The report on medicinal importance, tissue culture studies and phytochemistry of Bergenia ciliata (Haw.) Sternb

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ABSTRACT

Bergenia ciliata (Haw.) Sternb. is very promising herb with great medicinal importance. The plant contains important secondary metabolites like bergenin, catechin, arbutin, gallicin, gallic acid, tannic acid, gallic acid, glucose, mucilage, wax, albumen and mineral salts. It is locally known as “Zakhhmehayat”. The ethanolic leaf extracts of the plant have shown promising activity against malarial parasites: Plasmodium falciparum and P. berghei. Bergenia shows anti-inflammatory activity, anti-tussive activity, antioxidant activity, anti-cancer activity, anti-ulcer activity and anti-diabetic activity. The rhizomes of this plant are of pharmaceutical importance, therefore, the whole plants are dug out resulting in very less chances of regeneration and is put under ‘‘vulnerable’’ category in the western Himalaya. Keeping in view the importance of this medicinal plant, the present review summarises the literature available regarding the said plant on the following aspects– its medicinal uses (traditional and modern), in vitro culture studies for its conservation programme and the phytochemical studies with particular focus on HPLC/HPTLC studies carried in the said plant till date. The review would be beneficial in carrying forward further research in the said plant in terms of establishing a better in vitro protocol and enhancing the important secondary metabolites from the plant using various mutation breeding, molecular and genetic engineering techniques.

Keywords: Bergenia ciliata, HPLC/HPTLC, in vitro culture, medicinal importance

I. INTRODUCTION

The vast literature on B. ciliata mentioning its medicinal importance from vedic literature to the modern medicine is available. The current paper tries to summarise the research available on B. ciliata on the following aspects– its medicinal uses (traditional and modern), in vitro culture studies for its conservation programme and the phytochemical studies with particular focus on HPLC/HPTLC studies carried in the said plant till date. B. ciliata, commonly known as “Zakhmehayat”, is a perrenial rhizomatous herb upto 35cm tall, leaves are few in number, glabrous, suborbicular to orbicular or broadly obovate. Inflorescence is one sided raceme or corymbose, often subtended by an ovate leafy bract. It bears white or pink flowers and its fruit is capsule [1]. The plant occurs in temperate regions from Kashmir region to Bhutan. It is found in the Himalayas between the altitudes of 2000 and 2500 meters, commonly on the rocks in forest of hilly regions. Generally it grows wild at 8000-10000 ft. elevation in the Himalayan regions and also found in the Khasi hills and other areas in North-
East Himalaya at about 4000 ft. altitude. The first record of the plant dates back to the Vedic period where its medicinal properties have been mentioned in Vedas, Charaka Samhita and Sushruta Samhita [2]. Since then the vast research has been carried out in the said plant and its medicinal properties have been well established using the modern techniques. As the rhizomes of this plant are of pharmaceutical importance, the whole plants are dug out resulting in very less chances of regeneration. It is considered amongst the high valued temperate medicinal herbs and is put under “vulnerable” category by IUCN (2016) in the Western Himalaya and by Conservation Assessment and Management Plan Workshop Process, WWF, India, ZOO/CBSG, India and Uttar Pradesh Forest Department in 1997 [3].

II. MEDICINAL APPLICATIONS OF B. CILIATA

2.1. Traditional uses

*B. ciliata* (Haw.) Sternb. (syn. *B. ligulata*) is a very promising herb with great medicinal importance. In Swat and Kashmir areas, it has been reported to be used in fever, diarrhoea and applied to bruises and boils. One teaspoonful of the juice of dried rhizome of *B. ciliata* along with an equal amount of honey has been taken orally 2-3 times a day by post-partum women; against the digestive disorders as carminative and tonic as well. Rhizomes have been taken orally by human adults as an anti-helmintic [4,5,6]. In North West and trans-Himalayan region of Jammu and Kashmir State, the boiled roots with added salt are used as decoction for the treatment of asthma. In Ayurvedic system of medicine, the drug is reported to possess astringent, tonic, anticancer, and laxative properties. It has also been used as a poultice and is locally known as “Zakhhmehayat”. The extract is reported to be mildly diuretic in lower doses but exhibits anti-diuretic action at higher doses. The juice of the rhizome of *B. ciliata* is used as an anti-tussive for cold and cough also by the local people of the Sikkim and Darjeeling districts of West Bengal [3]. The root, rhizome and leaf powder are used by local tribes for piles treatment [7] and is found to have potential in the prevention and treatment of cancer [8].

