Suman Chandra
Hemant Lata *Editors*

High Altitude Medicinal Plants

Botany, Conservation and Cultivation

Springer

High Altitude Medicinal Plants

Suman Chandra • Hemant Lata
Editors

High Altitude Medicinal Plants

Botany, Conservation and Cultivation

Springer

Editors
Suman Chandra
School of Pharmacy
The University of Mississippi
University, MS, USA

Hemant Lata
School of Pharmacy
The University of Mississippi
University, MS, USA

ISBN 978-3-031-99405-0 ISBN 978-3-031-99406-7 (eBook)
https://doi.org/10.1007/978-3-031-99406-7

Cover Image: Sinopodophyllum hexandrum (Berberidaceae). Photo credit: Dr. Yang Niu and Dr. Hang Sun (authors of Chapter 25)

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

15 Asteraceae Plants from Eastern Himalayan Region of India: An Overview on Potential Ethnomedicinal Application and In Vitro Conservation

Krishna Kant Pachauri and Rakhi Chaturvedi

Abstract

Eastern Himalayan region of India is one of the significant ecological regions across the globe and recognized as the 12th biodiversity hotspot by IUCN. The area is exceptionally rich in medicinal plant biodiversity and contains 500 species in Arunachal Pradesh, 140 species in Darjeeling Hills, and 36 species in Sikkim. Interestingly, almost all medicinal plants from this region are used by indigenous people for healing purposes. Asteraceae family plants are predominantly used by local communities like Apatani (19 species, 11 genera), Adi (8 species, 8 genera), Monpa (8 species, 5 genera), and Lohit (6 species, 5 genera) tribes of Arunachal Pradesh. The *Ageratum conyzoides, Artemisia nilagirica, A. maritima, Spilanthes acmella, S. paniculata, Blumea balsamifera, B. lacera, Vernonia cinerea, Eclipta prostrata,* and *Chrysanthemum indicum* are a few of the most commonly used medicinal plants from this family. The extracts of these plants have shown antimicrobial, antioxidant, antihelminthic, analgesic, anticancer, antimalarial, and anti-inflammatory activities. Various secondary metabolites including alkaloids, terpenoides, sesquiterpene lactones, N-alkylamides and phenolics have been reported as active ingredients from these plants. Extensive medicinal utilization and overexploitation of these plants lead to habitat depletion and pose an inevitable extinction threat. In vitro conservation strategies, such as tissue culture, micropropagation, synthetic seed technology and cryopreservation, are necessary to enhance the long-term stability of these plants. Considering the importance of Asteraceae plants, the current chapter highlighted the in vitro conservation strategies for sustainable utilization of these plants and their potential ethnomedicinal applications.

K. K. Pachauri · R. Chaturvedi (✉)
Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, Assam, India
e-mail: rakhi_chaturvedi@iitg.ac.in

S. Chandra, H. Lata (eds.), *High Altitude Medicinal Plants*,
https://doi.org/10.1007/978-3-031-99406-7_15

15.1 Introduction

Medicinal plants have been the source of herbal remedies for local communities since antiquity and are even considered responsible for the origin of modern therapeutic system (Salmerón-Manzano et al. 2020). According to the WHO report (2002), 80% of the world's population depends on medicinal plants to cure various ailments. Approximately 80% of commercially available synthetic drugs are derived from herbal products (Bauer and Brönstrup 2014). The minimal side effects and affordability are prime reasons for the upsurge in traditional health care systems (THCS). In developing countries, like India, a large section of the rural population (80%) depends on this healthcare system (Jeelani et al. 2018).

India is one of the 12 megadiverse countries, representing 11% of the world's flora and sharing approximately 2.4% of the global land area. The country has a forest cover of about 22.5% of the total geographical area flourishing more than 17,000 angiosperm plant. Eastern Himalayan, Western Ghats, Indo-Burma, and Sundaland are four major biodiversity hotspots in India. Eastern Himalayan region is located at the juncture of the Indo-Malayan, Palearctic, and Sino-Japanese realms (Kandel et al. 2019). The high altitudes of eastern Himalaya lie in the eastern Nepal, Bhutan, and a few Indian territories like Darjeeling hills, Sikkim, and Arunachal Pradesh. Of these, Arunachal Pradesh (83,743 sq. km) and Sikkim (7096 sq. km) occupies a large area of this biodiversity hotspot (Tangjang et al. 2011). The area is exceptionally rich in biodiversity containing 500 species of medicinal plants in Arunachal Pradesh, 140 species in Darjeeling hills and 36 species in Sikkim (Banik et al. 2020). The Eastern Himalayan region also supports one of the world's richest alpine flora. Around one-third of alpine species are endemic to this region. The region's variable climate and changing altitudes create a diversity of habitats, from the cold deserts at very high altitudes to the evergreen tropical and temperate forests at low altitudes.

15.2 Asteraceae Family Plants

The Asteraceae family is one of the most diverse plant groups, comprising 1600–1700 genera and 24,000–30,000 plant species across the globe except Antarctica (Panda and Luyten 2018). It is India's fourth most prominent family of angiosperms after Poaceae, Orchidaceae and Leguminosae, representing more than 1050 plant species from 177 genera. The plants of this family are annual and perennial, vines, succulents, shrubs, and trees in nature. Northeast and Himalayan regions are the primary centres of these plants, containing 72.67% of total Asteraceae in India (Mitra and Mukherjee 2017; Abraham and Thomas 2016). As a global biodiversity hotspot, the region is extremely rich in medicinal herbs, and Asteraceae is the most dominant family in this area (Tangjang et al. 2011). *Ageratum conyzoides, Artemisia nilagirica, A. maritima, Spilanthes acmella, S. paniculata, Blumea balsamifera, B. lacera, Vernonia cinerea, Eclipta prostrata,* and *Chrysanthemum indicum* are some of the most commonly used local medicinal plants from this family (Mylliemngap and Arya 2021).

15.2.1 Traditional Medicinal Uses

Using medicinal plants to treat various ailments is an essential part of the health care system in India. Ayurvedic, Siddha, and Unani are three different traditional therapeutic systems of our country. The people from high altitudes of Arunachal Pradesh and Sikkim have a strong hold on herbal remedies. The local communities living there for thousands of years have built a vast knowledge base about the use of medicinal plants. These plants have been integrated into the culture of this region. The indigenous people of different tribes largely depend on medicinal plants due to their remoteness and inaccessibility to the urban areas. Asteraceae family plants are predominantly used by various tribes including Apatani (19 species, 11 genera), Adi (8 species, 8 genera), Monpa (8 species, 5 genera) and Lohit (6 species, 5 genera) from Arunachal Pradesh and Lepcha, Bhotia (4 species, 4 genera) from Sikkim (Kala 2005; Tangjang et al. 2011; Jeyaprakash et al. 2017; Chakraborty et al. 2017; Namsa et al. 2009; Tamang et al. 2023) (Fig. 15.1).

Various phytochemicals including alkaloids, terpenoids, sesquiterpene lactones, N-alkylamides, and phenolics have been reported as active ingredients from these plants (Fig. 15.2). The extracts have shown antimicrobial, antioxidant, antihelminthic, analgesic, anticancer, antimalarial, and anti-inflammatory activities (Fig. 15.1). Various medicinal plants from this family have recently been thoroughly studied to find bioactive components against multiple ailments. The popularly available genera from the Asteraceae family in the eastern Himalayan region are *Ageratum*, *Artemisia*, *Spilanthes*, *Blumea*, *Vernonia*, *Eclipta*, and *Chrysanthemum.* Table 15.1 summarizes the bioactive components and the medicinal uses of the aforementioned plants.

