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An Overview of Pharmaceutical Applications and *In vitro* Micropropagation Techniques for Rare and Endangered Plant Species

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Many rare and endangered plant species possess valuable secondary metabolites with pharmacological applications. These bioactive compounds are often integral to traditional medicine systems, highlighting the cultural significance of these plants. The health benefits of many medicinal species are not fully validated by contemporary scientific research, and some may be

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facing extinction due to habitat loss, overharvesting or climate change. This situation highlights the urgent need for effective conservation strategies of the species and sustainable cultivation methods. Micropropagation is a valuable technique for producing large numbers of plants from a single explant, significantly aiding in the conservation and commercial cultivation of rare species. Among the various types of explants, shoot tips and nodal segments have been identified as the most effective explants for micropropagation. These explants can be induced to generate multiple shoots in Murashige and Skoog (MS) medium containing Benzyl aminopurine (BAP). Thidiazuron (TDZ), Kinetin (KIN), or 2,4-Dichlorophenoxyacetic acid (2,4-D) are commonly used in MS medium to promote shoot and root development in both direct and indirect organogenesis processes. Rooting of the plantlets was typically achieved using MS medium either supplemented with Indole-3-butyric acid (IBA) or devoid of auxins, depending on the species and the specific requirements for rooting.

Keywords: Endangered species; medicinal plants; micropropagation; pharmacological uses.

1. INTRODUCTION

The Southeast Asia and Asia-Pacific region hosts nine biodiversity hotspots. In India only four biodiversity hotspots present and in these regions several thousands of species are habituated. Among these species some of the plants are in endangered condition due loss of habitat, population fragmentation, industrialization, urbanization, introduction of new species and loss of genetic variation [1,2]. Endangered species exist few in numbers only. In other words, these species are under threat of being extinct in condition [3]. Conserving the endangered species is necessary not only because of some species are of traditional herbs but also have medicinal properties by nature [4,5]. “The factors Influencing for the propagation of these rare plant species are low seed germination, relict species, torn areas, harsh climatic conditions, eaten by animals and birds” [6]. One of the ways to conserve their extinct condition is can be done by micropropagation methods. Micropropagation techniques play a crucial role in the conservation and multiplication of rare or threatened endangered plant species [7,8,9]. These techniques allow for the rapid and efficient production of a more number of plants using explants such as shoot tips and nodal buds [10,11]. This method helps to overcome challenges associated with conventional propagation methods, such as seed sterility or rudimentary seeds [12]. This method allows the efficient production of plants from rare and endangered species, aiding in their conservation and propagation for medicinal purposes. The review emphasizes the importance of identifying medicinal plants at risk of extinction, documenting their traditional uses, and detailing micropropagation methods that can be employed to ensure their survival.

2. *ARTEMISIA HOLOLEUCA* BIEB. EX BESS

Artemisia is a diverse genus of plants that includes approximately 200 to 300 species [13], belonging to the Asteraceae family. These plants can be classified as annuals, perennials or shrubs. Some species of *Artemisia* are cultivated for their medicinal properties, such as *Artemisia annua*, for treating malaria. Others, like *Artemisia vulgaris*, are valued for their insect-repellent qualities, while *Artemisia absinthium* (often referred to as wormwood) has ornamental uses as well as applications in traditional herbal medicine [14,15]. *Artemisia hololeuca* is a plant species in the genus Artemisia. It is commonly known as white sagebrush and is native to regions in Europe and Asia. The genus exemplifies a wide range of uses, making it significant in various fields, including herbal medicine, horticulture and pest management. *Artemisia hololeuca* Bieb located in the Rostov region is in threat of extinction in the native place of Europe (Fig. 1) [16,17,18].