2.2. Uses in Modern medicine

The ethanolic leaf extracts of the plant have shown promising activity against malarial parasites: *Plasmodium falciparum* and *P. berghei* [9]. The plant contains bergenin, catechin, arbutin, gallicin, gallic acid, tannic acid, gallic acid, glucose, mucilage, wax, albumen and mineral salts [10]. Secondary metabolites isolated from this plant have pharmacological effects in humans so are used as medicines [3]. The ethyl acetate extracts of the plant have shown anti-microbial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Klebsiella pneumonia* [11]. Bergenin shows anti-inflammatory activity, anti-tussive activity, antioxidant activity, anti-cancer activity, anti-ulcer activity and anti-diabetic activity [7]. It possesses anti-urlopathic property and is used as diuretic and anti-calcium medicine. It is an antidiabetic drug, astringent, cardiotonic, expectorant, antipyretic, antidote to poison, anti-inflammatory, wound healer and anti-haemorrhoidal and it allays burning sensation and excess thirst [2].

2.2.1. Application of bergenin
One of the main compounds obtained from Bergenia species is a polyphenol - bergenin. It is found in colourless crystal form isolated from Bergenia species such as B. ligulata, B. ciliata, B. stracheyi, B. crassifolia etc. It has two analogues (nor-bergenin and acetyl-bergenin) with additional pharmacological effects [12,13]. Bergenin is reported to decrease lipid peroxidation of cell membranes by its anti-oxidative property and its activity is more than vitamin C and protects against ascorbic acid depletion in several different body tissues [10]. Bergenin effects inflammation by balancing secretion of cellular messengers (cytokines) from pro-inflammatory and inhibitory cells of the immune system. It inhibits the release of inflammatory cytokines like Interleukin-2 (IL-2), gamma interferon (IFN-gamma), and Tumor Necrosis Factor-alpha (TNF-alpha). It also promotes the release of anti-inflammatory messengers like Interleukin-4 and Interleukin-5 [14]. Bergenin also plays a role in the breakdown of fat. It is becoming a popular component of thermogenic fat-burning dietary supplements. It does not directly stimulate lipolysis but appears to enhance the activity of lipolytic adrenergic hormones like norepinephrine. It also opposes the lipogenic activities of insulin. The mechanism appears to be through the enhancement of norepinephrine to phospholipids of adipose cells. The compound has moderate activity against an enzyme namely protein tyrosine phosphatase 1B. This enzyme negatively regulates insulin and leptin signaling and some of the positive effects of insulin and leptin may be enhanced by its inhibition [15]. Bergenin is reported to have protective effect on the liver against damaging environmental poisons. On experiments with animal models, bergenin successfully reduced the damage caused to liver by toxins like carbon tetrachloride and secreting less indicators (aspartate amino transferase and alanine amino transferase). It is found to replenish and protect the glutathione antioxidant system in liver cells burdened with tasks of neutralising or eliminating environmental toxins. Bergenin has germicide activity against E. coli and Pseudomonas aeruginosa. It is effective against many types of fungus by blocking a crucial enzyme called yeast alcohol dehydrogenase which is required for fermentation reactions. Bergenin has been used in curing arrhythmia and has anti-hepatitis C virus (HCV) activity. However, it is reported to have weak anti-HIV activity in vitro. It is also not effective at attacking HIV-I reverse transcriptase and HIV integrase [16].

2.2.2. Application of arbutin

Arbutin is another medicinally and economically important compound obtained from Bergenia spp. It is also known as arbutoside hydroquinone β-D-glucopyranoside. It is a derivative of hydroquinone and belongs to the group of simple phenols. It is normally present in both ether and glycoside form [10]. Pharmacologically, it possesses antibacterial properties, inhibits tyrosinase enzyme and prevents the formation of melanin biosynthesis in human skin. It is used in treating skin discolorations such as melasma, freckles, hyperpigmentation or other disorders. It is also used as a skin lightening agent, therefore, has great demand in cosmetic industry [17,18].