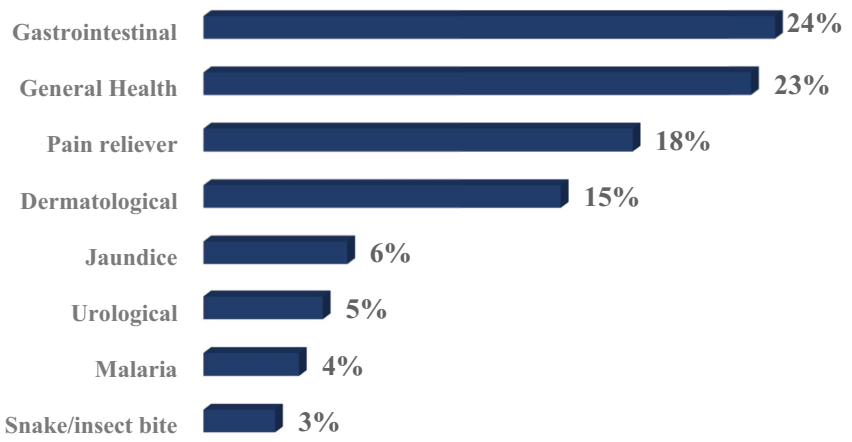

Fig. 15.1 Percentage of Asteraceae family plants used for treating various ailments (Mylliemngap and Arya 2021)

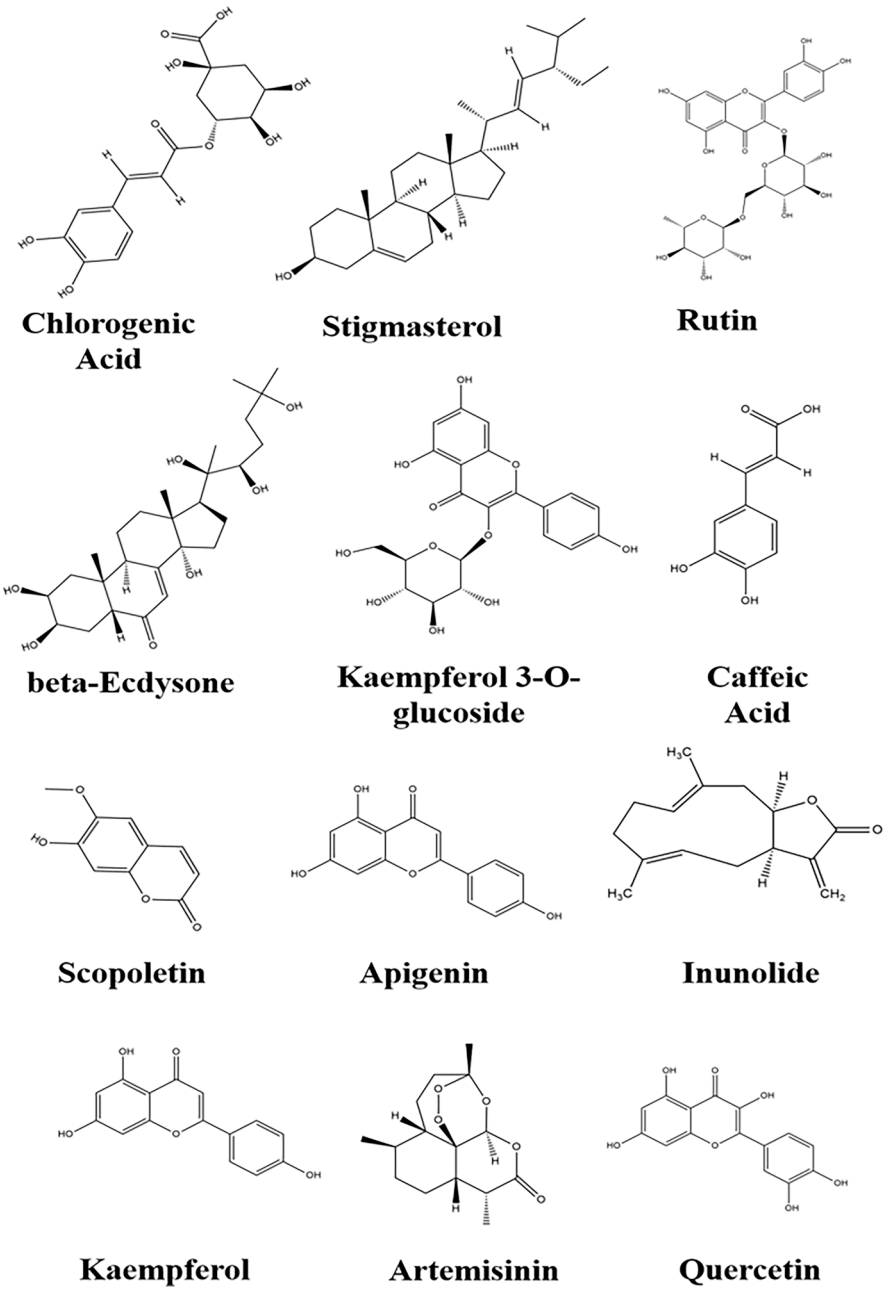

Fig. 15.2 Important bioactive metabolites from different plants of Asteraceae family from eastern Himalaya

Table 15.1 Ethnomedicinal plants belonging to Asteraceae family from Arunachal Pradesh, their important bioactive compounds and associated traditional medicinal applications

Sr. no.	Plant species	Plant part utilized	Extract	Important bioactive constituent	Medicinal uses	References
1.	*Artemisia maritima*	Whole plant	Methanolic	Epigallocatechin gallate, morin, ellagic acid, catechin hydrate, rutin, pyrogallol	Antioxidant, antimicrobial, antidiabetic, cytotoxic	Zaman et al. (2022)
2.	*Artemisia indica*	Aerial part	Aqueous	Artemisia ketone, germacrene B, borneol, chrysanthenyl acetate, *p*-cymene, α-thujone and β-pinene	Anti-tumor, antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, insecticidal and antiviral	Rashid et al. (2013)
3.	*Artemisia nilagirica*	Leaves	Ethanolic	Artemisinin, ferulic acid, luteolin, caffeic acid, quercetin, apigenin	Antifungal, anticancer, antioxidant	Albaqami et al. (2022)
4.	*Artemisia annua*	NO Data	Ethanol (80%)	Rutin, cynaroside, isorhamnetin, chrysosplenol D and casticin, scopolin and scopoletin, arteannuin B, artemisinin, dihydroartemisinic acid and artemisinic acid	Antimalarial, anticancer, hemorrhoids and bronchitis	Fu et al. (2020)
5.	*Artemisia vulgaris*	Whole plant	Ethanolic	Apigenin, chrysoeriol, diosmetin, eriodictyol, eupafolin, kaempferol, kaempferol-3-glucoside, homoeriodictyol, kaempferol 3-rhamnosid, rutin, vitexin, luteolin, quercetin, tricine	Antimalarial, anti-inflammatory, anti-hypertensive, antioxidant, anti-tumoral, immunomodulatory, hepatoprotective, antispasmodic, antiseptic	Abiri et al. (2018)
6.	*Ageratum conyzoides L*	Ariel parts	Aqueous petroleum ether	Kaempferol, stigmasterol, β-sitosterol, polymethoxy flavones, precocene I and precocene II	Antimicrobial, anti-inflammatory, analgesic, antioxidant, anticancer, antiprotozoal, antidiabetic, spasmolytic, allelopathy	Xu et al. (2023), Kamboj and Saluja (2011) and Yadav et al. (2019)
7.	*Spilanthes acmella Murr.*	Leaf and flower	Methanolic	Spilanthol, dodeca-2(E), 4(E)-dienoic acid isobutylamide and scopoletin	Antimalarial, anti-inflammatory, anaesthetic, anti-toothache	Singh and Chaturvedi (2010, 2012)

(continued)

Table 15.1 (continued)

Sr. no.	Plant species	Plant part utilized	Extract	Important bioactive constituent	Medicinal uses	References
8.	*Spilanthes paniculata*	Leaf and flower	Methanolic	Deca-2E,6Z,8E-trienoic acid isobutylamide (Spilanthol), UDA	Antimalarial, anti-inflammatory, anaesthetic, anti-toothache	Rajendran et al. (2017)
9.	*Blumea balsamifera*	Leaves	Hexane	Borneol L, veridiflorol	Anticancer	Rawati et al. (2023)
		Leaves	Petroleum ether, chloroform, and methanol	Tamarixetin, rhamnetin, luteolin, quercetin, blumeatin	Free radical-scavenging activity	Nessa et al. (2004)
10.	*Blumea lacera*	Whole plant	Methanolic	Linolenic acid, oleic acid, phytol	Antioxidant, cytotoxic, antimicrobial, and antidiarrhea	Ashrafi et al. (2022)
11.	*Vernonia cinerea*	Leaves	Methanol (90%)	Gallic acid, rutin, quercetin, caffeic acid, ferulic acid	Antidiarrhea, stomachache, cough and bronchitis	Rajamurugan et al. (2011)
12.	*Eclipta prostrata*	Whole plant	Methanolic	Ecliptal, eclalbatin, stigmasterol, wedelolactone, eclalbasaponins VII–X, eclalbasaponins I–VI, protocatechuic acid	Hepatoprotective, antimicrobial, antioxidant, anti-inflammatory, antiviral, anti-plasmodial, anti- pyretic, antihepatitis B, cytotoxic, hypotensive, hypo-lipidemic, antivenin, immunomodulatory, and analgesic	Chung et al. (2017)
13.	*Chrysanthemum indicum*	Flower	Methanol (60%)	Chlorogenic acid, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, linarin, luteolin-7-O-β-D-glucoside	Antibacterial, antiphlogistic, antimutagenic, antioxidant	He et al. (2016)

