Phytochemical, pharmacological and tissue culture studies of some important species of the genus *Barleria* L. (Acanthaceae) - a review

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**ABBREVIATION**

ARTICLE HISTORY

Received: 03 February 2021
Accepted: 18 May 2021
Available online: 01 July 2021

**KEYWORDS**

Acanthaceae
Metabolites
Flavonoids
Barlerin
Iridoid glycosides

**ABSTRACT**

Ayurvedic sciences helped the early humans to overcome chronic dangerous diseases. There are thousands of varieties of herbs and medicinal plants used to overcome such dreadful diseases. The genus *Barleria* L. belongs to family Acanthaceae, a medicinally significant group of plants having diversified phytochemicals used for different pharmacological properties. It has been utilized since ancient times for medicinal purposes. It has many plant secondary metabolites such as terpenes, flavonoids, lignins, alkaloids, particularly the iridoid glycosides. The secondary metabolites extracted from *Barleria* spp. show potential pharmacological activities viz., anti-microbial, anti-inflammatory, anti-oxidant, anti-fertility, anti-arthritis and anti-ulcer activity. In view of these, present review is focused on the phytochemistry, pharmacology and tissue culture studies of some of the important species of the genus *Barleria* L.

**Introduction**

Since ancient time, mankind has depended on plants for his food, shelter, clothing and medicines. The science of Ayurveda allows us to become familiar with plants and their medicinal properties. The genus *Barleria* L. belongs to the family Acanthaceae and there are many reports proving the medicinal properties of this genus. A total of 300 species have been reported globally, mainly distributed throughout the Asia, South Africa and most of the tropical countries (1). Numerous phytochemicals reported from various species of *Barleria* which are responsible for medicinal properties (2). The *Barleria* species are usually seen as herbs or shrubs with simple leaves which are usually arranged in decussate manner and few species have sharp apex. They have diverse coloured tubular flowers with epipetalous stamens, arranged in solitary, axillary and cymes. Thorns/spines are present at the axial positions, which are modified bracts (3). In ayurvedic medicine, *Barleria* species are used for their anti-fertility, anti-diabetic, cytotoxic, immunoprotective properties (4). Especially, Iridoid glycosides which are present in *Barleria* play a major role in building up the pharmacological profile. In this review, we have discussed about the phytochemicals, pharmacological activities and plant tissue culture studies of some important species of genus *Barleria*.

