Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Review article https://doi.org/10.12980/jclm.4.2016J6-163

A review of therapeutic potential of *Ajuga bracteosa*: A critically endangered plant from Himalaya

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ARTICLE INFO

Article history:
Received 26 Aug 2016
Received in revised form 8 Sep 2016
Accepted 14 Sep 2016
Available online

Keywords:
Ajuga bracteosa
Critically endangered
Pharmacology
Antitumor
Himalaya

ABSTRACT

Medicinal plants are the nature's gift for the humanity to treat various ailments and to spend a prosperous healthy life. There are almost 300 species of *Ajuga*. Among them, *Ajuga bracteosa* Wall. ex Benth (*A. bracteosa*) is an important medicinal plant of Himalaya regions. Its medicinal potential is due to the presence of various pharmacologically active compounds such as neo-clerodane diterpenoids, flavonol glycosides, iridoid glycosides, ergosterol-5,8-endoperoxide and phytoecdysones. The aim of this review article was to gather information about *A. bracteosa* which is currently scattered in form of various publications. This review article tried to attract the attention from people for therapeutic potential of *A. bracteosa*. The present review comprises upto date information of botanical aspects, active ingredients, traditional uses, and pharmacological activities such as antitumor, antimicrobial, antimalarial, anti-inflammatory, cardiotonic activity, antiarthritic activity, antioxidant activity and *in vitro* production of secondary metabolites for pharmaceuticals. Due to remarkable medicinal potential and commercialization, this species is indexed into critically endangered category and it is facing extremely high risk of extinction. Conservation practices and management techniques should be carried out to protect this important species from extinction. Recent biotechnological approaches will be quite helpful for its conservation.

1. Introduction

From the ancient times the people are relying on medicinal plants for curing their ailments. History of medicinal plants is as old as human history. From centuries the history of pharmacy and pharmacognosy is interlinked. Herbal drugs are utilized worldwide for treatment of wide range of diseases, so medicinal plants play a crucial role in world health. Despite of great advancement in modern medicines, people are still dependent on plants for health care. It is approximated that directly or indirectly almost 25% of entire modern medicines are derived from plants. Medicinal plants show distribution worldwide but they are more abundant in tropics. According to World Health Organization, 60%–80% population of developing countries depends on plants for their primary health care. From the last decades the use of medicinal plants become so popular that many important plants are at risk of extinction due to over exploitation.

Genus *Ajuga* of family Lamiaceae has numerous pharmacologically vital groups of flowering plants. These species are rich in the territory of Western Himalaya and upper Gangetic plans[1,2]. *Ajuga bracteosa* (*A. bracteosa*) is an important medicinal plant of Himalaya region. It has tremendous medicinal potential because of the presence of active ingredients. As for as conservation status is concerned, *A. bracteosa* is indexed into critically endangered category[3]. There is possibility of extinction of this highly medicinal plant. So there is need of multidimensional approach to conserve this plant species through better management practices like *ex-situ* conservation as well as multiplication both through biotechnological as well as conventional methods that could provide the possible solution to the existing problem.

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

2. Geographic distribution

A. bracteosa is commonly known as kauri booti and it belongs to family Lamiaceae[4]. It is a perennial herb growing wild in the Punjab plains, upper Gangetic plains and from Kashmir to Nepal in Western Himalaya[2]. *A. bracteosa* is a prised medicinal, aromatic, villous, soft and decumbent herb which is about 10–30 cm in height[5]. It is established on grassland, exposed slopes and open field in temperate and subtropical region of the world[6] at an elevation of 1 300 to 2 400 m[7].

3. Taxonomic hierarchy and vernacular names

A. bracteosa belongs to kingdom plantae, division Tracheophyta, class Magnoliopsida, order Lamiales and family Lamiaceae. *A. bracteosa* is known from different vernacular names. It is commonly called "Bungle" in English, "Nilkanthi" in Sanskrit and "Jan-i-adam" in Kashmiri. It is well distributed in the northern areas of Pakistan, where it is called as kauri booti due to its vicious taste[8].

4. Morphological description

It is a perennial prolixly branched evergreen herb (Figure 1) that remains flattened to the pulverised. Its yellowish flowers are congested in axillary spirals. *A. bracteosa* has usually woodier rootstock, leaves often larger up to 9.0 cm × 3.5 cm but usually much less with a more crenate to lobed margin, calyces 3.0–4.5 mm, corollas 10 mm, stamens usually exserted, and nutlets clearly longer and broader (eflora of Pakistan).