14.	*Chromolaena odorata* (Syn. *Eupatorium odoratum L.*)	Leaves	Ethanolic	Tamarixetin, trihydroxymonomethoxyflavanone, pentamethoxyflavanone, pentamethoxyflavanone, eupatilin, p-Coumaric acid, p-Hydroxybenzoic acid, protocatechuic acid (3,4-dihydroxyl benzoic acid)	Anti-inflammatory, antioxidant, antibacterial, anthelminthic, antifungal, anticonvulsant, antiprotozoal, cytotoxic, antipyretic, analgesic and antispasmodic	Phan et al. (2001) and Omokhua et al. (2016)
		Leaves	Methanolic	4-*O*-Methylsakuranetin, odoratin, ombuin, rutin, rhamnetin methyl caffeate, isovanillic acid, nonacosan-1-ol, β-sitosterol		Giang et al. (2024)
		Leaves	Aqueous	α-pinene, cadinene, (+)-camphor, limonene, β-caryophyllene, cadinol isomer		Inya-agha et al. (1987)
15.	*Bidens pilosa*	Whole plant	70% ethanol	Rutin, hyperoside, 4,5-O-dicaffeoylquinic acid	Anti-malarial, anti-diabetic, anti-tumoral, antimicrobial, hepatoprotective, antioxidant	Cortés-Rojas et al. (2013)
		Roots	Chloroform	1-phenyl-1,3-diyn-5-en-7-ol-acetate, antimalarial		Brandão et al. (1997)
16.	*Inula racemosa*	Roots	Ethanolic	Alantolactones, isoalantolactone, alantolides, sitosterol, daucosterol, inunolide, dihydroisoalantolactone, d-mannitol	Precordial chest pain, cough and dyspnoea, cardioprotective	Rathore et al. (2022) and Kalachaveedu et al. (2018)
17.	*Gynura bicolor*	Leaves	Ethyl acetate and water	5-p-trans-coumaroylquinic acid, 4-hydroxybenzoic acid, rutin, kampferol-3-*O*-rutinoside, 3,5-dicaffeoylquinic acid, kampferol-3-*O*-glucoside guanosine, chlorogenic acid	Anticancer, anti-hyperglycemic, antioxidant and anti-inflammatory	Teoh et al. (2016)

(continued)

Table 15.1 (continued)

Sr. no.	Plant species	Plant part utilized	Extract	Important bioactive constituent	Medicinal uses	References
18.	*Eupatorium cannabinum L.*	Whole plant	Chloroform and ethanol	β-Ecdysone, eupatorin, eupatilin, quercetin, rutin, caffeic acid	Antitumor, anti-inflammatory, hepatoprotective, immunomodulatory	Grigore et al. (2018)
19.	*Sonchus arvensis*	Leaves	Chloroform	Ascorbic acid, gallic acid, catechin, quercetin, myrecetin, kaempferol	Analgesic, sedative antibiotic, and antipyretic,	Seal (2016)
20.	*Sonchus oleraceus*	Leaves	Methanol (70%)	Caftaric acid, chicoric acid, chlorogenic acid	Antioxidant	Ou et al. (2013)

15.2.2 Geography and Climate of High Altitudes of Eastern Himalaya

The Indian portion of the Eastern Himalayas covers an area of 524,190 sq. km contributing more than 52% of the total eastern Himalayan region (Panda 2022). The high altitudes of eastern Himalaya are in Arunachal Pradesh (83,743 sq. km), Sikkim (7096 sq. km), and Darjeeling district (3149 sq. km) of West Bengal. The region owes a great altitudinal variation from foothills (<100 m) through timberline (4000 m) up to Mt. Khangchendzonga (8548 m). The annual temperature lies between less than 0 and 31 °C with 2000–8000 mm of annual rainfall. The region is thinly populated with lowest population density with 17 people per sq. km. in Arunachal Pradesh region (ISFR 2019).

15.3 Biodiversity Conservation

Eastern Himalaya is one of the significant biodiversity hotspots among 34 globally recognized biodiversity hotspots. A large number of medicinal plants are present in the region due to the unique topography and high degree of precipitation. According to an ethnobotanical survey conducted by Tangjang et al. (2011), medicinal plants have become an integral part of the traditional healthcare system of the indigenous people of Arunachal Pradesh. Over 500 medicinal plants have been identified and used by different tribes in the region for treating various ailments. Asteraceae family plants are highly used among the available medicinal plants. Recently, many of these herbs have become endangered and included in the Red-listed plants in this region. At present, some plants like *Inula racemosa* Hook F. (Rathore et al. 2022), *Spilanthes acmella*, (Sharma and Shahzad 2013), and *Artemisia vulgaris* (Govindaraj et al. 2008) are threatened and critically endangered perennial herbs throughout the Himalayan region. The overexploitation and unskilled harvesting of these plants may lead to their natural habitat depletion and pose an inevitable extinction threat. The conservation of these rich medicinal resources is necessary to stop their disappearance.

The primary objective of conserving these medicinal plants is to maintain the ecosystem stability and sustainable utilization of the phytochemicals for product development. The plant species can be conserved by two means: In situ and ex situ. In situ conservation methods, such as creating national parks, sanctuaries, and biosphere reserves can only preserve natural breeding. Ex situ conservation includes collecting, preserving, and maintaining plants in botanical gardens, gene banks, in vitro banks, seed banks and cryopreservation, keeping their characteristics and genetic information identical to the wild population (Priyanka et al. 2021) (Fig. 15.3). These methods are beneficial in case of heavy loss or sharp decline in the plant species due to habitat destruction or climate change in the region. Ex situ conservations, like gene banks, can provide excellent planting materials and authentic raw drugs. Still, these methods can never be sufficient for the sustainable utilization of medicinal plants. The conservation of these high-value medicinal plants through

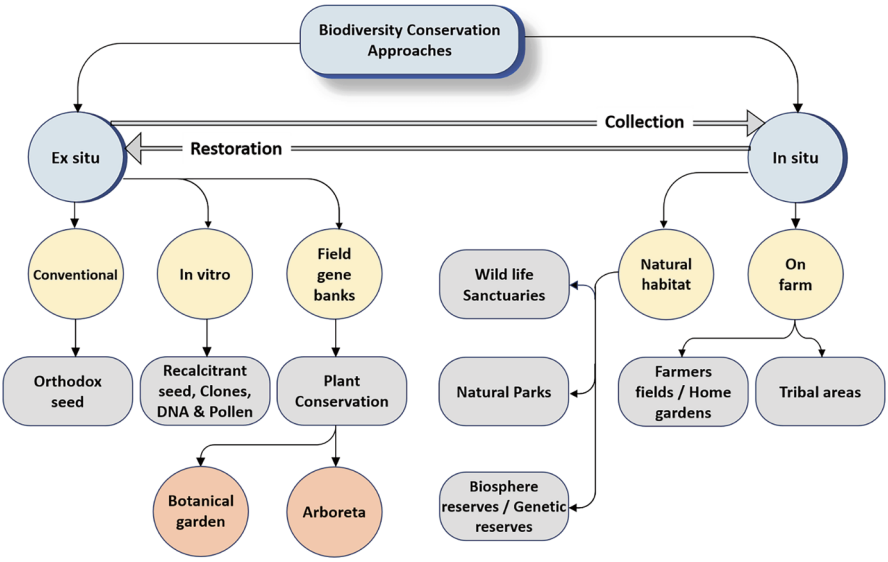

Fig. 15.3 Different methods of biodiversity conservation

modern biotechnological tools is necessary to safeguard them from extinction threats. In vitro conservation strategies pertaining to tissue culture, micropropagation, bioreactor mediated metabolite production, cryopreservation, and synthetic seed technology are most relevant methodologies to enhance the long-term stability of these plants. Micropropagation could be an excellent technique for in situ conservation, as it quickly generates many good-quality plants. This technique can improve the plant population and restore natural habitats after destructive harvesting or reproductive barriers (Krishnan et al. 2011).