**Phytochemistry of Barleria species**

Macroscopic and microscopic studies on *Barleria* helps us to understand the chemical compositions present in the plant body right from leaves, stem and roots. There are many pharmacologically active compounds present in the plant body of *B. prionitis*. The decoctions of the *B. prionitis* leaves reveal the presence of metabolites like alkaloids, tannins and oils. Tannins are also present in the bark of *B. prionitis* (5). Glycosides like scutellarein 7-rhamnosylglucoside, is reported in *B. prionitis* (6). Acetyl Barlerin A with molecular formula C<sub>24</sub>H<sub>18</sub>O<sub>13</sub> and Barlerin B with molecular formula C<sub>25</sub>H<sub>20</sub>O<sub>12</sub> are isolated and compared with the standard spectroscopic data and they are found to be iridoids isolated from *B. prionitis* (7). Later, Barlerin is also reported to be present in the methanolic extract of *B. dinteri* (8). From the ethanolic extracts of *B. prionitis*, a compound known as Balarenone was isolated. Spectral studies helped to find the molecular formula as C<sub>26</sub>H<sub>33</sub>O<sub>9</sub>. From the ethanolic extract of *B. prionitis* showed the presence of Barlerinoside, which is a phenylethanoid glycoside. Spectral data revealed that the molecular formula of the compound is C<sub>38</sub>H<sub>53</sub>O<sub>16</sub> with chemical name β(3,4-dihydroxyphenyl)ethyl-α-L-rhamnopyranosyl-(1″→3″)-α-L-rhamnopyranosyl-(1″→3″)-β-D-glucopyranosyl-(1″→6″)-O-(3,4-dihydroxycinnamoyl)-β-D-glucopyranoside (10).
In *B. lupulina* the aerial parts are subjected to metabolite analysis using ethanol and hexane. During chromatography technique, CH:Cl<sub>2</sub>-methanol at ratio of (80:20) fractions showed presence of shanzhiside methyl ester along with Barlerin (8-O-acetylshanzhiside methyl ester) and Acetyl Barlerin (6,8-O,O-diacylshanzhiside methyl ester) which are chief iridoid glucosides present in *Barleria* spp. (11). In aerial parts of *B. lupulina*, minor iridoid compounds like ipolamiidoside and 6-O-acetylshanzhiside methyl ester are isolated and they are characterised using spectroscopic analysis (12). Compounds like 6-O-p-methoxy-cis-cinnamoyl-8-O-acetylshanzhiside methyl ester, 6-O-p-methoxy-trans-cinnamoyl-8-O-acetylshanzhiside methyl ester, 6-O-p-cis-coumaroyl-8-O-acetylshanzhiside methyl ester and 6-O-p-trans-coumaroyl-8-O-acetylshanzhiside methyl ester are isolated from leaves of *B. lupulina* (13). In the flowers of *B. lupulina* lupinoside is isolated which is an iridoid diglucoside. Chemically it is 8-O-acetyl-2′-O-(β-gluco-pyranosyl) mussyanoeside with molecular formula C<sub>32</sub>H<sub>33</sub>O<sub>16</sub> (14). In the ethanolic extract of *B. lupulina*, iridoid glycosides like 8-O-acetylpolamidic acid, 8-O-acetyl-6-O-(p-methoxy-cis-cinnamoyl)shanzhiside and 8-O-acetyl-6-O-(p-methoxy-trans-cinnamoyl)shanzhiside were isolated and iridoid rich component having other active compound showed potent alkaline phosphatase activity (15). Spectral analysis conducted on the aerial extracts from *B. lupulina* showed the presence of chakyungulupulins A and B, which are the 4,8,8-trimethylcyclclooct-2–ene derivatives. The molecular formula of chakyungulupin A and B was found to be C<sub>21</sub>H<sub>20</sub>O<sub>12</sub> (16). In the aqueous extract of *B. lupulina*, 4-ethylcatechol, 4-vinylcatechol and 4-methylecatechol were isolated (17). The ethanolic extracts from *B. lupulina* showed the presence of Barlerulins, which are iridoid glycosides. NMR spectroscopic observations reveal that Barulpulin A has molecular formula C<sub>26</sub>H<sub>27</sub>O<sub>13</sub>, Barulpulin B has molecular formula with C<sub>27</sub>H<sub>27</sub>O<sub>13</sub>, and Barulpulin C, D has the same molecular formula C<sub>27</sub>H<sub>27</sub>O<sub>13</sub> (18). Another iridoid glycoside namely Barulpulin C methyl ester was isolated from aerial parts of *B. lupulina*. NMR data suggests that the molecular formula of the compound is C<sub>34</sub>H<sub>30</sub>O<sub>12</sub> (19). Compounds like cyclobutane, 1,1-dimethyl-2-octyl, 1-Hentetracontanol, 1,2-Benzenedicarboxylic acid, diisooctyl ester, 1-Hentetracontanol, 2-Hexyl-1-octanol were isolated from the essential oils of *B. lupulina* (20).

In *Barleria buxifolia* Linn., roots were subjected to benzene extraction and it is seen that pigments like Barleriaquinone. The molecular weight and chemical formula of Barleriaquinone is found to be 238.0659 and C<sub>13</sub>H<sub>20</sub>O<sub>5</sub>. Spectroscopic studies help us to understand that the chemical name of the isolated quinone is 1-hydroxy-7-methylantherquinone (21). Spectroscopic analysis helps us to find out that different compounds are present in *B. strigosa*. Striogoside is a compound isolated having a chemical name 4-hydroxyphenethyl 4-O-β-D-glucopyranosyl-(1→3)-O-α-L-rhamnopyranoside with molecular formula C<sub>26</sub>H<sub>30</sub>O<sub>14</sub> and another iridoid compound with chemical name 10-O-trans-coumaroyl-erhantheside with molecular formula C<sub>26</sub>H<sub>30</sub>O<sub>11</sub> (22). From the methanolic extracts of *B. strigosa* parvifloroside A and B were isolated, which are phenylethanoid glycosides. The molecular formula of parvifloroside A and B is found to be C<sub>30</sub>H<sub>26</sub>O<sub>14</sub>, these compounds show great anti-oxidant properties (23).