Fig. 1. *Artemisia hololeuca*

Table 1. Photochemical properties and medicinal uses of rare endangered species

Sl. No	Scientific name	Native place	Photochemical constituents	Medicinal uses
1	*Artemisia hololeuca* Bieb. ex Bess	Rostov region in Europe	Above parts - carotene, alkaloid, flavonoid, cumarin, Root -alkaloid	Malaria, diarrhea and constipation
2	*Orchis catasetum*	Europe and Asia	Glucoside-loroglossin	Cure dysentery, diarrhea, chronic fever, cough, stomachache, wounds
3	*Viola uliginosa*	Poland	Alkaloid, glycoside, saponins, methyl salicylate	Uterotonic, hemolytic, inhibition of neurotensin action, anti-HIV and cytotoxic, Antibacteria and insect larvae
4	*Helianthemum inaguae*	Canary Islands	Tannins, fatty acids, glucoside, inulin, levuline, polyphenols, flavonoids, kaempherol	Chronic diarrhea and dysentery, ulcers, eyes inflammation
5	*Citrus halimii*	Malaya and Peninsular Thailand	Vitamin C, flavonoids, citric acid, malic acid, oxalic acid, succinic acid, and malonic acid	Antioxidant, anti-cancer, antiviral, anti-inflammatory activities, effects on capillarity, and cholesterol-lowering ability
6	*Malus niedzwetzkyana*	China	Flavonoids	Cardiovascular disease, cancer
7	*Acanthopanax seoulenses* Naka (siberian ginseng)	Cheongnyangni, Seoul, Korea	Flavonoids like quercetin, quercitrin, rutin and hyperin	kidney disease, Alzheimer's disease, attention deficit-hyperactivity disorder (ADHD), chronic fatigue syndrome, diabetes, high cholesterol,
8	*Leucojum aestivum*	Bulgaria	Alkaloid Galanthamine, Nivalin	Alzheimer's disease Poliomyelitis, muscular dystrophy, myasthenia, myopathy, and paralysis in newborns.
9	*Tuberaria major*	Western and southern Europe	Ascorbic acid and phenolic compounds	Antioxidant, anti-inflammatory, antimicrobial, and anti proliferative / anti-tumoural.
10	*Daphne cneorum*	Central and southern Europe	Coumarins, flavonoids, lignins, steroids and different classes of terpenes	Antimicrobial, antioxidant, analgesic, anti-inflammatory, cytotoxic, anti-ulcerogenic, abortive,hypocholesterolemic and hemostatic effect

2.1 Medicinal Properties

The plants contain a variety of bioactive compounds, including essential oils, terpenoids and sesquiterpene lactones. The above-ground portion of the plant contains carotene, alkaloids, flavonoids, and coumarin, while the root portion may contain traces of alkaloids (Table 1) [19]. it is most commonly used for treating menstrual and digestion related problems and also to get rid of intestinal worms. The leaves of this species are slightly bitter and very aromatic, so they are added to certain dishes in small quantities to stimulating digestive system. *Artemisia* bitters are known to have digestive benefits and can help stimulate appetite. The water infusion of the bark and leaves is commonly used for treating asthma [20, 21].

2.2 Micropropagation

“The cultivation of *A. hololeucain vitro* has not been specifically studied yet. However, there *are established technologies for cultivating other species within the genus* Artemisia, such as *A. vulgaris*, *A. annua*, and *A. nilagirica* var. nilagirica. Each species may require different growth medium compositions and hormone treatments for optimal multiplication and rhizogenesis. *A. vulgaris* shows high multiplication rates on MS medium with an addition of 1.0 mg/L 6-(γ,γ-Dimethylallylamino) purine (2-iP) for multiplication and indolyl acetic acid (IAA) for rhizogenesis” [22]. *Artemisia annua* produced shoots when cultured in MS medium supplemented with 1.0 mg/L of BAP. *A. nilagirica* var. nilagirica requires a combination of BAP and 2-iP for shoot regeneration and IBA for rhizogenesis [23]. Additionally, TDZ is recommended for obtaining callus from *Artemisia* plants [24]. Further research may be needed to determine the specific requirements for culturing of *A. hololeuca* under *in vitro* condition.