15.3.1 In Vitro Methodologies for the Conservation of Plants

Ex situ conservations through in vitro methods are implemented when in situ conservations are insufficient, and sometimes, these are the only methods to save the species. It acts as a backup to in situ conservation for certain threatened plant species that might be lost if not conserved through "off-site conservation." Although these techniques have been explored for a long time, only a few practices, such as seed banks and in vitro methodologies, have been successfully implemented (Li and Pritchard 2009).

Plant biotechnology advancements related to in vitro culture and molecular biology have produced practical tools to improve and assist plant diversity conservation and management. In vitro conservation is essential, particularly for plants that do not produce viable seeds as in case of Banana and *Stevia* (Cruz-Cruz et al. 2013). In vitro methods of ex situ conservation, like micropropagation, slow growth

maintenance, bioreactor mediated biomass and metabolite productions, cryopreservation and synthetic seeds, are excellent tools for providing good quality pathogen-free raw material and reducing the burden over wild flora (Kulak et al. 2022).

15.3.1.1 Micropropagation

Micropropagation (syn.: in vitro propagation) is the most frequently used term for aseptically producing true-to-type or clonal plants using a variety of tissues, cells, or cell organs. It includes inoculating small section of tissue or organ (explant) in a closed sterile vessel on a defined artificial medium under controlled physical and chemical conditions. The idea of micropropagation was first visualized by an Austrian botanist Gottlieb Haberlandt (1854–1945) in 1902 by successfully culturing the tomato roots, tobacco and carrot calluses. Unlike most animal cells, the totipotent nature of plant cells provides a potential to generate whole plant from an individual cell or group of cells expressing the entire genome (Loberant and Altman 2010). The controlled conditions include the specific growth medium with or without plant growth regulators (PGRs), photoperiod, temperature, and humidity of the environment. Various PGRs including auxin, cytokinin, and gibberellin are supplemented in the medium to control the growth and morphogenesis of the explants. A small section of meristematic (shoot-tip, apical, axillary or lateral buds) and non-meristematic tissues (roots, internodal section, leaf, petiole) are used as an explant (Deepa and Thomas 2020).

Apart from large-scale clonal propagation, the application of micropropagation has been expanded to several areas, like germplasm conservation, generation of virus free plants, genetically engineered plants, somatic hybridization, somatic embryogenesis, large biomass production through bioreactor technology and drug production through callus and root suspension cultures. Several medicinal plants in the eastern Himalayan region are under rare, endangered or threatened categories. Some of them are *Inula racemosa* (Kaur et al. 2010), *Spilanthes acmella* (Singh and Chaturvedi 2010), *Ageratum conyzoides* L. (Sharma 2008), *Artemisia annua* (Gulati et al. 1996), and *Vernonia cinerea* (Maheshwari and Kumar 2006). Unlike conventional vegetative propagation methods, viz. stem cutting and grafting, micropropagation provides the required number of true-to-type or clonal plants in less time with minimal labor requirements, irrespective of seasonal and regional variations. It is a reliable conservation method for important medicinal plants, and some of the conservation protocols are summarized in Table 15.2.

15.3.1.2 Cryopreservation

The in vitro strategies of ex-situ plant conservation can be used for 1–5 years. However, the cryopreservation method can help to store the plants or their tissues for >5 years. The in vitro generated plant tissues can be used as a starting material for cryopreservation. In this method, the plant tissues are stored at very low temperature (Approx. 165–196 °C) after treating them with a suitable cryoprotectant. Liquid nitrogen or nitrogen vapors are used to maintain these low temperatures for a long time (Kaviani and Kulus 2022). In liquid nitrogen, the metabolic processes like enzymatic activities, respiration etc. are seized and cell division process is also

Table 15.2 Summary of the in vitro conservation methods of Asteraceae plants from Arunachal Pradesh

Sr. no	Plant species	Explant	Medium composition	In vitro methodology	References
1	*Artemisia maritima*	Stem and leaf	MS + 2,4-D (11.31 µM) + BAP (6.66 µM)	Callus induction	Nabi et al. (2022)
		Nodal segment and shoot-tip	MS + BAP (6.66 µM)	Shoot regeneration	
		Green callus from stem	MS + TDZ (4.54 µM) + IBA (12.30 µM)	Somatic embryogenesis	
2	*Artemisia nilagirica*	Nodal segment	MS + IAA (14.27 µM)	Callus induction	Shinde et al. (2016)
		Callus induced from nodal segment	MS + BAP (2.50 µM) + 2iP (7.50 µM)	Adventitious shoot regeneration	
3	*Artemisia annua*	Shoot-tip and nodal explant	MS + BAP (4.44 µM) + IBA (0.49 µM)	Micropropagation	Hailu et al. (2013)
		Leaves	MS + TDZ (2.72 µM) + IBA (0.49 µM)	Somatic embryogenesis	Alejos-Gonzalez et al. (2013)
4	*Artemisia vulgaris*	Nodal explants	MS + 2iP (4.9 µM)	Micropropagation	Sujatha and Kumari (2008)
		Shoot-tip	MS + BAP (4.44 µM)	Micropropagation	Govindaraj et al. (2008)
		Shoot-tip and nodal segment	MS + BAP (4.44 µM) + kinetin (2.32 µM)	Micropropagation	Sujatha and Kumari (2007)
5	*Ageratum conyzoides*	Nodal segment	MS + BAP (13.32 µM) + IAA (17.13 µM)	Micropropagation	Sharma (2008)
6	*Spilanthes acmella*	Nodal segment	MS + BAP (5.0 µM)	Micropropagation	Singh and Chaturvedi (2010)
		Leaf	MS + BAP (10.0 µM) + NAA (1.0 µM)	Callus induction	Pandey and Agrawal (2009)
		Callus	MS + BAP (10 µM)	Shoot generation	

7	*Spilanthes paniculata*	Flowerheads	MS + 2,4-D (1 μM) + NAA (1 μM) + BAP (5 μM)	Callus induction	Rajendran et al. (2017)
8	*Blumea balsamifera*	Shoot tips	MS + BA (4.44 μM)	Micropropagation	Soriano and Cangao (2009)
9	*Blumea lacera*	Nodal segment and leaf	MS + BAP (13.32 μM) + kinetin (4.65 μM)	Micropropagation and adventitious shoot regeneration	Swaraz et al. (2020)
10	*Vernonia cinerea*	Leaf and nodal explants	MS + BAP (8.88 μM) + NAA (8.06 μM)	Shoot regeneration	Seetharam et al. (2007)
11	*Chrysanthemum indicum*	Apical meristems	MS + BAP (4.44 μM) + IAA (0.57 μM)	Micropropagation	Zafarullah et al. (2013)
12	*Chromolaena odorata*	Nodal explants	MS + BAP (2.22 μM) + IAA (0.57 μM)	Micropropagation	Mani et al. (2020)
13	*Bidens pilosa*	Stem and leaf section	MS + BAP (4.44 μM) + 2,4-D (2.04 μM)	Callus induction	Ramabulana et al. (2021)
14	*Inula racemosa*	Seeds	MS + BAP (4.44 μM)	Micropropagation	Kaur et al. (2010)
15	*Gynura bicolor*	Leaf	MS + BAP (2.22 μM) + 2,4-D (9.05 μM)	Micropropagation	Liu et al. (2011)
16	*Sonchus arvensis*	Leaf and petiole	MS + BAP (2.22 μM) + 2,4-D (4.52 μM)	Callus induction	Wahyuni et al. (2020)
17	*Sonchus oleraceus*	Stem	MS + NNA (7.42 μM)	Callus induction	Zhao et al. (2012)

BAP 6-Benzylaminopurine, *2,4-D* 2,4-Dichlorophenoxyacetic acid, *TDZ* Thidiazuron, *IBA* Indole-3-butyric acid, *IAA* 3-Indoleacetic acid, *2iP* N6-(2-Isopentenyl)Adenine, *Kinetin* 6-Furfurylaminopurine, *NAA* 1-Naphthaleneacetic acid, *NNA* Nitro-L-arginine

effectively arrested. These physiological and biochemical changes in the plant tissue under low temperatures make long-term storage possible. Cryoprotectants, such as dimethylsulphoxide (DMSO), sorbitol and mannitol, are used before freezing the tissue. The principal role of cryoprotectants is to avoid any physical and chemical injury during storage. The cryoprotecting agents lower the freezing point by increasing the viscosity of the cytosol. These compounds increase the plasma membrane stability and modulate water distribution inside and outside the cell (Kaviani and Kulus 2022).