From the ethanolic extracts of *Barleria trispinosa* an iridoid compound is isolated with molecular formula C<sub>25</sub>H<sub>30</sub>O<sub>16</sub> having chemical name 6-α-L-rhamnopyranosyl-8-O-acetylshanzhiside methyl ester, which is illustrated using NMR spectroscopy (24). In *Barleria acanthoides* there are diversified compounds isolated from the n-butanol extracts. Spectral analysis suggests the presence of compounds called Barleriside A and B. Molecular formula of the isolated compounds are C<sub>34</sub>H<sub>30</sub>O<sub>19</sub> and C<sub>34</sub>H<sub>30</sub>O<sub>15</sub>. Chemical name of the Barleriside A is found to be 8,4′-di hydroxy-6-O-(β-D-glucopyranosyl)flavone and Barleriside B is 1-O-caffeyl-6′-(3′,4′-dihydroxyphenyl)ethyl-O-α-L-rhamnopyranosyl-(1→3) β-D-glucopyranoside (25). In the n-butanol fraction of methanolic extract from *B. acanthoides* showed presence of neoglanign diglucoside called Barlericin. High resolution fast-atom bombardment mass spectroscopic results suggested that the molecular formula of the isolated Barlericin is C<sub>34</sub>H<sub>30</sub>O<sub>16</sub> (26). Mixture of 4-hydroxy-trans-cinnamic acid alkyl esters and oleanolic acid (triterpen compound) were isolated from the methanolic extract of *Barleria cristata* (27). The list of important phytochemicals present is represented in Table 1.

**Analytical methods for standardization of phytochemicals in Barleria species**

Development of validated analytical methods is very important to analyse the phytochemicals in the polyherbal extracts. Chromatographic techniques were employed to screen the biochemicals in the plant extracts of *Barleria*. Important metabolites like Barlierin, Acetylbarlierin and Shanzhisidemethylster from *Barleria* spp. were analysed using HPTLC. It is seen that mobile phase having chloroform-ethylacetate- methanol-acetic acid at concentration of 3:3:3:1 showed best results at 233 nm. Upon statistical data analysis it was seen that highest content of barlierin was found in the shoot regions of *B. prionitis*. Acetylbarlierin is found highest in the leaves of *B. lupulina* and Shanzhisidemethylster content is found highest in leaves of *B. prionitis* (31). HPTLC and RP-HPLC methods were developed for *B. cristata*. An optimised HPTLC technique was developed with the help of solvent system having methanol: ethyl acetate: toluene: formic acid at (1: 1: 7.5: 0.2) to separate and quantify the significant anti-oxidants like quercetin, apigenin and naringenin at 308 nm. In RP-HPLC method, potassium dihydrogen phosphate along with methanol at 30:70 was used as solvent system and the detection of biochemicals was done at 330 nm. These standardised analytical methods helped in evaluating the phytochemical markers in plasma (32). Various saponins were identified in the leaf extracts of *B. cristata*. It is seen that in the optimised mobile phase having chloroform: glacial acetic acid: methanol: water at 6:4:3:2:4:2:0 ratio was used for analysis. Anisaldehyde-sulphuric acid reagent is used to obtain the chromatogram and later saponins were analysed from plates at 500 nm (33).
In *B. prionitis*, HPTLC technique was developed to quantify the various biochemicals. Saponins were analysed with optimized solvent system having chloroform:glacial acetic acid: methanol: water at ratio of 6.4:3.2:1.2:0.8. Chromatograms were compared with gallic acid, and it is seen that extracts showed presence of tannins. Flavonoids were qualitatively estimated using ethyl acetate: formic acid: glacial acetic acid: water at 10:1:1.1:2.6 ratio as solvent system. It is seen that leaf showed the highest percentage in the presence of saponins, tannins and related metabolites when compared to roots (34). GC-MS analysis was employed to analyse the biomolecules present in different extracts of *B. buxifolia* L. and *B. montana*. A total of 26 compounds were analysed which comes under broader class of flavonoids, alkaloids, terpenoids and tannins from ethanolic extract of *B. montana*. It is seen that injector at 280 °C and helium gas with the flow at 0.8 ml/min is used in improved protocol (35). Similarly in *B. buxifolia*, 30 compounds were analysed from methanolic extract of leaf. It is seen that injector at 230 to 250 °C and helium gas with the flow at 1ml/min was used in improved protocol (36).

**Pharmacology of Barleria species**

**Anti-viral property**

Cytopathic effect assay is conducted against respiratory syncytial virus (A2 strain) using 6-O-cis-p-coumaroyl-8-O-acetylshanzhiside methyl ester and 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester and it is found that 1:3 ratio of the cis and trans isoforms of above mentioned compounds isolated from *B. prionitis* showed best half maximal inhibitory concentration (IC₅₀) and half best maximal effective concentrations (EC₅₀) at 42.2 µg/ml and 2.46 µg/ml respectively (37). Plaque inhibition assay suggest that the anti-viral activity of *B. lupulina* extracts against Herpes simplex virus type 2 strain G, and some medical isolates of HSV-2 is very much promising and its activity is compared with *Clinacanthus nutans* which is also an Acanthaceae member. It is seen that *C. nutans* anti-viral activity is not up to the mark when compared to *B. lupulina*. Yield reduction assay also suggests that *B. lupulina* has promising viricidal effects (38).