Figure 1. *A. bracteosa.*

5. Active ingredients

Phytochemically, *A. bracteosa* contains various compounds such as neo-clerodane diterpenoids, flavonol glycosides, iridoid glycosides, ergosterol-5,8-endoperoxide and phytoecdysones[2,9-11]. These chemical compounds were either synthesized or isolated from the plant. Cytotoxicity level was evaluated using skin carcinoma cell line and it was found that ergosterol-5,8-endoperoxide and neo-clerodane diterpenoids were not cytotoxic at higher concentration used for antiplasmodial activity.

6. Biological properties

A. bracteosa is utilized in medicine since ancient times and has various applications. In ethno medicine its use is reported as anthelmintic, astringent, antibacterial, antifungal, anti-inflammatory, hypoglycemic and it also remediates intestinal ailments[2]. This plant is traditionally used to treat phlegm and fever in China[12]. It is recommended in Ayurveda to treat rheumatism, amenorrhea, gout and palsy[13]. *A. bracteosa* contains diverse important compounds such as neo-clerodane diterpenoids, withanolides, iridoid glycosides and phytoecdysteroids[2]. Previous investigations of *A. bracteosa* have reported the inhibition of lipoxygenase, acetylcholinesterase and butyrylcholinesterase[11,14], cancer chemopreventive[15] and antispasmodic actions[7]. It is known that most of the plant derived compounds have substantial analgesic properties. Based on this, compounds which are derived from medicinal plants have very little side effects[16].

7. Pharmacology

7.1. Antitumor activity

Cancer is still one of the most devastating disease throughout the world including Pakistan. The risk prevalence and differences in the comparative risk were explained by various factors, mainly dietary factors[17]. Hartwell[18] listed more than 3 000 plant species that have reportedly been used in the treatment of cancer, but in many instances, the "cancer" is undefined, or reference is made to conditions such as hard swellings, abscesses, calluses, corns, warts, polyps, or tumors, to name a few. Plants have served as important sources of effective anticancer agents and over 60% currently used anticancer agents were isolated from natural sources, including plants, marine organisms and microorganisms are related to them[19,20]. Mothan *et al.*[21] reported *in vitro* anti-proliferative activities (IC_{50} values in mg/mL ± SEM) of the crude methanolic extracts tested against three human cancer cell lines by using methanolic extract of *A. bracteosa*. The phytochemicals which are screened include essential oil, terpenoids, flavonoids, and iridoid.

7.2. Antimicrobial activity

During early and in established contagion, ethanolic extract of leaves of *A. bracteosa* (250, 500 and 750 mg/kg per day) established a dose-dependent chemosuppression along with

significant ($P < 0.05$) repository activity. Among various concentrations, concentration of 750 mg/kg per day showed maximum 68.8% chemosuppression in respiratory tract and 77.7% chemosuppression during early contagion were found. ED_{50} of ELEAB was 300 mg/kg body weight of mice[22].

The elevated problematic issue in recent years is the *Plasmodium* resistance to the traditional antimalarial drugs pressures the necessity to look for novel and newer antiplasmodial constituents with new mode of action and efficacy. The medicinally important *A. bracteosa* has been curtained for its antiplasmodial effectiveness and it was found that the extract possess significant *in vitro* antiplasmodial efficacy with an IC_{50} of 10.0 μg/mL[23].

7.3. Antimalarial activity

Malaria is another most devastating disease and various plants are currently being employed to treat and control of malaria have become tougher due to spread of insecticides resistant mosquito vectors and drug resistant parasites. In an attempt to find advance antimalarial drugs, various resources such as ethnopharmacological should attain more attention. More extensively studies are required for the safety issues of traditional herbal medicines along with their potential role of active ingredients are vital steps for accessible and properly standardize herbal medicines. Phytochemical characterization assist as base for the development of new compounds. Various phytochemicals were tested and it was found that they have the therapeutic potential for malaria[22].

