PHYTOCHEMICAL, ETHNOMEDICINAL AND PHARMACOLOGICAL OVERVIEW OF *Ajuga bracteosa* Wall. Ex. Benth : AN ENDENGERED MEDICINAL PLANT OF UTTRAKHAND, INDIA

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ABSTRACT

For live a happy and healthy life, we need medicinal plants. Ajuga has nearly 300 species. *Ajuga bracteosa* Wall. Ex Benth (*A. bracteosa*) is a Himalayan medicinal plant. Its medicinal potential comes from neo-clerodane diterpenoids, flavonol glycosides, iridoid glycosides, ergosterol-5,8-endoperoxide, and phytoecdysones. The purpose of this article aimed to compile existing literature on *A. bracteosa*. This review article aimed to raise awareness of therapeutic potential of the plant. Updated information on medicinal plant secondary metabolite production in vitro for pharmaceuticals is included in this review, as well as current information on botanical secondary metabolite production in vitro for pharmaceuticals. This species is critically endangered due to its medicinal potential and commercialization. Conservation and management measures should be implemented to save this endangered species. The current review focused on its phytochemical, traditional and scientific uses along with its recent biotechnological advances for its preservation.

Key Words: *Ajuga bracteosa* Phytochemistry, Ethnopharmacology, Pharmacology, Conservation

INTRODUCTION

Since ancient times, medicinal plants have been utilized to treat ailments. These plants are as old as mankind. To improve global health, medicinal plants are used to treat a wide range of ailments. Despite modern medicine advancements, people still rely on plants for health. Plants are responsible for over a quarter of all contemporary medicines, either directly or indirectly. Medicinal plants can be found all around the world, but they are more prevalent in the tropics. According to the WHO human population size has grown enormously over the last hundred years. This means increases in demand for food, water, home, electricity, roads, automobiles and numerous other commodities. These demands are exerting tremendous pressure on our natural resources. To meet this demand many significant plants are threatened/endangered with extinction. The loss of biodiversity actually constraint and counteract economic development. The entire ecosystem can be protected either by in situ or ex situ conservation of endangered species. Surveying, documenting, and mapping biodiversity are widely acknowledged as urgent tasks for plant conservation and sustainable use. In this review we tried to updated the knowledge and documented the distribution, phytochemistry, ethnomedicinal properties, pharmacognosy, pharmacology, in-situ and ex-situ conservation techniques of one such nearly endangered plant *Ajuga bracteosa* (Figure 1 a & b) belonging to the family Lamiaceae. It is a perennial herb that grows abundant in Himalayan province of India and Nepal [1,2]. In the temperate and subtropical parts of the world, it thrives on grassland, exposed slopes, and open fields at elevations ranging from 1200 to 2500 meters [3]. It has great medicinal potential due to its diverse active ingredients [4]. The biological significance, challenges to conservation, and opportunities for the plant were all discussed by the author Mubashir Hussain et al., [5]. But with the morphological and pharmacological studies, greater attention has been placed on phytochemical aspects in the current study. Along with its numerous current conservation approaches for its protection, the current review also emphasised the use of plants as cytotoxic, antimutagenic, antibiotic, and parasite agents.

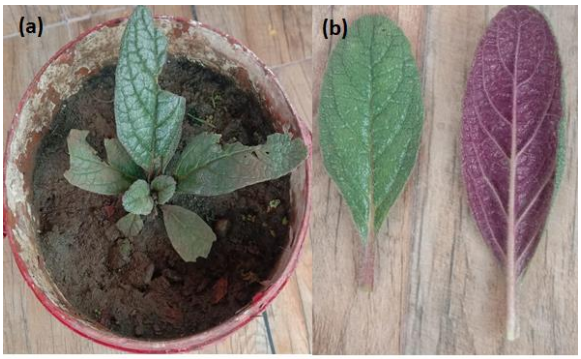


Figure 1(a) *Ajuga bracteosa* plant (b) *Ajuga bracteosa* leaf

Taxonomical Status and vernacular Name:

A. bracteosa comes to the kingdom Planate of the Tracheophyta division, the Magnoliopsida class, the Lamiales order, and the Lamiaceae family. *A. bracteosa* is known by many different names. It's called "Bungle" in English, "Nilkanthi" in Sanskrit, and "Jan-i-adam" in Kashmiri, Kauri booti in Urdu [6].