III. IN VITRO CULTURE OF B. CILIATA

The first work regarding tissue culture studies from genus Bergenia was reported by Furmanova et al. [19] who reported seed germination of B. crassifolia on MS medium. The hypocotyls formed friable green or light yellow
callus on MS medium+0.3mg/L NAA+1mg/L BA+80mg/L adenine sulphate. 0.5mg microrhizome cuttings from
in vitro raised plants were also able to form adventitious buds when cultured on ML medium+3mg/L
BA+0.1mg/L NAA and the roots were produced on the same medium. Duskova et al., [20] also reported in B.
crassifolia that sterile germinating plants were used to derive a tissue culture. The greatest stimulating effect on
the growth of the culture was exerted by NAA cultures in all concentrations tested, by IAA in concentrations of
1.0 and 10.0 mg/L, by IBA in a concentration of 0.1 mg/L, and by the combination of IBA + Kn. Further, TLC
analysis of callus extracts demonstrated the presence of bergenin, arbutin, hydroquinone, and methylarbutine.
Arbutin (0.25%), hydroquinone (0.05%) methylarbutin (0.28%) was confirmed by HPLC. Carmen [18] tried
young leaves, small gemma, petioles, rhizome and roots of B. crassifolia to obtain in vitro cultures but no
successful results were obtained. The cultures developed necrosis and death of cells occurred before callus
formation. However, Liu et al., 2009 was successful in obtaining plant cultures from leaves of B. crassifolia on
MS+0.5 mg/L NAA+ 0.1 mg/ L IBA+ 0.5 mg/L BA.

The complete tissue culture protocol from family Saxifragaceae was worked out by Abou Dahab [21] using
mature Hydrangea macrophylla plants which has ornamental importance. The highest percentage of
contamination free explants (100%) was obtained using chlorox (50%) and HgCl₂ (0.2%). MS medium + 2mg/L
BA resulted in the highest number of shoots/explant and increasing the number of subcultures significantly
increased the number of shoot per explants, the 3rd subculture giving the highest value. Shoot length was
significantly reduced by adding TDZ to the medium whereas MS medium + BA/Kn (2 mg/L) were the most
effective treatments in giving significant increases in shoot length. Culturing the explants on MS medium
supplemented with BA at 4 mg/L produced the highest number of leaves/explant. B₅ full strength medium was
reported to be the best medium for causing significant increases in the number of shoot per explant. The highest
number of roots/shootlet was recorded on MS ½ medium supplemented with 2 mg/L IBA and activated
charcoal. The tallest plantlets were obtained on MS medium supplemented with 3 mg/L IBA and activated
charcoal and were transferred in pots containing peat moss plus sand (1:1 v/v) with 100% survival rate.
Previously, the dormant buds of H. macrophylla were used to produce shoots when cultured on MS+ 10 µM
BA, zeatin or 2ip [22]. In a recent study by Liu et al., [23], effect of different plant growth regulators on shoot
regeneration were investigated jointly with selecting optimal basal media and ceftoxime concentrations in H.
macrophylla ‘Hyd1’. The highest frequency of shoot organogenesis (100%) and mean number of shoots per
explant (2.7) were found on B₅+2.25 mg/L BA+ 0.1 mg/L IBA+100 mg/L ceftoxime. Regenerated shoots were
rooted by culturing on perlite + B₅ ½ liquid media + 0.5 mg/L NAA. Rooted plantlets were transplanted to the
greenhouse with 100% survival rate. Genetic stability of 32 plantlets (one mother plant and 31 regenerants) was
assessed by 44 ISSR markers. Out of 44 ISSR markers, ten markers produced clear, reproducible bands with a
mean of 5.9 bands per marker.