**Anti-fertility activity**

Methanolic root extracts of *B. prionitis* reduced the fertility in male rats. It is seen that administration of 100 mg/l concentration of the plant extracts obstructed the spermatogenesis in rats. Anatomical studies helped to understand that the spermatids, cross sectional area of Sertoli cells and Leydig cells drastically reduced. Fertility tests conducted on male rats proved the anti-fertility effect of *B. prionitis* (39, 40). In northern Thailand folk medicine, *Barleria* species are used in anti-fertility and when a study conducted on wistar male rats proved that *B. strigosa* played significant role in inducing hormonal imbalance and ultimately reducing the sperm count and making rats infertile (41). Different fractions of crude methanol extract from *B. prionitis* root were eluted with 3:1 and 1:1 concentrations of trichloromethane with methanol. The fertility ability of the treated individuals decreased rapidly and the parameters like sperm motility, spermatogenic cells count and biochemical parameters like total protein, sialic acid and glycogen content were drastically changed in treated rats. Fraction eluted with 1:1 ratio of trichloromethane with methanol showed best results (42).

**Anti-inflammatory activity**

*B. lupulina* is used as anti-inflammatory agent and its extracts are used as antidote for snake poision (43). Methanolic extracts of leaves and shoots of *B. lupulina* showed best anti-inflammatory activity against carrageenan and serotonin induced paw oedema and the extract showed results which are equal to that of indomethacin, which is a standard drug at 300 mg/kg concentration which is ideal (44). Another study was carried out on different animal models having carrageenan induced paw oedema and ethyl phenylpropionate induced ear oedema. Myeloperoxidase activity (for neutrophil migration), trypan blue exclusion and MTT cytotoxicity assays demonstrates that methanolic extracts from *B. lupulina* showed best anti-inflammatory activities (45). Alkyl catechols, which are present in hot aqueous extracts of *B. lupulina* are found to be playing an important role in activating Nrf2 defence pathway which ultimately helps the organism in overcoming the inflammation (17). The pharmacologically active components having iridoid glucosides in the aqueous fraction (TAF) of *B. prionitis* showed best results with respect to anti-inflammation. The study was carried on different sets of rats which have induced the inflammation using carrageenan, histamine and dextran. Treatment of these effected rats with TAF fraction showed comparable results with respect to standard drugs like Ibuprofen. Leucocyte migration inhibition assay also suggested that TAF fraction of *B. prionitis* taken from methanol extract showed best results (46). Membrane stabilization activity and mast cell protection activity is studied after the administration of hydro alcoholic extracts of *B. prionitis* in rats. It is seen that ethanolic extract at 10 µg/ml concentration showed best results with respect to membrane stabilization and it is comparable to that of indomethacin, standard drug at 10 µg/ml concentration. The percentage of degranulation of mast cells is monitored and it is seen that 10 µg/ml concentration showed best results against the toxicant compound 48/80 (47). Carrageenan induced paw oedema model is established and effect of the anti-inflammatory activity of methanolic extract from *B. prionitis* is estimated. It is seen that methanolic extract at 500 mg/kg concentration showed best efficiency in reducing the inflammation (48). 8-O-acetyl shanzhiside methyl ester, shanzhiside methyl ester rich component from *B. prionitis* showed inhibition against myeloperoxidase, matrix metalloproteinase-9 and elastase enzymes and release of cytokines which prove the anti-inflammatory activity (49).

The effect of *B. cristata* leaf extracts on carrageenan induced paw oedema, acetic acid induced vascular permeability and castor oil induced diarrhoea was studied and it was seen that 500 mg/kg concentration of the extract showed best results in...
inhibiting the oedema, inhibited the vascular permeability. Indomethacin is used as a standard drug at 10 mg/kg in all studies (50). Leaves of B. cuspitate are studied for their wound healing property. The methanolic fractions at 10% w/w and 15%w/w concentrations were used for the study. It was seen that these fractions showed best results with respect to wound healing (i.e., decrease in the wound length) and comparable to that of the nitrofurazone which is the standard drug (51). Formalin at the concentration of 0.1 mg/kg is administered to the albino Wistar rats to induce the inflammation. It is seen that leaf ethanol extracts from B. montana at 300 mg/kg body weight concentration reduced the inflammation. The blood biochemistry parameters like levels of haemoglobin, red and white blood cells were also taken into consideration and the efficiency is compared with indomethacin at 25 mg/kg body weight concentration (52).