Morphological description: It is an aromatic medicinal, villous, soft, and decumbent herb that grows 15–30 cm tall [7]. It is perennial evergreen plant with prolixly branching stems that stay flattened. The flowers are yellowish with axillary spirals. It has a woody rootstock, leaves that can grow to be up to 8.5 cm 3.5 cm in length and are usually much smaller with a more crenate to lobed margin, calyces that are 3.5–4.5 mm in length.

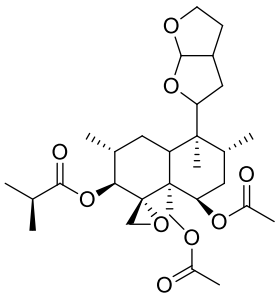
Active Phyto constituents:

Compounds produced by *A. bracteosa* have a variety of medicinal effects. Flavonoids, saponins, phenols, tannins, terpenoids, xanthoproteins, glycosides, and other compounds are among them. According to Zahra et al., ethanol extract had the highest level of flavonoid concentration while chloroform-methanol extract had the highest level of radical scavenging ability. Polyphenols like pyrocatechol, gallic acid, resorcinol, catechin, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, vanillic acid, coumarin, sinapinic acid, trans-cinnamic acid, rutin, and kaempferol were confirmed using RP-HPLC-based quantification [8].

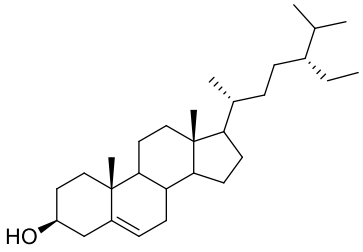
According to Viljoen et al., 6-deoxyharpagide and raptoside are iridoid glycosides present in the plant [9]. These compounds are optically active cyclopentonoids monoterpenes and could be used for defence action [10].

According to Rubnawaz et al., transgenic *Ajuga bracteosa* has a rich phenolic content and is hence a superior choice for phenolic-guided pharmacological activities [11]. According to studies, the constituent 20-hydroxyecdysone is present but its concentration varies depending on where it is found due to the action of various exogenous factors. One such exogenous factor, cold temperature, is ideally suited for consistent 20-hydroxyecdysone synthesis. Studies have also suggested that this steroid might also have therapeutic benefits for a number

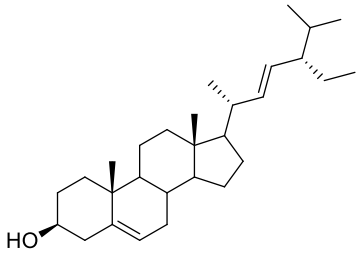
of respiratory illnesses as well as cardiometabolic and neuromuscular problems. According to M Iqbal et al lactone steroids withanoloide, which serve as cholinesterase inhibitors, is also present in the plant [12]. Dichloromethane extract of whole plant of A. bracteosa produced a variety of clerodane and neoclerodane diterpenoids. Neoclerodane diterpenoids have been shown to be effective as an anti-bacterial in tests [13,14]. As per report analysis the antimicrobial activity and insect anti-feedent activity can also be correlate with such diterpenoids [15,16]. Narendra Singh et al in the year 2006 isolated and identify phthalic acid ester from nonpolar hexane extract of the whole plant [17]. There are several other biologically active compounds (Figure2) were isolated and identified from the methanol extract of aerial part of Ajuga which are showing anti-mutagenic activity [1].



