *B. ciliata* were observed for their antimicrobial activity against *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, *Serratia marcescense*, *Nocardia tenerifensis*, *Streptomyces* sp., *Aspergillus niger* and *Fusarium oxysporum*. Methanol and ethyl acetate extract of the plant has shown similar effects against bacteria. They showed maximum inhibition against *Bacillus megaterium* followed by *Nocardia tenerifensis* and *Bacillus subtilis*. In contrast, the hexane extract showed maximum antibacterial activity against *Serratia marcescense* and *B. subtilis*. No activity was observed against fungi (Singh *et al*., 2017).

Verma *et al*., (2019) investigated the antimicrobial activity of ethanolic rhizome extract of plant against *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It showed the strongest activity against the clinical isolate of *S. typhimurium* followed by a clinical isolate of *E. coli*. The crude extract, n-butanol and ethyl acetate fraction of plant rhizome showed more than 100% inhibition as compared to roxithromycin which was used as a positive control against *Salmonella setubal* and *Salmonella typhimurium*. More than 60% inhibition was observed among different fractions against *Escherichia coli* while 30-58% inhibition was observed against *Bordetella bronchiseptica*, *Micrococcus luteus* and *Staphylococcus aureus* (Ahmed *et al*., 2016). Methanol and ethanol extracts of rhizome of the plant exhibited effective antimicrobial activity against *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* (Minimum Inhibitory Concentration = 6.25 µg mL^{-1}) (Dulta *et al*., 2021). Methanol, ethanol, n-hexane, aqueous and chloroform extract of rhizome of the plant showed antibacterial activity against the selected bacteria. The methanol fraction was observed for the strongest activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Bacillus subtilis* when compared with ethanolic and n-hexane extracts. The lowest antibacterial activity was indicated by the aqueous extract of the plant (Yousaf *et al*., 2018). In another study, neither crude extract nor a fraction of *B. ciliata* rhizome showed any inhibition against *Aspergillus niger* and *A. flavus*. However, the chloroform extract was strongly effective against *Bacillus atrophoeus* and *Bacillus subtilis*. The prominent activity was observed in ethyl acetate fraction against *B. atrophoeus* whereas moderate activity was revealed by n-hexane fraction against *Klebsiella pneumonia* (Khan *et al*., 2018).

Antidiabetic

The ethanol, aqueous, chloroform extracts of this plant showed a reduction in blood sugar level in streptozotocin (STZ) induced rats whereas hexane and butanol extracts showed no hypoglycemic activities. This supports the use of plant in folklore medicine to treat diabetes mellitus. However, the toxicological studies revealed that the plant have severe toxicity symptoms including erythema, edema, breathing problem, diarrhea *etc* (Islam *et al*., 2002). In other study, (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin isolated from *B. ciliata* showed significant dose dependent enzyme inhibitory activities against rat intestinal a-glucosidase and porcine pancreatic a-amylase that supports antidiabetic activity of *B. ciliata* (Bhandari *et al*., 2008).

Antimalarial

The ethanolic leaf extract of *B. ciliata* revealed *in vitro* and *in vivo* antimalarial activity against *Plasmodium berghei* which confirms the traditional usage of the plant for treating fever. (-)-3-O-galloylepicatechin and (-)-3-O-galloylcatechin reported from plant extract in previous studies may be responsible for this antiplasmodial activity (Walter *et al*., 2013). Ethanol extract of rhizome of *B. ciliata* exhibited considerable inhibition (IC_{50} < 5 µg/mL) of schizont maturation of strains of *Plasmodium falciparum* and also found safe to liver and kidney function (Gorki *et al*., 2021). Presence of phenols, flavonoids, steroids and diterpenes in medicinal plants has been known for their antimalarial activity (Tasdemir *et al*., 2006; Chandel *et al*., 2010 and 2012).

Anti-tussive

The methanol extract of the rhizome of *Bergenia ciliata* has shown significant anti-tussive activity in a dose dependent manner which was comparable to codeine phosphate, which is used as a standard anti-tussive agent (Sinha *et al*., 2001b). This supports the claim of use by traditional healers for the treatment of cough and other pulmonary infections.

Antiulcer activity

The methanol extract of rhizomes of *B. ciliata* showed highest inhibition activity when studied against isolates of *Helicobacter pylori* which causes sores and ulcers in stomach (Ali *et al*., 2020). Aqueous and methanol extracts of rhizome were admisintered in doses of 15, 30 and 60 mg/kg b/w 1 h after ulcerogenic treatment. The aqueous extract was more effective to decrease the ulcer lesion than the methanol extract because of cytoprotective effects due to enhancement of mucosal barrier (Kakub and Gulfraz, 2007). In earlier studies, it has been found that *H. pylori* contains urease enzyme which supports its colonization in the stomach. Bergenin inhibits the urease enzyme and that may be responsible for anti-pyloric activity (Phillips *et al*. 1993; Suerbaum 2002 and Arfan *et al*., 2012).