7.4. Anti-inflammatory activity

Seventy percent ethanolic extract of *A. bracteosa* possesses promising and significant anti-inflammatory activity. The mechanism of anti-inflammatory is supposed to be facilitated through the inhibition of COX-1 and COX-2. The study also indicates that isolated active ingredients (lupulin A, ajugarin I, deoxyharpagide withaferin A and reptoside) could be accountable for COX inhibitory and anti-inflammatory activity. The study confirms traditional use of *A. bracteosa* for the treatment of rheumatism and some other inflammatory disorders[24].

7.5. Cardiotonic activity

An alkaloidal fraction from *A. bracteosa* showed the cardiostimulant action on frog heart and rat ventricle. The activity was antagonized by dichloroisoprenaline, did not occur in hearts from reserpine-treated animals, and may result from liberation of catecholamine stores in the heart[25].

7.6. Antiarthritic activity

The substantial and promising antiarthritic activity of ELEAB is possibly facilitated through the inhibition of COX-1 and COX-2. The isolated active ingredients *i.e.* 6-deoxyharpagide, withaferin A, lupulin A, reptoside and ajugarin I, could be responsible for inducing the antiarthritic activity. The current opinion is the first trial providing the scientific evidence to support and rationalize traditional use of *A. bracteosa* for the treatment of rheumatism[13].

7.7. Antioxidant activity

Reactive oxygen species eagerly assault and persuade oxidative damage to various biomolecules such as DNA, proteins, lipids and lipoprotein. This damage is considered to be vital factor in different chronic diseases of human such as diabetes mellitus, cerebrovascular diseases, rheumatism, cancer and cardiovascular diseases[26]. The existing therapeutic approaches often have severe side effects such as strong host immune response and cytotoxicity to normal cells. Therefore, there is requirement of useful antioxidants to protect against chronic diseases. Antioxidants are considered as chemical substances that lessen and prevent oxidation. Antioxidants have the ability to protect from the damaging effects of radicals in tissue. They are supposed to counteract against heart disease, tumor, arteriosclerosis, cerebrovascular diseases and various other diseases[27]. Various antioxidant compounds have been found in different medicinal species, which are well known for their free radical scavenging assay.

Phenolic compounds may have direct contribution in the antioxidant activity. A strong relationship exists between antioxidant activities and phenolic compounds which have been produced in various plants *in vitro*[28]. The presence of phenolic compounds in various medicinal plants has been known to possess antioxidant potential.

The antioxidant activity of essential oil, plant extract or isolated compounds has grown due to the circumstance that antioxidants can scavenge the reactive oxygen species and hence may be beneficial in the prophylaxis and treatment of various diseases such as Alzheimer's disease, stroke diabetes, cancer, inflammation and arteriosclerosis[29-33]. The potential antioxidant activity of the oils was determined on the basis of scavenging activity of the stable free radical 2,2-Diphenyl-1-picrylhydrazl. Antioxidant activity was reported only from the oil of *A. bracteosa* and it showed 78% radical scavenging activity which is more as compared to high antioxidant activity of ascorbic acid[21]. This elevated antioxidant activity could be linked with high amount of oxygenated monoterpenes in the *A. bracteosa* essential oil[32].

7.8. In vitro production of secondary metabolites for pharmaceutical

Plant secondary metabolites are recognized as unique sources for pharmaceuticals, flavors, food additives and other industrial materials either as a raw material or as a part of final product. Among various classes of secondary metabolites, polyphenols comprise the major group of innate antioxidants[34]. Flavonoids and phenolics possess biological properties like anti-carcinogen, anti-aging, antioxidant and protection from brain dysfunctions, *viz*. Huntington's diseases, Parkinson's, Alzheimer's immune/ autoimmune and cardiovascular diseases[35].

Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative and anti-carcinogenic. The biological activities of phenolic compounds and flavonoids are associated to their antioxidant potential.

Callus culture and cell suspension cultures are the effective ways for the production of secondary metabolites which have various therapeutic potential. To study the production and growth kinetics, cell suspension cultures are proposed to be simple system to implement and evaluate the most favorable scheme for the production of medicinal compounds in good quantities[36]. Light regimes play a significant role in all the fundamental process of plant and fundamental building blocks like primary and secondary metabolism, growth and development[37]. By optimizing *in vitro* conditions like light regime, production of secondary metabolites can be proficiently stimulated. Various stimulatory effects of light regime have been observed for the accumulation of secondary metabolites such as artemisinin, anthocyanins, derivatives and flavonoids[38]. Light is very crucial as the inhibitory and stimulatory effects for the production of secondary metabolites. The antioxidant and antimicrobial activities and contents of total phenolics and flavonoids of *Ajuga chamaepitys* (L.) Schreb. subsp. chamaepitys (Lamiaceae) were investigated. The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu reagent and expressed as the gallic acid equivalent (GAE) (mg GAE/g of extract). The highest value was obtained in the ethyl acetate extract (57.02 mg GAE/g)[39].