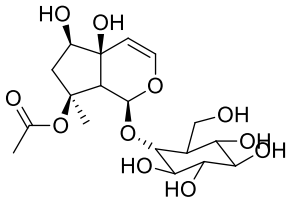
14, 15-Dihydroajuapitin



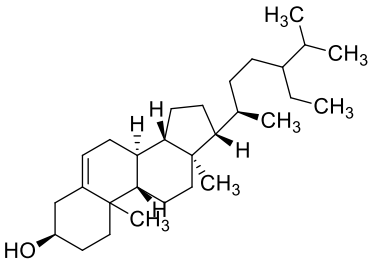
B-Sitosterol



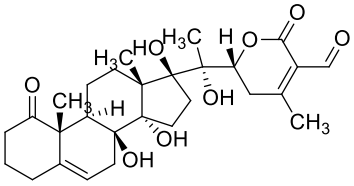
Stigmasterol



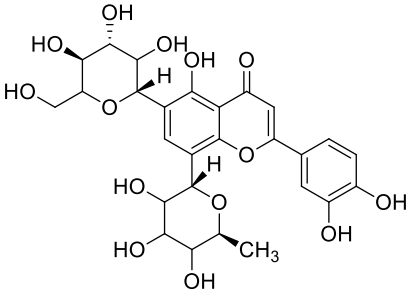
8-O-Acetylharpagide



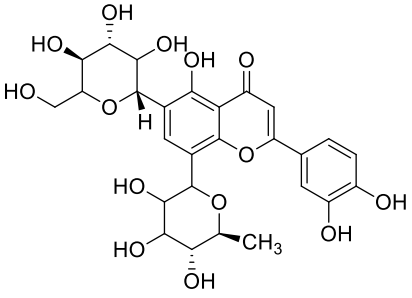
Arjunin D



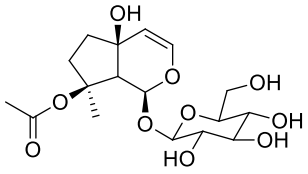
Argugin C



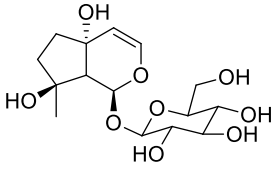
Carotinoid



Falvonoid



Reptoside



6-Deoxyhapagide

105

106 **Figure 2: Phytochemicals of *A. bracteosa***

Pharmacology

A. bracteosa has been evaluated exhaustively both in vitro and in vivo for diverse therapeutic claims. Previous studies explored that *Ajuga bracteosa* possessed numerous pharmacological activities due to its composition of secondary metabolite like diterpenoid, steroids and flavonoids etc. Studies on the therapeutic profile of these secondary metabolites focused on their immune system, anti-insect, anti-carcinogenic, anti-inflammatory, anti-arthritic, anti-malarial, and anti-carcinogenic properties (Figure 3).

Previous research on *A. bracteosa* found that it inhibited the enzymes like lipoxygenase, acetyl cholinesterase, and butyrylcholinesterase [17]. Phytoconstituents isolated form the plant have very lesser side effects as compared to synthetic drug [18].