Neuroprotectivity activity

Methanolic extracts of B. lupulina are studied for their neuro-pharmacological activity in mice, which are induced by propylene glycol. General behaviour tests for sound response, touch and pain responses are conducted on different sets of mice and rats. Along with these muscle relaxant tests like traction test, rotarod performance tests, 30° inclined tests are conducted. It is seen that in all the above tests conducted, on overall B. lupulina extracts show promising pharmacological activity when compared to standard neuroleptics (53).

Anti-oxidant activity

DPPH radial scavenging activity suggest that the availability of the anti-oxidant molecules are present in the methanolic extracts of whole B. prionitis and from the leaf extracts of B. greenii, as they showed ideal half maximal effective concentration. The efficiency is compared with that of ascorbic acid which is a strong anti-oxidant (54). The antioxidant property of the ethanolic extracts from B. prionitis helped in reversing the cataract which is induced with the help of galactose and selenite at 25 moles/kg body weight concentration. Ethanolic extract at 200 mg/kg and 400 mg/kg body weight concentration showed best results in reducing the oxidative stress (55). Ferric thiocyanate method and thiobarbituric acid methods also suggest that the ethanolic extract from B. prionitis have promising anti-oxidant activity (56). Ferrous reducing power, DPPH radial scavenging activity, nitric oxide radical scavenging activity, hydrogen peroxide radical scavenging activity, superoxide anion scavenging activity and ABTS’ radical cation decorolourisation assay were conducted on leaf and root extracts of B. noctiflora and root extracts showed best results and performed best anti-oxidant activity (57). The ethanolic extracts from B. cristata were checked for their anti-oxidant activity. DPPH assay, ABTS’ assay and FRAP assay were conducted to check the efficiency of the ethanolic extracts. It is seen that ethanolic extracts showed best results in all conducted assays and it is compared with that of butylated hydroxytoluene (BHT) which is a standard anti-oxidant (58). DPPH radical scavenging assay, nitric oxide radical scavenging assay was conducted on ethanolic extracts of B. gibsonii. The results suggest that the presence of pharmacologically significant anti-oxidant molecules which showed their efficiency in scavenging the DPPH radicals which was compared with standard anti-oxidant molecule, ascorbic acid. And scavenging of nitric oxide radicals from sodium nitroprusside were also seen which ultimately proved their promising anti-oxidant activity (59).

Immuno-protective activity

Immuno-protectivity of the methanolic extracts from B. lupulina was proved with the help of checking the parameters like WBC count, spleen WBC count, spleen weight and delayed type hypersensitivity reaction on footpad thickness. It is seen that 600 mg/kg concentration of the extract showed good results along with 300 mg/kg (60). Oral administration of the iridoid fraction of the methanolic extract from B. prionitis triggered the immune response in the rats. Different tests like neutrophil adhesion test, haemaglutinating antibody titre test, delayed type hypersensitivity response test, cyclophosphamide-induced immunosuppression test and macrophage phagocytosis by carbon clearance test were conducted to check the efficiency of the extracts. It is proved that shanzhiside methyl ester and barlerin rich iridoid component helps in immunomodulation (61).

Anti-cancer activity

The cytotoxicity effect of the Barleria quinones (Barleriaquinone-I, Barleriaquinone-II) isolated from B. buxifolia roots are studied and it is seen that Barleriaquinone-II showed best LD₅₀ when compared to Barleriaquinone-I in human breast adenocarcinoma cells (MC77) (62). The anti-cancer activity of the methanolic extracts from B. grandifolia were studied against cancer cell lines i.e., A- 549 (human lung cancer) cells, Dalton’s lymphoma ascites. Methanolic extracts showed best half-maximal inhibitory concentration against dalton’s lymphoma ascites with the value 137.2 µg/ml. Various parameters like tumor weight, viable cell count, tumor volume were taken into consideration and the methanolic extracts showed best anti-tumour activity (63).