3.*ORCHIS CATASETUM*

“Orchids are grown as ornamentals and valued as cut flowers not only because of their exotic beauty but also for their long shelf life” [25]. “Orchids are one of the beautiful flowers in flowering plants. It contains 800 genera and 25000 species” [26] and it has an incredible

range of diversity in size, shape and colour. Propagation of orchids by seed only give rise for the production of heterozygous plants whereas through *in vitro* micropropagation of tissue culture, give true to type of the plants are being produced in orchids especially in endangered species like *Orchis catasetum*, are threatened with the danger of extinction (Fig. 2) [27].

Fig. 2. *Orchis catasetum*

3.1 Medicinal Properties

"The main constituents of this species are trans-geranyl geraniol, 1,4-dimethoxybenzene, linalool, 2-phenylethyl acetate, geraniol, 7-*epi*-1,2-dehydro-sesquicineole, 1,8-cineole, benzyl acetate, limonene, methyl salicylate, (*E*)-β-farnesene, anisyl butyrate, *cis*-carvone oxide, cadin-4-en-10-ol, indole, α-pinene, and δ-cadinene. The roots of this species are rich in starch, mucilage, sugar, phosphate, chloride and glucoside-loroglossin and are used for medicinal purpose. In Unani, the roots of the plant are considered aphrodisiac and nervine tonic. The roots are cooling, emollient, aphrodisiac, rejuvenating and tonic and are used to cure dysentery, diarrhea, chronic fever, cough, stomachache and wounds on the body (Table 1)" [28,29].

3.2 Micropropagation

"Various explants, including shoot tip, root tip, stem, leaf, node, bud, inflorescence, and rhizome, as well as somatic embryo and thin cell layer are commonly utilized for the successful development of plant regeneration protocols in orchid species. The micropropagation through protocorm-like bodies (PLBs) is more efficient as compared to plantlet development from seeds or adventitious shoots" [30]. "But to get the efficient micropropagation using PLB, much effort has been taken to modify the culture media, by adding plant growth regulators such as BA, TDZ, BAP, NAA, IAA and GA_3. Cytokinins are the most important to improve the plant regeneration from PLBs. Healthy and sterilized protocorms of *Orchis catasetum* is prepared from a plant tissue culture having 0.2 mg/L of BA resulted in proper root and shoot induction. The medium containing 2.0 mg/L BAP individually or in combination with 1.5 mg/L NAA induced the roots on same shoots (100%)" [31]. "A combination of 0.5 mg/L BA and 0.5 mg/L NAA was found to be suitable for maximum protocorm-like bodies (PLBs) regeneration (20.40 per plantlet).The largest number of root (7.16 per plantlet) and leaf (10.10 per plantlet), also the highest plant height (114.20 mm per plantlet) and root length (193.40 mm per plantlet) were obtained on MS medium supplemented with 0.5 mg/L BA along with 0.5 mg/L NAA. The best induction of PLBs (15 per explant) is observed from the MS media containing 5.0 mg/L BAP within 6 weeks. The medium containing 0.5 mg/L KIN was also good for PLB formation in *Orchis catasetum"* [32].

4.*VIOLA ULIGINOSA*

Viola uliginosa was originally described in 1809 by Besser from Rza˛ska near Cracow, Poland (*Locus classicus*). *Viola uliginosa*. Besser is a species of flowering plant belonging to the family Violaceae, the swamp violet, is native to Europe (Fig. 3) [33].The main range of distribution of this species was in the Baltic Sea region. This species is considered an endangered or even threatened with extinction in the countries of Poland, Germany, Sweden, Russia and is declining throughout its range [34]. *Viola utiginosa* produces cyclotides, which are cyclic polypeptides that serve as defense agents against insect pest [35].

Fig. 3. *Viola uliginosa*

4.1 Medicinal Properties

"It contains phytochemicals like alkaloid, glycoside, saponins, methyl salicylate, mucilage and vitamin C (Table 1). It has biological activities for cyclotides, including uterotonic, hemolytic, inhibition of neurotensin action, anti-HIV and cytotoxic [35]. They are active against different bacteria and insect larvae with insecticidal and antimicrobial activities" [36].