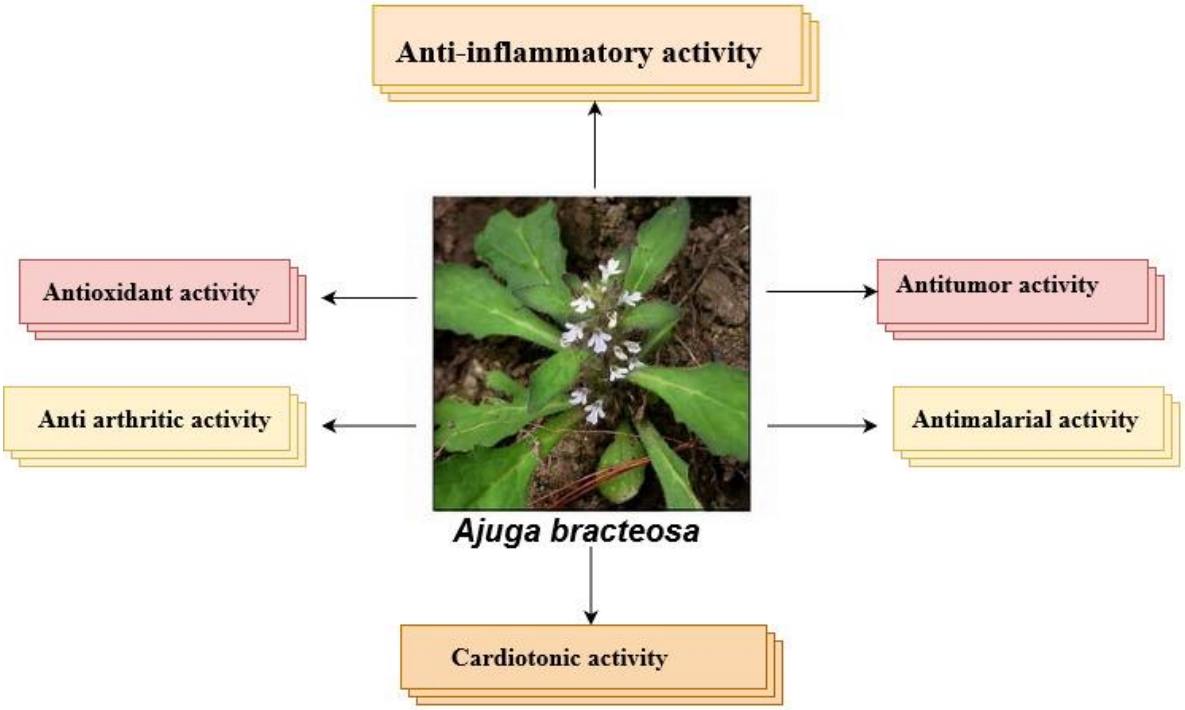


Figure 3: Pharmacological activity reported in the *A. bracteosa*

Antitumor/anticancer/antimutagenic activity: Cancer is still one of the world's deadliest disease, and India is no exception. Plants have long been key sources of effective anticancer medicines accounting for more than 60% of all currently used anticancer drugs. Cragg et al., 2005 & Newmanet al., 2003 Pal et al studied the various extracts of Ajuga for its in-vitro cytotoxicity activity [19,20]. In this experiment the author used the tumor cell lines MCF-7 and Hep-2. The methanol extract showed the maximum anticancer activity rather than its petroleum ether and aqueous extract. Whereas the traditional approaches support its decoction as anticancer potential [21]. The potentiality of the methanol extract is further evaluated for its antimutagenic activity. In-vivo antimutagenic study was done in mice model induced by Ethylmethanesulfonate. There are several compounds were isolated and quantified by HPLC method. Among those, compound 14,15-dihydroajugapitin showed maximum reduction (85%) of micronuclei followed by the compound β- Sitosterol and 8-O-acetylharpagide [17].

Antimalarial activity:

Malaria is a terrible disease that is currently being treated and controlled by using a variety of plants. The emergence of insecticide-resistant mosquito vectors and drug-resistant parasites has made malaria control increasingly challenging. These are important steps toward making herbal medicines more accessible and consistent. The characterization of phytochemical substances lays the groundwork for the creation of new ones. Ethanol leaf extract inhibit content of parasite in blood in BALB/c mice model and mean survival time is increased in dose dependent manner [22]. Apart from this studies several other studies also reported for anti-parasitic activity against *Leishmania tropica.* In the same study the author also reveals the maximum potentiality with n-hexane extract along with its insecticidal, anti-alzhiemer activity [23].

Anti-inflammatory activity: Inflammation though it is part of the body's defense mechanism but it includes a vast array of disorders and conditions that are characterized by inflammation including allergic reaction to autoimmune disorders or else any visceral organ inflammation or even in case of graft rejection. 70% alcoholic extract of *Ajuga bracteosa*

showed anti-inflammatory activity by the inhibition of Cyclooxygenase-I and Cyclooxygenase-2. The finding proved the active phytoconstituents (lupulin A, ajugarin I, deoxyharpagide withaferin A, and reptoside) might be responsible for anti-inflammatory property. Such investigation also support the folk uses of *Ajuga bracteosa* for inflammatory disease [24]. The anti-inflammatory activity may also be due to the presence of iridoids glycosides and the mechanism might be through COX-2 inhibition.

Analgesic activity: Significant and dose dependent analgesic activity was evaluated using the acetic acid induced writhing inhibition and tail method on mice. The mechanism of action assumed to inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues [25]. The anti-nociceptive activity is also supported by Khanavi et al [26]. which in further concrete the use of the plant as anantiarthitc and any other inflammation diseases.