Anti-arthritic activity

B. lupulina from the ancient days is used for treating arthritis. Scientifically it is proved that methanolic extracts from the leaves showed promising activity against arthritis. Study was carried out on different animal models of induced arthritis like formalin-induced arthritis, collagen type II-induced arthritis, monosodium iodoacetate induced osteoarthritis. It is seen that at 300 mg/kg and 600 mg/kg concentrations of the administered extracts showed comparable promising anti-arthritic activity with that of indomethacin at 10 mg/kg concentration (60). Artificially arthritis is induced using chemicals like formaldehyde and friend’s adjuvant (FCA) in the sprague-dawley rats. It is seen that ethanolic extracts of B. prionitis leaves showed decreased arthritis score and paw volume which was increased as a sign of
arthritits. Ethanolic extract of concentration 250 mg/kg showed best results with respect to all tests conducted. The efficiency of anti-arthritic activity is checked with the standard drug, diclofenac at 4 mg/kg body weight concentration (64).

Anti-Ulcer activity
Aerial parts (Leaves, shoot regions) of B. lupulina were subjected to methanolic extraction and their anti-ulcer properties are studied. It is seen that in pylorus ligated rats the methanolic extract of B. lupulina at 200 mg/kg concentration showed promising anti-ulcer activity and the results are comparable with famotidine at 30 mg/kg which is the standard anti-ulcer drug. The effect of B. lupulina extracts on different animal models where ulcer is induced using stress, alcohol and Indomethacin. It is seen that methanolic extracts of B. lupulina showed best results in alcohol induced stress even when compared with famotidine and omeprazole (65). Aspirin at 200 mg/kg concentration, ethanol at 1 ml/200 mg concentration with respect to body weight is administered to induce ulcer in rats. Iridoid fraction from B. prionitis at 200 mg/kg body weight concentration showed best efficiency in reducing the ulcers. Cold-restraint stress and pylorus ligated induced ulcer models were also developed and the activity of the iridoid fraction on them is checked. Many parameters like acid output, pH value and gastric juice amount were taken into consideration. It is concluded that B. prionitis extracts which are rich in iridoid fraction have promising anti-ulcer activity (66). In pylorus ligated induced ulcer, the efficiency of ethanolic extracts from B. gibsoni was checked for its anti-ulcer property. It is seen that acidity levels and ulcer lesions got reduced upon the administration of 500 mg/ml concentration of the extracts and its capacity to reduce ulcer lesions is compared with omeprazole at 20 mg/ml concentration.

Anti-diabetic activity
In the streptozotocin induced diabetic rats the anti-diabetic activity of the methanolic extracts of B. lupulina is studied and it was found that at 300 mg/kg body weight concentration showed decreased amount of glucose content in the blood and showed comparing efficiency with Glibenclamide at 10 mg/kg body weight concentration (67). In another study, animals were induced with alloxan monohydrate at 150 mg/kg concentration and ethanolic extracts of B. prionitis were administered. It is seen that ethanolic leaf extracts showed potent anti-diabetic activity and showed the reduced glucose levels by increasing the serum insulin levels when compared to root extracts (68). Ethyl acetate fraction from the ethanolic extract of B. noctiflora showed promising in-vitro and in-vivo anti-diabetic activity. The anti-diabetic property was compared with glibenclamide at 10 mg/kg. The ethyl acetate fraction showed reduced glucose levels in the blood which was isolated from high fat diet fed and streptozotocin induced diabetic rats at 40 mg/kg body weight concentration (69). The ability of inhibiting the α-amylase and α-glucosidase enzymes by ethanolic and petroleum ether extracts of B. cristata leaves proved its anti-diabetic activity. It is seen that 100 µl concentration of both extracts showed promising efficiency (70).

Hepatoprotectivity
In B. prionitis its iridoid rich fraction was used to study the hepatoprotectivity. The study is carried out on different rat models which are liver toxicated with drugs like carbon tetrachloride (100 µl/kg), galactosamine (300 mg/kg) and paracetamol (200 mg/kg). The median lethal dose of the drug in oral administration and intraperitoneal administration is studied. Serum parameters (alkaline phosphatase, alanine transaminase, aspartate transaminase) and hepatic parameters (lipid peroxidation) are taken into consideration for the study and this rich fraction of B. prionitis is proved to have hepatoprotective activity (71). The methanolic extracts of B. montana leaves were administered in the liver damaged rats and the serum enzyme parameters were analysed. It is seen that methanolic extracts at 500 mg/kg concentration successfully inhibited the enzyme levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) and the efficiency is compared with silymarin which is standard drug against liver disorders. Anatomical studies at tissue level also suggested that B. montana has capacity to repair the damaged liver tissues (72). Another study was carried out on liver damaged animal model which is caused with the help of carbon tetrachloride. It is seen that methanolic extracts from the aerial parts of B. montana at the concentration of 400 mg/kg and 800 mg/kg reduced the serum sensitive enzymes levels for the liver damage i.e., serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase (73).