4.2 Micropropagation

Tissue culture is a highly efficient method for plant asexual reproduction, offering advantages such as a large reproduction coefficient, fast propagation, and no limitations based on season or land factors. It is a key technology for promoting excellent clones and conserving germplasm of endangered or valuable plant species, contributing to biodiversity conservation [37,38,39]. "In this process, Petiole and leaves are used as explants and are cultured in MS medium supplemented with different concentrations of plant growth regulators like TDZ, KIN and 2,4-D. Rooted shoots are obtained on MS with 2% sucrose and 0.5 mg/L IBA. MS media supplemented with TDZ (0.5 or 1 mg/L) or with equal concentrations (2 mg/L) of KIN and 2,4-D followed by callus transfer on 1 mg/L TDZ which induce the direct and indirect (via callus) organogenesis" [40]. "In MS media supplemented with TDZ, 2 mg/L KIN, and 2 mg/L 2,4-D transferred to 1 mg/L TDZ, approximately 24% of the regenerants are obtained from direct and indirect organogenesis. However, in tetraploid plants, the frequency of indirect organogenesis significantly increases to around 70%" [41]. The process involves callus proliferation on MS medium with 2 mg/L KIN and 2 mg/L 2,4-D. Shoot formation is achieved by adding 1 mg/L TDZ to the callus proliferation media. Using a leaf as an explant is more efficient in this method as it allows for the involvement of the entire surface [42]. A lower concentration of TDZ (0.5 mg/L) can induce direct organogenesis from petiole explants. The survival rate is 95 % when it is acclimatized to the green house condition.

5.*HELIANTHEMUM INAGUAE*

Helianthemum is a flowering plant of Cistaceae family and serves as markers for truffle hunters (Fig. 4). There are over 200 cultivars available in this species. The local distribution of *Helianthemum* in the Canary Islands is characterized by the small number of individuals in their natural populations [43]. "*Helianthemum inaguae* is also a flowering plant which causes a constant loss of alleles results to the extinction of their habitats which reduce the ability of *Helianthemum* species, and ultimately to adapt to future changing circumstances. *Helianthemum inaguae* was the only one population that has been located in the South West of Gran Canaria" [44]. "So that it is considered as "in danger of extinction" and also included in legal catalogues of threatened plants (CNEA: Catalogo Nacional de Especies Amenazadas and CEAC: Catalogo de Especies Amenazadas de Canarias) for its preservation. According to IUCN (2001), it is included in the Critically Endangered (CR) list also" [45]. *Helianthemum inaguae* has a great potential in forage, in traditional medicine, for halting desert encroachment and stabilizing sand dunes through their excellent root systems development, and in the improvement of soil organic matter content.

Fig. 4. *Helianthemum inaguae*

5.1 Medicinal Properties

It has a bioactive photochemical constituents like tannins (helianthi tannic acid), fatty acids, essential oil, glucoside, inulin, levuline, polyphenols, flavonoids, kaempherol and carbohydrates (Table 1). The extract of leaf contains tonic, astringent properties and is used for the treatment of chronic diarrhea and dysentery, as well against various diseases like ulcers, eyes' inflammation, prurigo and also used to treat rashes, chronic rhinitis and sinusitis [46].

5.2 Micropropagation

"Shoot tips and nodal segments are used as explants. Multiple shoot production is obtained

using MS medium supplemented with different concentrations of BA, Kin and NAA. Especially BA 2 mg/L and Kin 1.5 mg/L is the best in shoot induction. BA stimulates multiple shoot formation while Kin is more efficient in the elongation process" [47]. Moreover, after a BA treatment, the lower concentrations of Kin (0.2 mg/L) stimulate shoots elongation. For callus formation, NAA propagation medium either with BA or Kin used in a high proportion of the explants. Rooting was observed in MS medium supplemented with IBA or without any plant growth regulator. During the acclimatization process, 72% survival rate was obtained [48].