8. Traditional uses

A. bracteosa is highly medicinal plant and it is the most valuable species among all the species of genus *Ajuga*. *A. bracteosa* is traditionally used to treat fever and phlegm in China[12]. It is recommended in Ayurveda to treat gout, palsy, amenorrhea and rheumatism[13]. Leaves of *A. bracteosa* are stimulant, diuretic and locally used to treat malaria[40,41]. *A. bracteosa* is regarded an alternative to cinchona[42] (Table 1).

Table 1
Traditional methods of applications of *A. bracteosa.*

Conditions	Methods of applications
Headache	Paste of the leaves is applied to cure headache[43]
Abdominal pain	Powder of the whole plant is given to treat abdominal pain[43]
Indigestion	Powder of whole plant is also used to treat indigestion[43]
Astringent	Whole plant is used as astringent[3]
Tonic	Whole plant is also used as tonic[3]
Internal colic	Whole plant is used to treat internal colic[44]
Pimples	Barks juice is used to treat pimples[44]
Jaundice	Leaves extracts are used to treat jaundice[45]
Hypertension	Whole plant is used to treat hypertension[45]
Sore throat	Whole plant is used to sore throat[45]
Cold	Decoction of root is taken[44]
Leprosy	Root powder is ingested[44]
Blood purification	Leaves extract is used for blood purification[46]
Diabetes	Decoction of leaves is used to treat the diabetes
Fever	Decoction of leaves is used to treat the fever
Swollen wounds	Plant extract is used is used to cure swollen wounds[47]
Bites of insects	Plant extract is used is used to cure bites of insects[47]
Eye trouble	Plant extract is used is used to cure eye trouble[47]
Bladder disease	Plant extract is used is used to treat bladder disease[47]

9. Conservation status

Conservation status, *viz.*, critically endangered, endangered, vulnerable and least concern are recorded on the basis of density in accordance with International Union for Conservation of Nature Red List Database. Alam and Ali[48] revealed several threats to Pakistan's biodiversity including habitat loss, deforestation, grazing, invasive species, illegal trade, industrial pollution, growing demand for natural resources and the lack of adequate training. Medicinally important species like *A. bracteosa* is critically endangered not only locally, but also in the whole region[49]. Looking at the broad spectrum of *A. bracteosa* for various purposes particularly in pharmacology, it is useful to cultivate this plant at large scale. *A. bracteosa* is indexed in critically endangered category, so consistent efforts should be made to protect this plant species to become extinct. A multidimensional approach is required to maintain and includes selection of better quality genotype and *ex-situ* as well *in-situ* conservation followed by multiplication both by conventional as well as biotechnological methods that could provide solution to the existing problem.

10. Threats towards its extinction

Wild plants have been collected from decades[50]. For many years, *ex-situ* management has been ignored of wild plants. The time of collection and lack of awareness about its part used lead to be the mismanagement of the species. Currently various obstacles exist for gathering, sustainable cultivation and use of medicinal plants. These include lack of clear resource and custodianship, little understanding of sustainable management parameter and knowledge of market requirement[51]. Tali *et al.*[52] assessed the threatened status of *A. bracteosa* in accordance with International

Union For Conservation Of Nature guideline. The population size, area of occupancy, extent of occurrence and various other threats of this species were recorded. The most common operative threats are landslides and overexploitation for the local use.

11. Biotechnological methods

Tissue culture is a method of growing and multiplication of plant cells, tissues, organs, seeds or other plant parts in a sterile environment on a defined solid or liquid nutrient medium[53,54]. Plant tissue culture is a form of vegetative propagation used for the large-scale production of plants known as micropropagation[53]. Plant tissue culture technology offers the possible for proficiently propagating genetically uniform, disease-free and enormous amounts of plants since its notion[55]. The formation of synthetic seeds through somatic embryogenesis is an emerging trend to conserve the medicinally important plants and some other plants whose seeds are not viable like *Citrus reticulata* L.[56].