Anti-bacterial property
Compounds like Balarenone, pataline, 13,14-seco-stigmasta-5,14-diene-3-α-ol which were isolated from the crude extract of B. prionitis showed anti-bacterial property especially against Bacillus cereus (ATCC 14579) and Pseudomonas aeruginosa (ATCC 27853) (9). Different types of plant extracts using soxhlet apparatus were developed from the dried leaves of B. prionitis; among them ethanolic extract at 10 mg/ml showed best anti-bacterial activity against Staphylococcus aureus followed by Salmonella typhi, Vibrio cholera, Escherichia coli (74). Different fractional extracts made out of hexane and ethyl acetate from B. prionitis showed anti-bacterial activity (75). Different solvent extracts like methanol, ethanol, chloroform, di-chloromethane, acetone and water of B. montana Nees are studied for their anti-bacterial property. Anti-bacterial study is carried out on species like Bacillus subtilis, Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus vulgaris. It is seen that acetone extracts showed best anti-bacterial property in the decreasing order against Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi. The efficiency is compared with the standard drug streptomycin (76). The efficiency of the methanolic extracts of B. montana at 100 mg/ml and 200 mg/ml concentration were tried on different species of
gram-positive bacteria like Bacillus subtilis, B. cereus, B. pumilis, Staphylococcus aureus and gram-negative bacteria like Escherichia coli, Psuedomonas aeruginosa, P. vulgaris, Serratia marcescans. It is seen that the efficiency was moderate and that is compared with 10 µg/ml chloramphenicol, which is the standard drug (73). The essential oil extracted from B. lupulina having many important phytochemicals like cyclobutane,1,1-dimethyl-2-octyl, 1-Henitetracontanol, 1,2-Benzenedicarboxylic acid, diisooctyl ester, 2-Hexyl-1-octanol showed its anti-bacterial affect against the species like Bacillus pumilus and Staphylococcus aureus (20).

**Anti-fungal activity**

Minimum inhibitory concentration and minimum fungicidal concentrations of the extracts of petroleum ether, dichloromethane from B. albobestellata showed best results against Candida albicans (ATCC 10231). In the same way the ethanolic extracts of B. greenii leaves showed highest minimum inhibitory dilution and proved its fungicidal activity and that efficiency is similar to that of its root and stem which ultimately helps the harvesters to go with plant part substitution to replace the less available parts (54). Methanolic extract of B. montana at concentration of 100 mg/ml and 200 mg/ml showed moderate anti-fungal activity against fungal species like Aspergillus niger, Rhizopus stolonifer, Sacharomyces cerevisiae and Penicillium chrysogenum. The efficiency is compared with that of 10 µg/ml nystatin (73). Hot water extracts from the aerial parts of B. grandiflora showed promising anti-fungal activity against A. fumigatus. It is seen that the extracts down regulated the metabolic pathways which are very much important for the survival and pathogenicity of the A. fumigatus (77). Natural dye extracted from aerial parts of B. prionitis showed anti-fungal activity against species like A. flavus, A. niger, A. parasiticus, Fusarium moniliforme and Penicillium canescens. The minimum inhibitory concentration of the natural dye against the mentioned fungal species ranges like 23.25, 22.75, 23.5, 22.5 and 23 µg/ml. The fabrics which are made out of this natural dye is recommended for textile purposes (78).

**Anti-diarrhoecal activity**

Iridoid glycoside component isolated from B. prionitis was studied for its anti-diarrhoeal activity. Different tests like PGE2-induced enteropooling, charcoal transit test was conducted and it is seen that iridoid component successfully inhibited the enteropooling and fluid transition. 100 mg/kg concentration of the extract inhibited the castor oil induced diarrhoea and the efficiency is compared with loperamide, which is a standard drug (79).

**Anti-hypersensitive activity**

Methanolic extract of the leaves of B. prionitis is employed for studying the anti-hypersensitive activity. Hypersensivity is induced in the rat with the help of Deoxycorticosterone acetate (DOCA) salt at the concentration of 20 mg/kg body weight. It was observed that methanolic extract at 400 mg/kg showed best results in reducing hypertension (80).