Cryopreservation is highly evolved from the conventional two-step cooling to modern one-step methods, such as Desiccation, Pregrowth or Preculture, Pregrowth-Desiccation, Encapsulation-Dehydration, Vitrification, Encapsulation-Vitrification, Droplet-Vitrification, Cryo-Plates and Cryo-Mesh (Deepa and Thomas 2020) (Table 15.3). The development of any cryopreservation protocol is highly dependent on the stress tolerance capacity of the plants. Almost every cryopreservation method follows the six most common steps (Nagel et al. 2023).

1. *Pretreatment*—The pretreatment of in vitro generated tissues is necessary to increase their resistance against low temperatures.
2. *Treatment with cryoprotectant*—Treatment with penetrating (Glycerol, DMSO, ethylene glycol (EG) and propylene glycol (PG)) or non-penetrating cryoprotectants (sorbitol, mannitol, sucrose, dextran) is necessary to enable the cells to withstand freezing temperatures.
3. *Cooling*—Slow or fast cooling is necessary before the tissues are stored.
4. *Storage*—After cooling, tissues must be stored in a liquid nitrogen chamber.
5. *Rewarming*—It is a crucial step before recovery of the plant where the samples are rapidly transferred to a water bath at 35–40 for 2–3 min.
6. *Recovery*—In this step, the solutions or materials have been removed, and the viability of the stored tissues has been evaluated.

Inula racemosa is an important medicinal plant from the Asteraceae family. The plant is critically endangered and widely distributed throughout the eastern Himalayan region of India. Although the plant has been reported to have several medicinal properties, including antispasmodic, hypotensive, anti-inflammatory, digestive, cardiotonic, alexipharmic, aphrodisiac and antiseptic, roots of this plant are primarily used for cough treatment by the indigenous people. The plant has become critically endangered due to unskilled, illegal and unscientific harvesting to fulfil the increasing pharmaceutical and stakeholder demands (Rathore et al. 2022). In another report, Kaur et al. (2010) have developed a strategy to conserve this plant through in vitro propagation and cryopreservation. They obtained a high multiplication in MS medium supplemented by BAP (4.44 μM). The in vitro generated shoot tips were cryopreserved through vitrification, and a 64% survival rate was obtained after cryopreservation.

Two separate studies have also been conducted on the cryopreservation of transformed roots and callus of *Artemisia annua.* According to Teoh et al. (1996), the root tips of *A. annua* were conserved in a cryoprotecting mixture containing 8%

Table 15.3 Modern one-step methods of cryopreservation

Sr. no.	Cryopreservation method	Application procedure	References
1	Desiccation	In this process, tissue sample is dehydrated using sterile air (laminar airflow) and kept in a liquid nitrogen container.	Engelmann (2004)
2	Pregrowth or preculture	In this technique, the samples are cultivated in the presence of cryoprotectants like sugars (fructose, glucose, sucrose) or sugar alcohols (mannitol, sorbitol) and frozen directly into liquid nitrogen.	Engelmann (2004)
3	Pregrowth-desiccation	The rice embryos were precultured in a cryoprotectant (sucrose, sorbitol) and then air dried up to 10% water content. After drying, the samples were plunged into the liquid nitrogen	Zhang et al. (2001)
4	Encapsulation-dehydration	This technique is based on the artificial seed synthesis method. Plant material is encapsulated in alginate beads and precultured in sucrose rich medium. The sample is partially dried in up to 20–30% (fresh weight basis) and then frozen rapidly in liquid nitrogen.	Gonzalez-Arnao and Engelmann (2006)
5	Vitrification	The explant is precultured in a medium with high sucrose and salt concentrations. After preculture, sample is treated with loading solution (LS) followed by the application of PVS[a] (plant vitrification solution) for dehydration of the sample in the next step.	Roque-borda et al. (2021)
6	Encapsulation-vitrification	This method is a combination of encapsulation, dehydration, and vitrification. The samples are first encapsulated using alginate beads, and then further treated LS and PVS	Engelmann (2004)
7	Droplet-vitrification	Initial steps like preculture, LS, and PVS treatment are similar to those in the vitrification process. The cooling and rewarming are different in this method. The explant is covered in a drop of PVS on an aluminium sheet and immersed in liquid nitrogen. During rewarming, the explant is covered in aluminium foil and kept in a sucrose solution at room temperature for 20–30 min.	Roque-borda et al. (2021)
8	Cryo-plates and cryo-mesh	Aluminium cryo-plates containing 10–12 oval shaped tiny wells are used in this method. There are two types of cryo-plates: vitrification cryo-plates (V type) or dehydration cryo-plates (D type). The explant is encapsulated in alginate beads and dehydrated using PVS (V type) or air drying in laminar air flow (D type). The protocol for both methods has similar steps except the dehydration step.	Vujović et al. (2024) and Roque-borda et al. (2021)

[a]*PVS* a mixture of different cryoprotectants at different ratios. PVS1, PVS2, PVS3, PVS4 are available. LS 0.6 M sucrose, 2 M glycerol

(v/v) DMSO and 20% (w/v) sucrose at 25 °C for 1 h, followed by cooling to 4 °C at a rate of 0.09 °C/min then further cooling to −35 °C at 0.72 °C/min. After this cooling, the samples were plunged into the liquid nitrogen. An average of 65% of root tips showed regrowth after rewarming and recovery. In another study conducted by Chenshu et al. (2003), the leaf-disc generated callus of *A. annua* was conserved through cryopreservation. The in vitro generated callus was precultured in MS medium supplemented with 5% (v/v) DMSO. After preculturing a mixture of cryoprotectants containing 15% (v/v) EG, 15% (v/v) DMSO, 30% (v/v) glycerol and 13.6% (w/v) sucrose were applied before plunging the callus into the liquid nitrogen container. A high survival rate of 87% was achieved by rewarming the callus in a water bath at 25 °C and reloading in a 34% (w/v) sucrose solution. These cryopreservation methods of conserving plants like *I. racemose* and *A. annua* are practical techniques with high recovery rates. They can be applied to the other plants of the Asteraceae family and other families as well.

15.3.1.3 Synthetic Seed Technology

The idea of synthetic seed technology was first presented by Murashige (1977) in a symposium on tissue culture at Gent, Belgium. Initially, he proposed the encapsulation of in vitro generated somatic embryos. A successful synthesis of artificial seeds was reported by applying the water-soluble resin and polyoxyethylene glycol over carrot somatic embryos (Kitto and Janick 1982). Later, synthetic seeds of alfalfa were also synthesized by encapsulating the somatic embryos in alginate hydrogel (Redenbaugh et al. 1984). These seeds are produced by encapsulating the in vitro or in vivo generated explants like somatic embryos, calli, nodal segments, adventitious shoots, shoot tips, Protocorm-like bodies (orchids), hairy roots and adventitious roots. This technology is helpful for plants with non-viable seeds or limited seed supply (Gantait et al. 2015). Apart from the explant selection, the encapsulating agent is equally important in synthetic seed technology. Different gels, like agar, gelrite, alginate, carrageenan, carboxy methyl cellulose and sodium pectate, have been used for encapsulation; alginate has proved to be the best encapsulating agent. Sodium alginate is the most frequently used gelling agent due to its low cost and non-toxic nature. The encapsulated seeds are further exposed to $CaCl_2 \cdot H_2O$ solution to harden the hydrogels (Gantait et al. 2015).

Sudarshana (2013) reported the successful encapsulation of somatic embryos of *Artemisia vulgaris* L. in 2% alginate. In this process, $CaCl_2 \cdot H_2O$ (75 mM) was used to harden the hydrogel covering over the somatic embryos, and these embryos can be stored for 4 months at 4 °C temperature. These encapsulated embryos were cultured again on MS medium supplemented with GA_3 (4.33 μM) + IAA (2.85 μM) + Ascorbic acid (227.12 μM) with 90% regeneration frequency.

Sometimes, the generation of somatic embryos is not easy to produce. Therefore, other explants, like nodal segments, calli, adventitious shoots, shoot tips, roots, etc., are the best alternatives for encapsulation. In a study conducted by Shahzad et al. (2009), in vitro raised nodal segments of *Spilanthes acmella* were encapsulated into the 4% sodium alginate and hardened in $CaCl_2 \cdot H_2O$ (100 mM). Encapsulated nodal segments were stored at 4 °C temperature for 1–8 weeks. These nodal segments

were regenerated on MS medium with BAP (1.0 μM) and NAA (0.5 μM) with frequency of 87.8 ± 1.15%. The plants were generated from the alginate beads encapsulated nodal segments and successfully hardened with a 90% success rate. In another study, the axillary buds of *S. mauritiana* were encapsulated in 3% (w/v) sodium alginate and stored at 4 °C temperature. Approximately all synseeds retained their viability and showed maximum sprouting on ½ MS medium (Sharma et al. 2009).