Cardioprotective activity: As per WHO cardiovascular diseases are becoming critical issues leading to death (globally 31%). One cannot put finger in one reason. Cardiovascular disease (CVD) is a class of diseases that associated with either heart or blood vessels or both. CVD includes coronary artery diseases (angina, myocardial infraction), stroke, hypertensive heart disease, cardiac myopathy, cardiac arrhythmia, congestive heart failure etc. The underlying multiple mechanisms vary depending upon the state of the disease. Atherosclerosis is one of the main reason for Coronary artery disease, stroke and peripheral artery disease. This may be caused by hypertension, obesity, high blood cholesterol, diabetes etc. On the frog heart and rat ventricle, an alkaloid fraction of Ajuga bracteosa demonstrated cardio stimulant activity. The bioactivity was inhibited by dichloroisoprenaline which did not occur in reserpine-treated heart [27]. Report also supported the activation of mas 1 receptor 20-hydroxy which may contribute to such activity. Antioxidant and antiinflamatory & antihypertensive action of the drug could also be a strong reason for cardioprotective activity. Shaukat et al further confirm the antihypertensive efficacy which strengthens the cardio-protective activity of the plant [28]. Oral administration of the plant's aqueous extract and coumarin exhibits a substantial antihypertensive effect. IL-6 and TNF- serum concentrations also sharply declined. The phytochemicals' antihypertensive action was identified via molecular docking research.

Antioxidant activity: ROS assault and cause oxidative damage to a variety of biomolecules, including DNA. In chronic disorders such as diabetes, cerebrovascular disease, rheumatism, cancer, and cardiovascular disease, this damage is critical [29]. Current therapeutic procedures are known to cause acute immune reactions and cytotoxicity in normal cells. Antioxidants are necessary for the prevention of chronic disorders. Antioxidants are substances that help to prevent and minimize oxidation. Antioxidants can protect cells from free radical-induced oxidative damage. They are used to treat heart disease, cancer, arteriosclerosis, cerebrovascular diseases, and other illnesses [30]. A wide range of medicinal species contain antioxidant chemicals known for their free radical scavenging abilities. Antioxidant activity may be mediated by phenolic compounds. There is a strong relationship between antioxidant activity and phenolic chemicals produced in plants [31]. Antioxidants can scavenge reactive oxygen species and hence may be advantageous in the prophylaxis and treatment of diseases such as alzheimer's disease, stroke, diabetes, cancer, inflammation, and arteriosclerosis [32, 33]. The antioxidant activity of *A. bracteosa* oil was found to be 78 percent, which is higher than ascorbic acid's strong antioxidant activity [34]. The antioxidant activity of the oils was assessed using the 2,2-Diphenyl-1-picrylhydrazl stable free radical as a standard. Aerial and root parts of the plant was also reported for flavonoid and phenolic contents. Studies supported for its antioxidant activity of methanol extract of the plant. The author also revealed the anti-inflammatory, analgesic, antidepressant and anticoagulant activities of the extract [35]. Rehman *et al* also supported the antioxidant activity of the plants [36].

Antiarthritic activity: There are several studies that supported the relationship of total phenolics and total flavonoid with anti-arthritic, anti-inflammatory, antioxidant activity. The anti-arthritic effect of Ajuga bracteosa showed the inhibition of cyclooxygenase –I and cyclooxygenase-II. The isolated active compounds, 6-deoxyharpagide, withaferin A, lupulin A, reptoside, and ajugarin I, are responsible for antiarthritic effects [24].

Others activity: Methanol extract of *A. bracteosa* also shows activity against Hepatitis C Virus [37]. As per the report transgenic regenerants of *A. bracteosa* reported for in vitro antibacterial, antihemolytic, cytotoxic, anticancer, and leishmanial activity [38]. Sadia

Nazer et al also supported the synergistic antibacterial activity of the plant when formulated as Silver Nanoparticles [39]. Antibacterial activity is also supported by Khaista Rahman et al [40]. The author Vohra et al supported the volatile oil constituents obtained from leaf as antimicrobial against *Staphylococcus aureas, E. coli* etc [41]. The author also claimed the presence of Limonene, α-humulene, β-Myrcene, Elemol, Camphene, β-Caryophellene, α-phellendrene by gas chromatography might be act as antimicrobial. Kokab Hafeez et al reported α-glucosidase inhibitory activities of several nonpolar and polar extracts of *A. bracteosa*. The author postulated such α-glucosidase inhibitory activity may also useful as hypoglycemic agents in the management of postprandial hyperglycemia [42].