**Marketed medicinal formulations from Barleria spp.**

Barleria species are used in formulation of herbal toothpastes. Vicco vajradanti (81) is a herbal toothpaste made from B. prionitis, which have capacity to reduce the salivary glucose and pH. It is also seen as a chief ingredient in Patanjali Dantkanti (82). B. prionitis is the chief component in Sahacharakashayam, an ayurvedic polyherbal formulation which is used as an anti-oxidant (83). Whole plant is of B. prionitis is used in the polyherbal formulation of Maharasnadikwath. This liquid extract showed great anti-arthritic potential with anti-oxidant activity (84).

**Tissue culture Studies in Barleria spp.**

Biotechnological approach helped the mankind to solve his problems in effective manner. Tissue culture is one of such biotechnological tool which helped to conserve the medicinal plant species and their medicinally important metabolites. Barleria species being threatened in the wild, there is an immense requirement for the tissue culture technique for filling the gap in the demand for the cell lines and the raw materials. The information regarding the in-vitro development of Barleria is very much limited.

It is seen that when nodal explants of B. prionitis cultured in the nutrient medium having 0.4 ppm thidiazuron (TDZ) along with 1.5 ppm BAP showed best results in inducing the shoot development and the subculturing multiplication media having 5 ppm 6-benzylaminopurine (BAP) along with 2 ppm α-naphthaleneacetic acid (NAA) showed good proliferation capacity (85). Another study also revealed that nodal explants were grown on the MS media supplemented with 1.5 ppm BAP with 0.4 ppm TDZ showed highest number of shoots and highest mean shoot length (86). Nodal explants having axillary buds were used as explants for the shoot induction and bud break in B. prionitis. It is seen that MS media supplemented with BAP at 8.88 μM concentration showed best results. It is seen that BAP at 4.44 μM concentration showed best multiplication efficiency (87). BAP at 1.5 ppm along with NAA at 1 ppm concentration showed best response in the development of shoot from the shoot tip explant in B. prionitis (88). MS media supplemented with 1 ppm BAP and 0.5 ppm TDZ showed best results with respect to the development of shoots from the nodal explants of B. prionitis (89).

Development of callus is important task and it helps in the establishment of cell cultures. Callus was developed from different explants of B. prionitis. 0.0125 ppm of 2,4-D along with Kinetin (KN) showed good results in development of callus from leaf explant and at 0.025 ppm of 2,4-D along with KN showed good results in development of callus from shoot explants (90). MS medium supplemented with 2 ppm of IAA along with BAP showed best results with respect to the leaf explants of B. lupulina Lindl. and developed callus was also screened for their potential medicinal properties (91). Callus was developed from the leaf explants which were cultured on the MS medium having 2 ppm of IAA along with 1.5 ppm of
BAP (92). Compact green callus was developed from the leaf explants of Barleria lupulina Lindl. It is seen that MS media having 0.1 ppm NAA along with 0.5 ppm BAP showed best response (93).

In Barleria prionitis rooting efficiency was checked under the influence of different auxin concentrations. It is seen that Indole butyric acid at 0.4 ppm concentration showed best root inducing capacity of the in-vitro grown shoots (86). Half strength MS media supplemented with 2.46 μm Indole butyric acid (IBA) showed good response in rooting with good mean number and the length (87). 0.5 ppm of IBA supplemented in the half strength MS media showed best results in development of roots from the in-vitro grown micro shoots of Barleria prionitis (89).

Conclusion
Biotechnological studies reveal that Barleria L. have potential drugs which help in treating chronic illness. Scientifically there are many reports which support its requirement in curing many diseases which are chronic in nature. The phytochemicals present helps to overcome different health issues like inflammation, microbial infections, diabetes, hepatic tissue related issues, cancer, arthritis, immune deficiency etc. Most importantly the metabolites from Barleria are used in creating the organism in fertile. So, consuming the crude extracts without proper dose and doctor concern may lead to cause different fertility related issues. So, with the present scientific evidence it is the responsibility of the scientific community to explore further medicinally important metabolites and use them for the benefit of the humankind.

Acknowledgements
Authors are thankful to the Head of the institution, CHRIST (Deemed to be University), Bengaluru for all the encouragement and support extended for carrying out the research work.

Authors’ contributions
PN conceived the idea and edited the manuscript. SWN gathered the data and wrote the manuscript.

Conflict of interests
The authors declare that there is no conflict of interest.

Supplementary files
Table 1. Some important phytochemicals present in different species of Barleria.

Fig. 1. Molecular structure of important metabolites in the genus Barleria.

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