15.4 *Spilanthes paniculata* Wall. ex DC.

Spilanthes paniculata wall ex. DC. is an indigenous plant of Arunachal Pradesh belonging to the Asteraceae family. It is a rich source of various bioactive N-alkylamides and is widely used for food and medicinal purposes by indigenous tribes in the region. The flowerheads of this plant are used mainly for immediate toothache relief, therefore also called an anti-toothache plant. Apart from the anti-toothache activity, the plant also possesses several other biological activities like immunomodulatory, bio-insecticidal, larvicidal, anaesthetic, and anthelminthic (Rajendran and Chaturvedi 2017). The spilanthol (Deca-2E,6Z,8E-trienoic acid isobutylamide) and dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamide are the most essential therapeutic compounds available in hexane extract of flower and roots respectively. These metabolites are primary ingredients in beauty care, anti-ageing, and cosmetic application, and they reduce muscle tension and decrease visible wrinkles (Tiwari et al. 2011).

The rising global interest in *Spilanthes* and its therapeutic and cosmetic products increases the demand for plant raw materials. However, the natural habitats of this plant are diminishing at an alarming rate due to overharvesting and uncontrolled cattle grazing. Although *S. paniculata* is predominantly propagated through seeds, the geographical and seasonal restrictions can not be ignored. Obtaining pathogen free plant is also one of the major limitations of seed cultivation in the natural habitat. To overcome these limitations and fulfil continuously increasing raw material demand, micropropagation is a valuable strategy for large scale production of plants and secondary metabolites with consistent quality and quantity of the desired products.

15.4.1 Axillary Bud Proliferation and Callus Generation in *S. paniculata*

It is the most popular approach of micropropagation, where true-to-type plants are produced with uniform characteristics to the source plant (Fig. 15.4). Researchers have reported the axillary bud proliferation in different species of *Spilanthes*. *S. acmella* is a well-studied plant species in the context of micropropagation and tissue culture studies (Singh and Chaturvedi 2010). Only a few reports are available related to the micropropagation of *S. paniculata*. Mahendran et al. (2006)

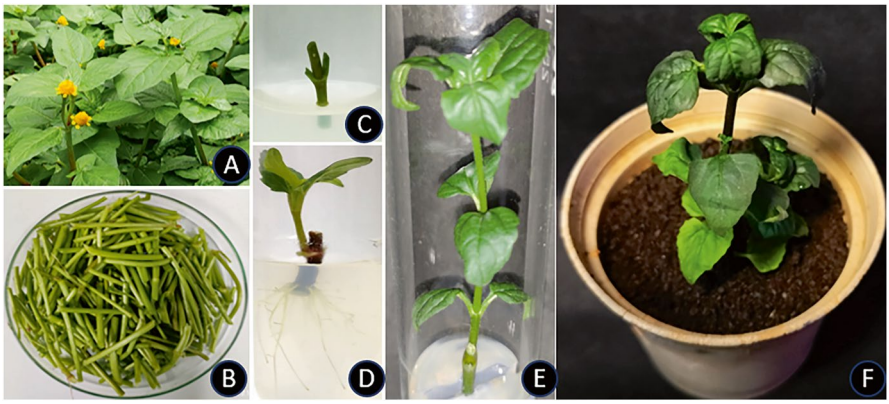

Fig. 15.4 Nodal segment culture of Spilanthes paniculata,. (a) Mother plant,. (b) Nodal segments collected from the mother plant. (c) Nodal segment inoculated on MS medium, (d) and. (e) Nodal segment after 1 and 3 weeks of culture. (f) Hardening of the rooted plant

established an efficient protocol for high frequency shoot generation in *S. paniculata* using nodal segment as an explant. In this study, Murashige and Skoog (MS) medium added with BAP (13.32 μM) showed a high number of shoot multiplication. On average, 35 shoots were obtained from one nodal segment inoculated as an explant. The generated shoots showed the best rooting response in half-strength MS medium (only major salts reduced to half) supplemented with IAA (0.57 μM) and NAA (0.54 μM).

Majority of reports related to the tissue culture studies on *S. paniculata* are based on callus generation or callus mediated shoot multiplication. Varsha and Ghulam (2015) reported that leaf, nodal and internodal segments were used for callus generation in MS medium supplemented with different plant growth regulators. The maximum callus proliferation response (84.62%) was obtained from the internodal segment in a medium supplemented with BAP (7.77 μM). In another report by Rajendran and Chaturvedi (2017), callus from the leaf-disc as an explant was generated as a method of conservation and sustainable biomass generation and utilization (Fig. 15.5). The half-strength MS medium supplemented with 5% sucrose, BAP (4.82 μM) and 2,4-D (1.8 μM) was the most suitable medium for biomass and metabolite production. The callus mediated shoot proliferation of *S. paniculata* has also been investigated. The MS medium added with NAA (1 μM) and BAP (5.0 μM) shows shoot differentiation from the leaf-disc generated callus. An average of 6.5 ± 2.6 shoots per explant were obtained from each explant responded. Further increasing the BAP concentration showed a drastic decrease in the shoot proliferation per explant (Pandey et al. 2014).

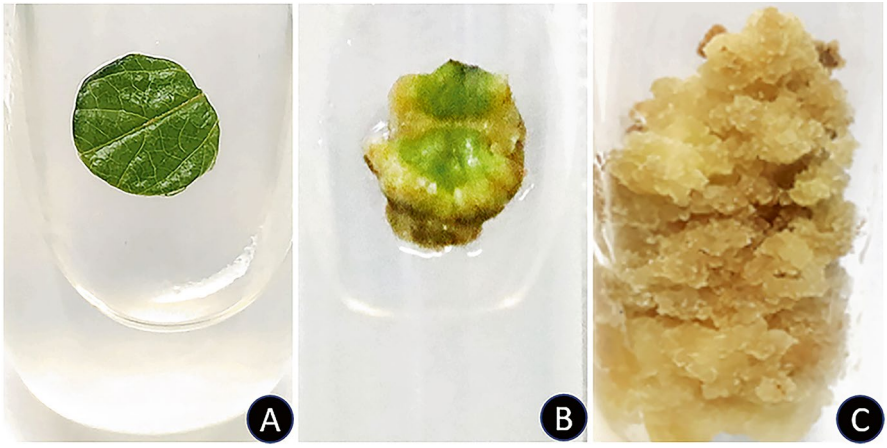

Fig. 15.5 Callus proliferation using leaf-disc as an explant. (a) Leaf-disc inoculated on MS medium supplemented with optimized growth regulators concentration. (b) Leaf-disc culture after 2 weeks of culture. (c) Callus proliferation after 5 weeks of culture

15.4.2 Adventitious Root Proliferation in *S. paniculata*

The pharmaceutical and cosmetic industry requires a large amount of biomass to extract essential metabolites like spilanthol from the different plants of *Spilanthes* sp. These metabolites can be directly used in various formulations or to generate pharmaceutically active derivatives. Regularly increasing applications of these metabolites increase the demand for raw plant materials. Adventitious root culture is an excellent alternative to produce natural plant biomass without posing any excess burden over natural flora. The roots originated from any other part of the plant except the radicle region, called adventitious roots. These roots are developed through complex molecular mechanisms based on exogenous and endogenous physiological factors. These roots are genetically more stable and have high biosynthetic capability. Adventitious roots are generated via direct organogenesis from the explant or indirectly through callus formation. These roots can be induced from the plant's petiole, leaf, internode and nodal segment by providing a suitable growth medium supplemented with or without auxin. Providing the auxin externally into the medium changes the endogenous auxin level of the explant, leading to the adventitious root formation (Khanam et al. 2022).