6.*CITRUS HALIMII*

"Citrus fruit is one of the major horticultural crops grown worldwide and they are the most traded horticultural commodity in the world" [49]. The new species *Citrus halimii* has been discovered in Malaya and Peninsular Thailand. *Citrus halimii* is a rare and endangered species native to Thailand and Malaysia (Fig. 5.) In the propagation process, difficulties arise in producing identical cultivars through traditional hybridization due to the similarity of cultivars. It is in extinction condition, mainly in Southeast Asia [50, 51].

Fig. 5. *Citrus halimii*

6.1 Medicinal Properties

"In this species, Organic acids are present such as citric acid, malic acid, oxalic acid, succinic acid and malonic acid provide calories, and are easily metabolized" [52]. It also exerts antioxidant properties [53]. It is also a good source of vitamin C and flavonoids (Table 1). It is used for potential antioxidant (prevents aging), anti-cancer, antiviral and anti-inflammatory activities and cholesterol-lowering ability [54,55].

6.2 Micropropagation

Micropropagation is indeed a valuable technique used to overcome heterozygosity and produce homozygous plants, especially in endangered species like *Citrus halimii*. It is worth noting that there are currently no regeneration studies available for *Citrus halimii* through either organogenesis or somatic embryogenesis pathways.

7.*MALUS NIEDZWETZKYANA*

Apples belong to the genus Malus, which consists of a varying number of species, typically ranging from 8 to 78, depending on the classification criteria used. This variability is due to the ease with which many species within the genus can be hybridized [56]. The production of 87,236,221 tones of apples per year worldwide underscores the significant role play in global food production. Conservation of biological diversity is crucial for ensuring food security and improving nutrition, as it helps maintain genetic diversity within apple species and other crops. This diversity can lead to the development of new varieties with improved traits, resilience to diseases, and adaptation to changing environmental conditions, ultimately contributing to a more sustainable and secure food supply [57]. Nearly 387 plant species including the rare, endemic, and endangered species like *Malus niedzwetzkyana* (Fig. 6), are listed in the Red Book of Kazakhstan and also in the International Red List.

7.1 Medicinal Properties

It is rich in fiber and has phytochemical compounds like polyphenols and flavonoids and are good basis of antioxidant (Table 1) [58,59]. They have bundles of fibres which are soluble as well as insoluble, together with cellulose and hemicellulose, with pectin as the main soluble fibre [60]. *Malus*pectin has cholesterol-lowering properties and also good effect on glucose metabolism. It is used to cure cardiovascular disease and cancer.

7.2 Micropropagation

Biotechnology methods like microclonal propagation techniques are widely used to preserve rare plant species for the long-term preservation of genetic material and also enabling their large-scale reproduction and propagation. The explants of axillary buds of

annual shoots are used for shoot multiplication in Quoirin–Lepoivre (QL) culture medium containing three cytokinins (BAP, kinetin, and TDZ). "The optimized micropropagation technology achieved a high propagation rate of 28.77 new shoots per explant on QL medium with 0.5 mg/L BAP and 0.01 mg/L IBA. Subsequently, all shoots developed the roots on 0.5× QL media supplemented with 10 mg/L sucrose and 1.5 mg/L IBA, resulting in an average of 11.8 roots per explant. This successful protocol demonstrates the effectiveness of the specific growth factors and conditions used in promoting shoot and root development in the micropropagation process" [61].

Fig. 6. *Malus niedzwetzkyana*

8. *ACANTHOPANAX SEOULENSES* NAKAI

The *Acanthopanax seoulenses* Nakai is a rare and endangered species and is in danger of extinction which found at Cheongnyangni, Seoul, Korea (Fig. 7). In this species, only two trees are conserved at Hong Neung Botanical Garden in Korea Forest Research Institute, Korea due to limitation of the distribution area and seedling propagation difficulties. It has a conservation value, to protect 359 species as part of the "National Strategy on Biological Diversity" by the Ministry of Environment (MOE), Korea. The immature zygotic embryos are embedded in seeds, leading to a prolonged germination process of almost 2 years for the embryo to mature. So that micropropagation technique used to help the conservation of this species.