Shreshta and Pant [24] reported tissue culture of B. ciliata (Haw.) Sternb. They inoculated leaf explants on MS
media supplemented with or without phytohormones. The hormonal series maintained were in the range of 0-2
mg/L for BA and 0-1.5 mg/L for NAA. The best growth of callus and plantlets were reported on BA 1.0 mg/L+
NAA 1.0 mg/L and BA 1.5 mg/L + NAA 1.0 mg/L. Verma et al. [25] reported that profuse multiple shoots and roots were obtained from the cultures derived from nodal segments of B. ciliata on MS + BA + IAA combinations. Parveen et al., [26] studied in vitro regeneration in B. ligulata and best seed germination and seedling formation was observed on modified MS medium (salts of MS medium + vitamins of Nitsch and Nitsch medium). The shoot tips obtained from in vitro germinated seedlings were cultured on various media viz. MS (both full and half strength), modified MS, Gamborg’s, Nitsch & Nitsch and White’s media to score better shoot multiplication response for effective micro-propagation. The media were supplemented with varying concentration of Kn (2.5-15μM) and IAA (7.5 μM). The results revealed very little shoot regeneration on MS full strength, B₅ and Nitsch & Nitsch media. The modified MS medium was found to be best medium for direct shoot regeneration, supplemented with Kn and IAA (18.2 shoots). They reported no response in shoot formation on MS ½ and White’s media. The in vitro raised leaves resulted in formation of indirect shoots at BA + NAA and Kinetin + IAA with maximum shoot length on 5 μM of NAA combined with 2.5 μM of BA, and also on 7.5 μM of BA combined with 2.5 μM of IBA. Zeatin also induced indirect shoot formation (6-9 μM) but when used in combination with auxins, it did not show any shoot multiplication. The field leaf explants did not show any response in terms of shoot formation, however, few phytohormone combinations showed slight callus formation. TDZ alone or in combination with 2,4-D/IBA/IAA was unable to initiate any shoot multiplication in B. ligulata [26,27]. The complete tissue culture protocol of B. ciliata was established by Rafi et al., [28] who reported MS ½ medium as the best media for germination of seeds and best response for callus formation was obtained on B₅ ½ medium with TDZ (2 mg/l) and NAA (2 mg/l) after 6 weeks. Maximum number of 54 shoots were obtained on B₅ ½ medium with BA (2 mg/l) and IAA (1 mg/l). The complete plantlets were regenerated on MS ½ basal medium on which direct rooting took place. The complete plants were acclimatised in first in lab conditions and then transferred to green house conditions with survival rate of 65%.

IV. PHYTOCHEMICAL STUDIES OF B. CILIATA

For the first time, a highly sensitive and precise reverse phase HPLC method with an optimised extraction procedure for the quantitative estimation of bergenin and (+)-afzelechin in different parts of B. ligulata was developed [29]. The method gives more than 99% recovery of the active constituents. The rhizomes of the plant were found to contain higher concentrations of bergenin (0.907% w/w) and (+) -afzelechin (0.168% w/w) than other parts of the plant. The presence of these constituents was also estimated in polyherbal formulations containing this plant. Later on, a reproducible HPTLC method for the simultaneous determination of bergenin and gallic acid in B. ligulata was developed [30]. The method involved separation of the components by TLC using ethyl acetate–formic acid–acetic acid–water (100 + 11 + 11 + 27) as the solvent system. The sensitivity of the method for bergenin was 0.30 mg, whereas for gallic acid it was 0.25 mg. Rawat et al., [31] developed RP-HPLC method coupled with photodiode-array detection for bergenin and gallic acid and validated for simultaneous determination of these compounds in three Bergenia species: B. ligulata (Wall) Eng., B. ciliata (Royle) Raizada, and B. stracheyi Engl. B. ciliata and B. stracheyi were reported to contain most bergenin, 3.275% and 3.277% respectively; B. ligulata contained 2.419% bergenin. The similar findings has also been
revealed by Srivstava and Rawat, [32] who reported maximum bergenin in *B. stratchyei* (5.99%) followed by *B. ciliata* (5.73%) and *B. ligulata* (5.68%) using HPTLC. Bergenin was also reported be present in genus *Viburnum* for the first time [33]. The roots were defatted with the petroleum ether and extracted thoroughly with the rectified spirit in a soxhlet apparatus. Various derivatives of bergenin *viz* bergenin diethyl ether, its penta-acetate and diethyl ether triacetate were prepared and method of preparation and characterization by mass spectroscopy and NMR was reported for the first time.

The phytochemical investigation on the *in vitro* culture of *B. ciliata* (Haw.) Sternb. was studied [24]. The study revealed that the *in vitro* cultured callus of *B. ciliata* was capable of synthesizing bergenin in quantity comparable to that of the wild plant. Leaf explants were cultured in MS basal media supplemented with or without phytohormones. The hormonal series maintained were in the range of 0-2 mg/L for BA and 0-1.5 mg/L for NAA. Bergenin content of *in vitro* grown tissues of *B. ciliata* was compared with that of wild plants collected from three different localities of Nepal. The best growth of callus and plantlets occurred in the media containing BA 1.0 mg/L + NAA 1.0 mg/L and BA 1.5 mg/L + NAA 1.0 mg/L. Production of bergenin was high in the media supplemented with 1.0 mg/L BA + 1.5 mg/L NAA (3.40 µg/g) and 2.0 mg/L BA + 1.5 mg/L NAA (3.05 µg/g) under experimental conditions. The bergenin content in the wild plants collected from Langtang, Jumla and Godawari was found to be 4.28 µg/g, 4.53 µg/g and 3.64µg/g respectively.