The role of tissue culture in genetic engineering and biotechnology was first time exemplified by Kanta and Maheshwari in 1962. Plant tissue culture has become an important tool in the study of basic areas of plant biology and biochemistry[54]. Biotechnological advancement not only provides alternative methods for *in vitro* preservation of tropical fruits and recalcitrant seeds but also provides tools for disease free germplasm conservation, lower labor cost and limiting disease-transfer[57,58].

Jan *et al*.[8] reported callus induction from various explants of *A. bracteosa viz*., leaf, petiole and leaf nodes. Maximum callus induction response was obtained when Murashige and Skoog medium was supplemented with 6-Benzyl amino purine after 19 days of inoculation. Callus derived from leaf explants showed maximum response for regeneration. Maximum multiple shoot regenerated on Murashige and Skoog medium when supplemented with 5 mg/L BAP after 28 days of inoculation. The results of Jan *et al*.[8] are in contrast with Srivastav *et al*.[59] who reported maximum callus induction when Murashige and Skoog medium was supplemented with combination of BAP and Indole-3-acetic acid after 10 days of inoculation. Another technique of conservation is cyto-storage or normal storage of important plant species under *in vitro* condition to preserve germplasm. Another common technique of *in vitro* storage is utilization of alginate encapsulation of explants to produce synthetic seeds[60].

12. Challenges in conservation, sustainable use and way to forward

A. bracteosa was considered as highly medicinal plant. Due to high demand, most of natural population of *A. bracteosa* is under severe pressure at the present scenario. Due to excessive exploiting of this species, it is declining day by day. Due to the various known uses of this herb, there is a high demand at local and international level pharmaceutical industry. During the last decade, this species has attained a considerable attention because it is critically endangered and will be extinct in upcoming years if exploited at the same rate. So there is need of sustainable use of this highly medicinal species.

A multidimensional approach is required to maintain and includes selection of better quality genotype and *ex-situ* as well *in-situ* conservation followed by multiplication both by conventional as well as biotechnological methods that could provide solution to the existing problem. The importance of any medicinal plant depends on its active ingredients which is present in that species. It would be desirable to carry out cultivation of elite clones. The superior clones can be approved by the use of various molecular markers techniques and chemo-profiling. The conventional methods of propagation as well as plant tissue culture techniques can be used to multiply and raising the commercial plantation for conservation. Tissue culture can be employed as substitute to conservative methods *in vitro* propagation with the purpose of increasing the developmental rate of preferred genotypes and commercial micropropagation[61]. Gene transfer, selection and regeneration of transformants is now a day's employed by the plant tissue culture techniques[62]. This technique is helpful in the production of millions of healthy, vigorous and disease free plants in less time, space, labor and with less cost. Besides *in vitro* propagation, cell suspension culture is useful for the large scale production of secondary metabolites.

Post-harvest handling is another factor upon which the quality of plants is dependent. The collectors of herbal material pay less attention to quality of material during harvesting, handling and storage. It has been found that herbal drugs samples which are stored harbor mycotoxin producing fungi. Cultivation practices also need to be addressed. Due to genetic and environmental differences wild harvested plants vary in consistency and quality. Regional environmental conditions also influence the efficacy of medicinal plants. Some of the factors such as temperature, photoperiod, soil characteristics and rain fall have severe effects on the production of active constituents. Therefore, consistent efforts should be taken for the sustainable management of medicinal plants such as *A. bracteosa* at community level.

13. Conclusion

A. bracteosa is highly medicinal plant of Himalaya region but it is indexed in the critically endangered category. This plant has immense potential because of its efficacy towards various diseases. Some progress has been made, but still consistent efforts are required to explore the individual compounds isolated from *A. bracteosa* to validate and understand its traditional uses and clinical practices. It is also important plant species with respect to its ethnomedicinal importance, so this importance exert significant pressure on plant regarding its use. This pressure posed serious threat towards its extinction. So there

is urgent need to conserve this species and sustainable harvesting is usually required. A multidimensional approach is usually required to maintain and includes selection of better quality genotype and *ex-situ* as well *in-situ* conservation followed by multiplication both by conventional as well as biotechnological methods that could provide possible solution to the existing problem.

Conflict of interest statement

We declare that we have no conflict of interest.

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