A study was conducted on adventitious root formation from leaf-disc and nodal segment in our laboratory. The leaf-disc (10 mm dia.) and nodal segments (2–3 cm) were inoculated on an optimized MS medium supplemented with suitable concentrations of auxins, like IAA and IBA (Fig. 15.6). After inoculation, both the explants were kept in a photoperiod of 16/8 h light/dark at 25 °C temperature. After 7–10 days of incubation, the roots were induced from both the explants. After 4 weeks of culture in the semi-solid medium, roots were inoculated in the 250 ml Erlenmeyer flasks containing 100 ml of a similar composition suspension medium. The shake

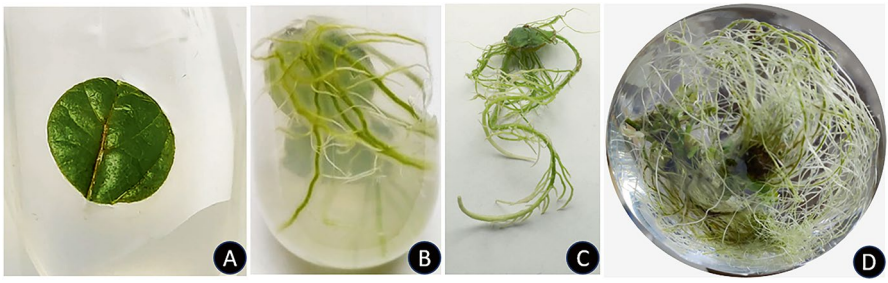

Fig. 15.6 Figure shows stages of adventitious root induction and proliferation from the leaf-disc tissue culture of *Spilanthes paniculata.* Subfigure A depicts a leaf-disc inoculated on an optimized semi-solid medium. Subfigure B shows inititiation and priliferation of adventitious roots from the leaf-disc explant after 4 weeks of culture. Subfigure C showing adventitious roots isolated and washed from semi-solid culture medium. Subfigure D displays adventitious roots inoculated in suspension medium of the similar composition

flasks were kept on an incubator shaker at 120 rpm, 25 °C and 16/8 h light/dark. After 3 weeks of culture in the liquid medium, the root biomass was harvested and further subjected to the analysis of the bioactive metabolites through chromatographic and spectrometric analysis.

15.4.3 Bioreactor Cultivation of Adventitious Roots of *S. paniculata*

The primary concern in the commercial production of phytochemicals is to convert the laboratory scale production to pilot or commercial scale. The in vitro production of plant biomass in the bioreactors is an attractive tool with better scaling-up possibilities. Unlike the traditional tissue culture system, biomass cultivation in the bioreactors also provides a real time monitoring and control of crucial process parameters like medium pH and temperature, dissolved oxygen and carbon di-oxide concentration inside the reactor vessel (Baque et al. 2012). The adventitious root cultivation has checked in different bioreactor forms, but every configuration has several advantages and disadvantages. The production of these cultures in the bioreactor is a significant challenge due to the difference between the nature of plant and microbial cells. Various factors affecting biomass and secondary metabolites production in bioreactors are impeller design, volumetric mass transfer coefficient (k_La), mixing time, agitation speed, liquid medium rheology and cell density. A suitable impeller design (Marine type, Rushton turbine, helical-ribbon type) is necessary in a stirred tank reactor for proper nutrient mixing and gaseous exchange. Modifications to the glass vessel, sparger and impeller design can be made to enhance the overall plant biomass productivity. Various explants such as

Fig. 15.7 Bioreactor cultivation of adventitious roots of Spilanthes paniculata. (a) A 3 litre capacity photobioreactor setup with controlling unit. (**b) A suspension root culture in bioreactor after 1-week of inoculation.** (**c**) Harvested adventitious root biomass after 3 weeks of inoculation

undifferentiated cells (callus) and differentiated tissues (root, shoot and embryos) can be used for biomass proliferation in bioreactors (Ruffoni et al. 2010).

An in vitro root culture study was performed on *S. paniculata* to produce the medicinally important N-alkylamides viz. (2E,6Z,8E)-N-(2-Methylpropyl) deca-2,6,8-trienamide (Spilanthol) and N-Isobutyl-(2E,4E,8Z,10E/Z)-dodecatetraenamide (DTAI). The roots were induced from the leaf-disc and nodal segment of the plant and cultivated into stirred tank bioreactor (STR) configurations (3 L capacity). Aeration, agitation, and inoculum size were optimized for bioreactor cultivation. Total biomass and metabolite production was higher in the bioreactor than in shake-flask cultures. The production of N-alkylamides was also observed as growth associated through growth kinetics studies in the bioreactor. The study results showed approximately 10× biomass growth, and this process can be scaled up further to a pilot plant scale or commercial scale (Fig. 15.7). Cell suspension culture in a bioreactor will pave the way for scaling up the biomass and metabolite production of medicinal plants in the future.

15.5 Conclusions

The indigenous communities of the eastern Himalayan region of India are majorly dependent over medicinal plants to cure most of their health issues. Asteraceae is one of the most widely used plant family for medicinal purposes in this region. Traditional practitioners and villagers use these species to treat cancer, snake/insect bites, malaria, kidney, jaundice, dermatological, pain relief, and gastrointestinal issues. These plants reportedly contain numerous bioactive metabolites like artemisinin, quercetin, rutin, spilanthol, apigenin etc. Although most of the plants of this family have been reported for at least one or more medicinal properties, *Artemisia*, *Spilanthes*, *Eclipta* etc. are a few important and frequently used genera. Climate change, alien species invasion, and extensive use of these plants for food and medicinal purposes destroy their natural habitats and may lead to extinction. At present, some Asteraceae herbs like *Inula racemosa* Hook F. (Rathore et al. 2022), *Spilanthes acmella*, (Sharma and Shahzad 2013), *Artemisia vulgaris* (Govindaraj et al. 2008) come under threatened and critically endangered categories throughout the Himalayan region. In vitro conservation of these plants through micropropagation (nodal segment and leaf-disc culture), bioreactor cultivation, cryopreservation, and synthetic seeds technology are excellent strategies to preserve the species.

The culture medium, plant growth regulators (PGRs), explant, and physical conditions are essential parameters that must be optimized for in vitro propagation. Bioreactor cultivation of plant biomass is also an emerging technology where large-scale production of plant biomass can be performed to meet food and pharmaceutical requirements. Finding the optimum bioreactor configuration for cultivating roots, embryos, calli, etc. is a significant challenge in this technology. The primary advantages of in vitro conservation strategies are genetic stability and an extended storage period. The explants can be stored for 3–5 years through micropropagation and more than 5 years through cryopreservation using liquid nitrogen. Synthetic seed technology is also another vital method of plant conservation. In this strategy, the somatic embryos, nodal segments, and calli are encapsulated through alginate as an explant. The current chapter includes detailed information on the medicinal aspects of Asteraceae plants from the eastern Himalayan region and their in vitro conservation methodologies. However, continuous and deepened research is required to conserve these plants of high medicinal importance. The information given in this book chapter here will help the researchers conserve medicinal plants from the Asteraceae and other plant families.

Acknowledgement We acknowledge. Ms Chetna Sharma for her kind assistance during the preparation of phytochemical structures.

References

Abiri R, Silva ALM, de Mesquita LSS, de Mesquita JWC, Atabaki N, de Almeida EB, Shaharuddin NA, Malik S (2018) Towards a better understanding of *Artemisia vulgaris*: botany, phytochemistry, pharmacological and biotechnological potential. Food Res Int 109:403–415. https://doi.org/10.1016/j.foodres.2018.03.072

Abraham J, Thomas TD (2016) Recent advances in Asteraceae tissue culture. In: Anis M, Ahmad N (eds) Plant tissue culture: propagation, conservation and crop improvement. Springer, Singapore, pp 161–195

Albaqami JJ, Benny TP, Hamdi H, Altemimi AB, Kuttithodi AM, Job JT, Sasidharan A, Narayanankutty A (2022) Phytochemical composition and in vitro antioxidant, anti-inflammatory, anticancer, and enzyme-inhibitory activities of *Artemisia nilagirica* (C.B. Clarke) pamp. Molecules 27(20):1–12. https://doi.org/10.3390/molecules27207119

Alejos-Gonzalez F, Perkins K, Winston MI, Xie D-Y (2013) Efficient somatic embryogenesis and organogenesis of self-pollination *Artemisia annua* Progeny and artemisinin formation in regenerated plants. Am J Plant Sci 04(11):2206–2217. https://doi.org/10.4236/ajps.2013.411274

Ashrafi S, Alam S, Islam A, Emon NU, Islam QS, Ahsan M (2022) Chemico-biological profiling of *Blumea lacera* (Burm. f.) DC. (Family: Asteraceae) provides new insights as a potential source of antioxidant, cytotoxic, antimicrobial, and antidiarrheal agents. Evidence-Based Complement Altern Med 2022:1–14. https://doi.org/10.1155/2022/2293415