8.1 Medicinal Properties

Four Flavonoids are extracted from the leaves of *Acanthopanax which are* quercetin, quercitrin, rutin and hyperin (Table 1) [62]. "It is used for treating the kidney disease, Alzheimer's disease, Attention Deficit-Hyperactivity Disorder (ADHD), Chronic Fatigue syndrome, Diabetes, high cholesterol, improving loss of sensation in extremities (peripheral neuropathy), Fibromyalgia, Rheumatoid arthritis, reducing the effects of a hangover, flu, colds, Chronic Bronchitis, and Tuberculosis" [63]. It is also used for treating the side effects of cancer chemotherapy. It is also used to boost the immune system, prevent colds, and increase appetite.

Fig. 7. *Acanthopanax seoulenses*

8.2 Micropropagation

"Somatic embryogenesis and plant regeneration study was conducted in this rare and endangered species" [64]. "MS medium supplemented with 3% sucrose and 0.1–0.2 mg/L abscisic acid (ABA), or MS medium with 3% sucrose and 0.1 mg/l ABA and 0.02% activated charcoal combinations served as a better medium for callus induction of somatic embryos production" [65]. "There is a difference in somatic embryo germination and plant conversion rates between two gelling agents, specifically agar-gelled medium. A plant conversion rate of 78±18.2% was achieved in agar-gelled medium, with 98% of the plants surviving in greenhouse conditions" [66].

Maximum frequncy of somatic embryos were induced in MS medium supplemented with 1.0 mg/L 2,4-D, 0.01 mg/L TDZ, 3% sucrose, and 0.3% gelrite in dark condition. The highest percentge of somatic embryos induction was recorded in 1/2MS medium with 3% sucrose and 7% Poly ethylene Glycol (PEG). GA_3 was required to induce normal SE germination and higher [67,68] and more rapid germination with increasing concentration, but hyperhydrated plants were observed frequently.

9. *LEUCOJUM AESTIVUM L.*

Leucojum aestivum,is a threatened and endangered species in the family of Amaryllidaceae (Fig. 8) [69]. It is used as a raw material for commercial production of galanthamine-based medicines in Bulgaria [70,71]. The plants are used for the treatment of neurological diseases, poliomyelitis, amnesia, but it is in extinction condition [72,73,74].

Fig. 8. *Acanthopanax seoulenses*

9.1 Medicinal Properties

It has a galantamine contents which is an alkaloid (Table 1). This is extremely valuable source for both pharmacy and medicine [75]. It is used for the treatment of Alzheimer's disease patients with mild and moderate stage. The ingredient isolated from the leaves and the flowers of this plant known as Nivalin, is recognized as drug for treating poliomyelitis. The drug is also used for muscular dystrophy, myasthenia, myopathy, and paralysis in newborns.

9.2 Micropropagation

There is a wide range of techniques available for conservation of plant genetic resources of this species includes seed germination, micropropagation, regeneration from callus, embryo rescue, micrografting and cryopreservation. In compared to vegetative method of propagation, micropropagation by bulb explant is the best choice for regeneration of this crop.

The different explants like bulb, stem, leaves and ovaries are used for rapid propagation of *Leucojum aestivum*. Leaves of *Leucojum aestivum* L. is the best for giving the highest regeneration activity. For direct organogenesis, MS medium containing 1 mg/L BAP and 1 mg/L kinetin was most favour for production of shoots in *Leucojum aestivum* L. Linsmaier and Skoog (LS) medium containing 0.5 mg/L NAA and 0.1 mg/L kinetin were also favourable for shoot regeneration. Compared to the apical meristem, the basal scales showed more active for organogenesis [76]. “The bulblets with low temperature stimulated rhizogenesis in 85% of the regenerants obtained. Callus formation is observed when the leaves are transferred to LS medium supplemented with 5 mg/L 2,4-D, 1 mg/L NAA and 1 mg/L BAP whereas lowest callus forrmation when BAP is replaced by 2 mg/L kinetin. Larger calluses were obtained in 25.6% of the inoculated scales, and small calluses in 41.0%” [77].