Relationship between secondary metabolites and Therapeutic uses:

Secondary metabolites from plants are recognized as unique sources for drugs, flavours, food additives, and other commercial components [43]. Polyphenols are the most potent inherent antioxidants among secondary metabolites [44]. Flavonoids and phenolics have anti-carcinogenic, anti-aging, antioxidant, and protective qualities against brain dysfunctions such as Huntington's disease, Parkinson's disease, Alzheimer's disease, immune/autoimmune, and cardiovascular illnesses [45]. Since the plant being studied is an important and endangered species too, plant tissue culture or other in-vitro techniques will be considered urgently necessary. Secondary metabolites derived from plants exhibit significant biological and pharmacological properties, including antioxidant and anti-carcinogenic properties. The biological activity of phenolic acids and flavonoids is proportional to their antioxidant capacity. Callus culture and cell suspension cultures are efficient methods for the synthesis of secondary metabolites with a variety of medicinal applications. To explore the synthesis and growth kinetics of medicinal compounds, cell suspension cultures are offered as a simple method for implementing and evaluating the most advantageous scheme for producing large amounts of medicinal compounds [46]. Light regimes are critical in all of a plant's core processes and building blocks, including primary and secondary metabolism, growth, and development [47]. Secondary metabolite production can be efficiently stimulated by optimizing in vitro conditions such as light regime. Numerous stimulatory effects of light regimes on secondary metabolite formation have been documented, including artemisinin,

anthocyanins, derivatives, and flavonoids [14]. Light is critical because it has both inhibitory and stimulatory effects on secondary metabolite synthesis.

Ethnomedicinal uses

Since ancient times, *Ajuga bracteosa* has been used in medicine for a variety of purposes. It is used in ethno medicine as an anthelmintic, astringent, antibacterial, antifungal, anti-inflammatory, hypoglycemic etc [48]. It is used to treat rheumatism, amenorrhea, gout, and palsy in Ayurveda [49]. In China, *A. bracteosa* is traditionally used to treat fever and phlegm [50]. The leaves of *A. bracteosa* are stimulant, diuretic, and are used locally to treat malaria [51]. Ahmad et al in the year 2014 reported the use of the plant leaves paste in headache [52]. The author also reported the that the whole plant traditionally uses in indigestion, abdominal pain etc. The author CP Khare reported multiples traditional uses [53] *viz*. diuretic, stimulant, fever, astringent, gout and rheumatism, amenorrhoea, aperient etc. The author also mentioned the use of juice of the leave as blood purifier, powders used in burns and boils. It was reported that the leaves can be used in fever as a substitute for cinchona. In an another report the plant showed traditional importance in jaundice and bites of insects [54]. Research also supported the use of root of *A. bracteosa* in the treatment of diarrhea, dysentery, and inflammatory disorders and decoction of leaves and bark in for cancer, sore throat, cough, pneumonia, and other respiratory issues [55, 56].

Conservation methodologies: Past and current

The IUCN Red List Database classifies that the species of *Ajuga bracteosea* is critically endangered or least concern based on density [57]. Medicine-valued species like *A. bracteosa* are under danger of extinction across the entire region. The wide range of uses for *A. bracteosa*, especially in pharmacology, warrants large-scale cultivation. Because *A. bracteosa* is listed as critically endangered, consistent efforts should be made to keep this plant species from becoming extinct. To maintain, a multidimensional approach is required, which includes genotype selection and ex-situ as well as in-situ conservation, followed by multiplication using both conventional and biotechnological methods, which could provide a solution to the existing problem.

For decades, wild plants have been collected for several purposes. Ex-situ management of wild plants has been ignored for many years [58]. The time of collection and a lack of awareness about its role in the species' management resulted in mismanagement. There are currently a number of impediments to the gathering, sustainable cultivation, and use of medicinal plants. These include a lack of clear resource and custodianship, as well as a lack of understanding of sustainable management parameters and market requirements [59]. As per International Union for Conservation of Nature's guidelines the possible reason for the plant to be endangered [60]. are species' population size, area of occupancy, extent of occurrence etc. There are several other threats like landslides and overexploitation by the local peoples are also considered as common operational threats.