The identification and qualitative analysis of arbutin using HPLC and UV-spectroscopic methods and quantitative determination of arbutin as well as other pharmaceutically interesting compounds: flavonoids, hydroxycinnamic acids, polyphenols and tannins in domestic cultivars was studied by Arok *et al.*, [34], considering the synergism of phytochemical agents in therapeutic effect in skin disorders. The arbutin content was found to be between 4.8 – 9.8 g/100g in the two *Bergenia* species and significant amount of total polyphenol (4.13 – 9.27 g/100g), tannin (3.70 -6.70 g/100g) and hydroxycinnamic acid (1.80 -2.42 g/100g) was measured. In the similar year, HPLC method for the determination and quantification of arbutin and hydroquinone in many different raw materials was also developed and validated [18]. The optimum conditions for the separation and detection of these two constituents were achieved on a LiChro-CARD 125-4 Superspher®100 RP-18 column with the water-methanol (gradient elution) mobile phase and recorded at 289 nm. This method was used in comparative qualitative analysis of arbutin and hydroquinone in 16 different raw materials from families Lamiaceae, Ericaceae, Saxifragaceae and Rosaceae. Sunita *et al.*, [35] extracted the powdered rhizomes with various solvents by successive soxhlet hot extraction process with increasing order of polarity and showed the presence of carbohydrates, alkaloids, steroids, glycosides, flavonoids and tannins. By charging alcohol extract in column two compounds were isolated by column chromatography technique and compounds were identified as Bergenin and Octyl ester of rhamnosyl bergenin. Boros *et al.*, [36] developed an efficient RP-HPLC method for the simultaneous identification and detection of arbutin, bergenin and gallic acid from *Bergenia* leaf samples which were extracted with a methanolic solvent mixture [methanol: water = 1:1 (v/v)]). Chromatographic separations were performed on a reversed phase Luna C18(2)-HST HPLC column. This chromatographic system provided increased speed and efficiency for separations, without the need for
ultra-high pressures and the method was validated using ICH guidelines. The level of gallic acid was significantly higher in *B. crassifolia* samples compared to *B. cordifolia*. However, the samples of the two *Bergenia* species did not differ substantially regarding the concentrations of arbutin and bergenin. *Bergenia* root extracts have shown very strong evidence for DPPH radical scavenging activity and α-glucosidase inhibitory activity and some evidence regarding α-tyrosinase and anticancer activities [37].

The ethyl acetate extracts of the plant have shown anti-microbial activities against gram-positive bacteria (*Staphylococcus aureus* and *Micrococcus luteus*) and gram-negative ones (*Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Klebsiella pneumonia*) [11]. In the same year, Saha and Verma, [38] investigated hydro-methanolic extracts of *B. ciliata* rhizomes and *T. chebula* fruits for antioxidant potential against sodium oxalate induced oxidative imbalance in the kidney of female Wistar rats. The findings revealed that sodium oxalate caused significant increase in lipid peroxidation with concurrent decrease in activities of SOD and catalase as well as in total reduced glutathione content in a concentration-dependent manner. The hydro-alcoholic extract when co-administered with sodium oxalate resulted in significant protection with maximum percent protection achieved by *B. ciliata*. The ethanolic leaf extracts of the plant have shown promising activity against malarial parasites: *P. falciparum* and *P. berghei* [9].

The recent study was conducted by Singh *et al.* [39] on *B. ciliata* leaf extracts (methanol, ethyl acetate and hexane) for evaluating antioxidant, antimicrobial activity and bioactive compounds. The extracts were analyzed for total phenolic and flavonoid contents along with the antioxidant and antimicrobial activities. Methanol was found to be the best solvent for extraction with highest total phenolic contents and the lowest IC50 values for DPPH and ABTS assays. Methanol extract also exhibited effective antimicrobial activity. The HPLC results also revealed methanolic extract to be the efficient solvent for extraction of all the three bioactive compounds tested viz. bergenin, catechin and gallic acid.

**V. CONCLUSIONS**

The current review is the first attempt to summarise the tissue culture studies and HPLC studies carried out in *B. ciliata* so far. The review would be beneficial in carrying out further studies in the said plant for upgrading its medicinally important constituents using the modern technology of mutation breeding and genetic engineering keeping in view its medicinal usage and its demand in medicinal and cosmetic industry.

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