Antioxidant

Reducing power and lipid peroxidation inhibition efficiency (TBARS assay) of mehanolic and aqueous extracts were evaluated and both extracts showed potential activity in preventing lipid peroxidation and thus might prevent oxidative damages to biomolecules. Further, it was found that both the extracts were able to protect DNA from oxidative damage (Rajkumar *et al*., 2010). In DPPH assay, fractions of *B. ciliata* also showed effective free radical scavenging activity. Crude extract showed potent antioxidant activity with IC_{50} 80.50 µg/ml. Among fractions, n-butanol fraction had maximum antioxidant activity (IC_{50} 142.62 µg/ml) and in this study it was also found that the extract does not cause any harm to DNA (Ahmed *et al*., 2016). The DPPH radical scavenging activity of mathanolic extracts of *B. ciliata* leaves were superior as compare to ethyl acetate and hexane extract. Although, all these extracts showed low DPPH radical scavenging activity when compared with the standard butylated hydroxytoluene (BHT) Similar trend was obtained for ABTS radical scavenging assay (Singh *et al*., 2017).

In another study, antioxidant activities of different solvent extracts of *B. ciliata* were evaluated against DPPH, H_2O_2, ABTS, total antioxidant capacity and reducing power assays. Methanol extract was found to have maximum antioxidant activity which was further followed by ethanol, n-hexane, aqueous and chloroform extracts respectively (Yousaf *et al*., 2018). In contrast, the chloroform fractions of *B. ciliata* showed a strong anti-oxidative potential as compare to ethyl acetate fraction (28.15±10.17µg/ml) and n-hexane fraction

(42.39±11.74µg/ml). It has been found that because of side effects of synthetic food additives which are used to prevent food, drugs and cosmetics degeneration there is a strong need for successful antioxidants products which are derived from natural sources. The compounds-rutin, morin and pyrogallol isolated from the rhizome of *B. ciliata* showed high antioxidant potentials with highest activity in pyrogallol as compared to other two against DPPH and ABTS free radicals. Whereas, when DPPH and ABTS radical scavenging activity of crude extract of *B. ciliata* was compared with subfractions of rhizome it was observed that crude extract had potent free radical scavenging activity *i. e.* 87.37 ± 2.45 and 83.50 ± 0.70% respectively (Zafar *et al*., 2019; Khan *et al*., 2018). Flavonoids in plant and its parts are one of the reasons for their capacity to act as antioxidants (Kumar and Pandey, 2013).

Anti-tumour

The methanol and aqueous extracts of *B. ciliata* rhizome were studied for anti-neoplastic activity. The extracts were tested for their cytotoxicity on human breast carcinoma cells, human hepatocellular carcinoma cells and human prostate cancer cell lines by the XTT assay. Both the extracts showed anti-neoplastic activity (Rajkumar *et al*., 2011). The crude extract and ethyl acetate fraction of plant showed dose dependent activity against H157 Lung Carcinoma and HT144 Malignant Melanoma (Ahmed *et al*., 2016). Bergenin have also been found to be effective against cervical cancer cells, colorectal cells and bladder cancer cells (Gao *et al*., 2017; Shi *et al*., 2019; Liu *et al*., 2020).

Anticholinesterase activity

The crude extract of rhizome of *B. ciliata* showed maximum anticholinesterase (acetylcholinesterase = 90.22 ± 1.15% and butyrylcholinesterase = 88.22 ± 0.71%) potentials as compare to that of other subfractions which indicate its role in treatment of neurological disorders (Zafar *et al*., 2019). Methanolic extracts of rhizomes of *B. ciliata* dose-dependently diminish streptozotocin induced behavioral deficits and biochemical anomalies in Wistar rats with Alzheimer's disease. The observed cognitive improvement after extract administration may be accredited to its antioxidant activity and refurbishment of cholinergic functions which supports the use of plant in neurological disorders (Barai *et al*., 2018).

Others

Co-treatment of wistar rats in which nephrolithiasis have been induced with ethylene glycol with hydro-methanol extract of rhizome of *B. ciliata* caused reduction in lipid peroxidation, increase in activities of antioxidative enzymes in kidney and reduction in calcification (Saha *et al*., 2014). This supports the very common use of rhizome in kidney stone problems. The methanol extract of *B. ciliata* rhizome significantly lowered the body temperature till 4 hours of administration in yeast induced pyrexia and effect was dose dependent which justify use of plant in treatment of fever (Sinha *et al*., 2000). According to Rajbhandari *et al*., (2009) extracts of *B. ciliata* showed good anti-herpes viral activity. Also, the methanol extract of the rhizome of *Bergenia ciliata* exhibited significant anti-inflammatory potential in two acute rat models (carrageenan- and serotonin (5-HT)-induced rat paw oedema) and a chronic rat model (cotton pouch-induced granuloma) (Sinha *et al*., 2001a).

Conclusion

This chapter deals with an up-to-date review of the pharmacological studies of *Bergenia ciliata*. It is an important medicinal plant of Himalayan states and is well known for its uses in the traditional systems of medicine. Traditionally, the plant is harvested from

the wild for its medicinal uses. *B. ciliata* is used in different parts of the world for the treatment of several diseases. The presence of various phytochemicals and pharmacological knowledge of this plant can be helpful to undertake further investigations on this plant. Some pharmacological and clinical studies of different chemical constituents of *B. ciliata* are found to be very promising, which calls for more-systematic research of this medicinal plant and its active principles.

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