Tissue culture as a source: Growing and multiplying plant cells, tissues, organs, seeds, or other plant parts in a controlled solid or liquid nutrient medium is known as tissue culture [61]. Plant tissue culture is a type of vegetative propagation used for large-scale plant production known as micro propagation [62]. Plant tissue culture technology has been used to make genetically uniform, disease-free, and huge amounts of plants since it was first thought up [63]. Somatic embryogenesis could be act as a new way to make synthetic seeds for such plants. This approach is very crucial and important for either medicinal plants that don't have seeds or else seeds are not good. Another approach of in vitro storage reported by Mishra et al is explants encapsulation in alginate to produce synthetic seeds could also be a better option for the plant *A. bracteosa* [64]. Biotechnology advancement not only provides alternative methods for in vitro preservation of tropical fruits and recalcitrant seeds, but also tools for disease-free germplasm conservation, lower labour costs, and disease-transfer limitation [65,66].

As *Ajuga bracteosa* is to be a highly important medicinal plant the majority of the natural population of the plant is currently under severe pressure due to high demand. This species is rapidly declining as a result of overexploitation. This herb is in high demand in the pharmaceutical industry at both the local and international levels. But the fact is that it is extremely endangered and, if it continues to be exploited at the current rate, will go extinct within the next few years. Therefore, long-term use of this incredibly healing species is

required to preserve for its numerous known uses. This species has received a lot of attention in the last decade. A multifaceted strategy is necessary for maintenance, which could offer a solution to the current issue. This strategy comprises the selection of higher-quality genotypes, as well as ex-situ and in-situ conservation, followed by multiplication utilizing both conventional and biotechnology means. Any medicinal plant's worth is based on the active components that are present in that species. Elite clone development would be desirable. Chemo-profiling and different molecular marker approaches can be used to find superior clones. Commercial plantations can be multiplied and grown for conservation using conventional propagation techniques as well as plant tissue culture procedures. To speed up the creation of favoured genotypes and commercial micro propagation, tissue culture can be employed as an alternative to traditional in vitro propagation techniques. Plant tissue culture techniques are now used for gene transfer, selection, and regeneration of transformants. The Cell suspension culture, in addition to in vitro propagation, is useful for large-scale secondary metabolite production. Another factor that influences plant quality is post-harvest handling. Herbal material collectors pay less attention to material quality during harvesting, handling, and storage. Mycotoxin-producing fungi have been discovered in herbal drug samples that have been stored. Cultivation practices must be addressed as well. Wild harvested plants vary in consistency and quality due to genetic and environmental differences. The efficacy of medicinal plants is also influenced by regional environmental conditions. Temperature, photoperiod, soil characteristics, and rainfall all have a significant impact on the production of active constituents. As a result, consistent efforts should be made at the community level to ensure the long-term management of medicinal plants. Shivanee et al reported that MS medium supplemented with IAA (2 mg/L) and BA (5 mg/L) induced 100 % shoot regeneration [65]. In this experiment leaf, petiole and root as an explants were selected. Leaf displayed quickest response followed by petiole while root was shown the slowest response. It was further experimentally proved that shoot induction is predominantly dependent on plant growth regulators added to the culture medium. Full- or half-strength Murashige and Skoog medium with or without auxin is used for in vitro rooting. An estimated survival rate of 82-100% was achieved when rooted shoots are acclimatized in the greenhouse [67]. Micro-propagation is a key technique used in our previous work [68] to conserve the plant. Leaf

explants in MS medium supplemented with Indole-3-acetic acid and Benzyladenine showed to be the optimum media for root and shoot regeneration.

Conclusion: *A. bracteosa* is a highly important medicinal plant of native to the Himalayas, but it is documented as critically endangered species. Because of its resistance to a variety of diseases, this plant has enormous potential. While significant progress has been made, further research is still required to identify and understand each of the isolated chemicals from *A. bracteosa* in order to validate and comprehend its and medical procedures. In terms of ethno medicine, it is a prominent plant species, and its significance places a lot of pressure on the plant in terms of its use. Its extinction was seriously threatened by this stress. Therefore, protecting this species is crucial, and ethical collecting practices are typically needed. In order to sustain, a multifaceted strategy that combines genotype selection, ex-situ and in-situ conservation, followed by multiplication utilizing both traditional and biotechnological means, is typically necessary. This could potentially offer a solution to the current issue.

Figure Captions

Figure 1(a) Ajuga bracteosa plant (b) Ajuga bracteosa leaf

Figure 2 Phytochemicals of *A. bracteosa*

Figure 3 Pharmacological activity reported in the *A. bracteosa*

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