10.*TUBERARIA MAJOR*

Tuberaria is a genus of about 12 species of family Cistaceae, native to western and southern Europe [78]. These species majorly found in dry, stony sites and close to the sea. In this, *Tuberaria major* is under endangered condition and the normal method of propagation is through seeds (Fig. 9).

Fig. 9. *Tuberaria major*

10.1 Medicinal Properties

This species has a composition of ascorbic acid and phenolic compounds (Table 1). It has excellent medicinal properties and used as an antioxidant, anti-inflammatory, antimicrobial, and anti proliferative and anti-tumoural.

10.2 Micropropagation

Micropropagation of the endangered species of *Tuberaria major* is done using seedlings as explants. Explants like apical shoots and nodal segments are also used. Explant type significantly influenced the proliferation frequency and mean number of shoots. Higher number of shoots was obtained when the explants were

cultured in half-strength MS medium supplemented with 0.2 mg/L BA (6.83 shoots) or Zeatin (ZEA) (6.55 shoots). The highest rooting frequencies of about 97–100% obtained in 1/2 MS medium with or without plant growth regulators. Apical shoot cuttings 0.5 cm were grown on MS medium and ½MS for 60 days with different growth regulators. The ½ MS medium containing 1 mg/L BAP provided the best results under in vitro condition. Shoots showing good growth and no vitrification or browning observed. These micropropagated plants were reintroduced into their natural habitat for normal development. Subsequent multiplication of nodal explants using Zeatin (ZEA) at 0.2mg/L, and successful ex vitro establishment of well-rooted plantlets on 1/2 MS medium results in large-scale propagation of*T. major* [79].

11. *DAPHNE CNEORUM*

It is a flowering plant belonging to the family Thymelaeaceae and is native to the mountains of central and southern Europe (Fig. 10). Unfortunately, this species is gradually disappearing due to its rare and endangered status, with occurrences limited to only two localities in Central Bohemia [80].

Fig. 10. *Tuberaria major*

11.1 Medicinal Properties

The Thymeleaceae family comprises 500 herbal species that serve as a substantial source of pharmacologically active compounds. These plants contain phytochemical constituents such as coumarins, flavonoids, lignins, steroids, and various classes of terpenes (Table 1) [81]. They are utilized for their antimicrobial, antioxidant, analgesic, anti-inflammatory, cytotoxic, anti-ulcerogenic, abortive, hypocholesterolemic, and hemostatic effects. Additionally, these plants are used as ingredients in cosmetic products, paints, and other applications.

11.2 Micropropagation

The crop is commonly propagated through cuttings, with micropropagation techniques being rarely utilized. *In vitro* regeneration through organogenesis is not considerably suitable for large-scale multiplication of plants due to phenolic issues.

Multiple shoots were produced on agar woody plant medium (WPM) supplemented with 0.2 mg/L of BAP, 0.1 mg/L IBA, 200 mg/L glutamine, and 200 mg/L casein hydrolysate. Rooting was achieved at a rate of 50% on 1/3 strength WPM medium supplemented with 2.83 mg/L IBA, while no rooting occurred in the presence of NAA. Organogenesis was observed in both types of explants induced on 6% agar woody plant medium containing 200 mg/L L-glutamine, 200 mg/L of casein hydrolysate, 30 g/L of sucrose, 0.2 mg/L BAP, and 0.1 mg/L IBA. A total of 7.3 shoots were obtained during the cultivation process when inducing organogenesis in the shoots from proximal and distal stem segments of this cultivar [82,83].

12. CONCLUSION

The conservation of endangered medicinal plants is of paramount importance, both for ecological integrity and for maintaining our traditional medicinal practices. Micropropagation techniques provide a viable pathway for the propagation and conservation of these species, potentially leading to sustainable practices that protect biodiversity. Continued research and conservation efforts are necessary to ensure that these valuable plants are preserved for future generations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Chatgptis.org was partially used for editing the abstract, conclusion and some part of Main MS.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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