Banik B, Das S, Das MK (2020) Medicinal plants with potent anti-inflammatory and anti-arthritic properties found in eastern parts of the Himalaya: an ethnomedicinal review. Pharmacogn Rev 14(28):121–137. https://doi.org/10.5530/phrev.2020.14.16

Baque MA, Moh SH, Lee EJ, Zhong JJ, Paek KY (2012) Production of biomass and useful compounds from adventitious roots of high-value added medicinal plants using bioreactor. Biotechnol Adv 30(6):1255–1267. https://doi.org/10.1016/j.biotechadv.2011.11.004

Bauer A, Brönstrup M (2014) Industrial natural product chemistry for drug discovery and development. Nat Prod Rep 31(1):35–60. https://doi.org/10.1039/c3np70058e

Brandão MGL, Krettli AU, Soares LSR, Nery CGC, Marinuzzi HC (1997) Antimalarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene and flavonoid compounds. J Ethnopharmacol 57(2):131–138. https://doi.org/10.1016/S0378-8741(97)00060-3

Chakraborty T, Saha S, Bisht NS (2017) First report on the ethnopharmacological uses of medicinal plants by Monpa tribe from the Zemith ang region of Arunachal Pradesh, Eastern Himalayas, India. Plants 6(1):A1–A3. https://doi.org/10.3390/plants6010013

Chenshu A, Wang X, Yuan X, Zhao B, Wang Y (2003) Optimization of cryopreservation of *Artemisia annua* L. callus. Biotechnol Lett 25(1):35–38. https://doi.org/10.1023/A:1021717828907

Chung IM, Rajakumar G, Lee JH, Kim SH, Thiruvengadam M (2017) Ethnopharmacological uses, phytochemistry, biological activities, and biotechnological applications of *Eclipta prostrata*. Appl Microbiol Biotechnol 101(13):5247–5257. https://doi.org/10.1007/s00253-017-8363-9

Cortés-Rojas DF, Chagas-Paula DA, Da Costa FB, Souza CRF, Oliveira WP (2013) Bioactive compounds in *Bidens pilosa* L. populations: a key step in the standardization of phytopharmaceutical preparations. Rev Bras 23(1):28–35. https://doi.org/10.1590/S0102-695X2012005000100

Cruz-Cruz CA, González-Arnao MT, Engelmann F (2013) Biotechnology and conservation of plant biodiversity. Resources 2(2):73–95. https://doi.org/10.3390/resources2020073

Deepa AV, Thomas TD (2020) In vitro strategies for the conservation of Indian medicinal climbers. In Vitro Cell Dev Biol Plant 56(6):784–802. https://doi.org/10.1007/s11627-020-10084-x

Engelmann F (2004) Plant cryopreservation: progress and prospects. In Vitro Cell Dev Biol Plant 40(5):427–433. https://doi.org/10.1079/IVP2004541

Fu C, Yu P, Wang M, Qiu F (2020) Phytochemical analysis and geographic assessment of flavonoids, coumarins and sesquiterpenes in *Artemisia annua* L. based on HPLC-DAD quantification and LC-ESI-QTOF-MS/MS confirmation. Food Chem 312:1–10. https://doi.org/10.1016/j.foodchem.2019.126070

Gantait S, Kundu S, Ali N, Sahu NC (2015) Synthetic seed production of medicinal plants: a review on influence of explants, encapsulation agent and matrix. Acta Physiol Plant 37(5):1–12. https://doi.org/10.1007/s11738-015-1847-2

Giang PM, Huong DTV, Thao VM, Thuy TTT, Trang VM (2024) Flavonoids from the leaves of *Chromolaena odorata* and their α-glucosidase inhibitory activity. Pharm Chem J 57:1621–1626

Gonzalez-Arnao MT, Engelmann F (2006) Cryopreservation of plant germplasm using the encapsulation-dehydration technique: review and case study on sugarcane. Cryo-Letters 27(3):155–168

Govindaraj S, Kumari BDR, Cioni PL, Flamini G (2008) Mass propagation and essential oil analysis of *Artemisia vulgaris*. J Biosci Bioeng 105(3):176–183. https://doi.org/10.1263/jbb.105.176

Grigore A, Neagu G, Dobre N, Albulescu A, Ionita L, Ionita C, Albulescu R (2018) Evaluation of antiproliferative and protective effects of *Eupatorium cannabinum* L. extracts. Turk J Biol 42(4):334–344. https://doi.org/10.3906/biy-1803-72

Gulati A, Bharel S, Jain SK, Abdin MZ, Srivastava PS (1996) In vitro micropropagation and flowering in *Artemisia annua*. J Plant Biochem Biotechnol 5(1):31–35. https://doi.org/10.1007/BF03262976

Hailu T, Abera B, Mariam EG (2013) In vitro mass propagation of Artemisia (*Artemisia annua* L.) cv: Anamed. Plant Tissue Cult Biotechnol 23(2):165–176. https://doi.org/10.3329/ptcb.v23i2.17518

He J, Wu X, Kuang Y, Wang T, Bi K, Li Q (2016) Quality assessment of *Chrysanthemum indicum* Flower by simultaneous quantification of six major ingredients using a single reference standard combined with HPLC fingerprint analysis. Asian J Pharm Sci 11(2):265–272. https://doi.org/10.1016/j.ajps.2015.08.010

Inya-agha SI, Oguntimein BO, Sofowora A, Benjamin TV (1987) Phytochemical and antibacterial studies on the essential oil of *Eupatorium odoratum*. Int J Crude Drug Res 25(1):49–52. https://doi.org/10.3109/13880208709060911

ISFR (2019) India State of Forest Report 2019 by Forest Survey of India. Ministry of Environment and Forests & Climate Change, Government of India, Dehradun, India. Available online https://fsi.nic.in/forest-report-2019. Accessed on 20 Jan 2024

Jeelani SM, Rather GA, Sharma A, Lattoo SK (2018) In perspective: potential medicinal plant resources of Kashmir Himalayas, their domestication and cultivation for commercial exploitation. J Appl Res Med Aromat Plants 8:10–25. https://doi.org/10.1016/j.jarmap.2017.11.001

Jeyaprakash K, Lego YJ, Payum T, Rathinavel S, Jayakumar K (2017) Diversity of medicinal plants used by Adi community in and around area of D'Ering Wildlife. World Sci News 65:135–159

Kala CP (2005) Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. J Ethnobiol Ethnomed 1:1–8. https://doi.org/10.1186/1746-4269-1-11

Kalachaveedu M, Raghavan D, Telapolu S, Kuruvilla S, Kedike B (2018) Phytoestrogenic effect of *Inula racemosa* Hook f—a cardioprotective root drug in traditional medicine. J Ethnopharmacol 210:408–416. https://doi.org/10.1016/j.jep.2017.09.001

Kamboj A, Saluja AK (2011) Isolation of stigmasterol and γ-sitosterol from petroleum ether extract of woody stem of *Abelmoschus manihot*. Int J Pharm Pharm Sci 3:94–96

Kandel P, Chettri N, Chaudhary RP, Badola HK, Gaira KS, Wangchuk S, Bidha N, Uprety Y, Sharma E (2019) Plant diversity of the Kangchenjunga Landscape, Eastern Himalayas. Plant Divers 41(3):153–165. https://doi.org/10.1016/j.pld.2019.04.006

Kaur R, Kashyap A, Majeed S, Chauhan NS, Bhardwaj SB (2010) In vitro propagation and conservation of *Inula racemosa* Hook. F. an endangered medicinal plant of temperate origin. J Adv Lab Res Biol 1(1):67–70

Kaviani B, Kulus D (2022) Cryopreservation of endangered ornamental plants and fruit crops from tropical and subtropical regions. Biology (Basel) 11(6):1–23. https://doi.org/10.3390/biology11060847

Khanam MN, Anis M, Bin JS, Mottaghipisheh J, Csupor D (2022) Adventitious root culture—an alternative strategy for secondary metabolite production: a review. Agronomy 12(5):1–18. https://doi.org/10.3390/agronomy12051178

Kitto SL, Janick J (1982) Polyox as an artificial seed coat for asexual embryos. Hortic Sci 17(3):488–488

Krishnan PN, Decruse SW, Radha RK (2011) Conservation of medicinal plants of Western Ghats, India and its sustainable utilization through in vitro technology. In Vitro Cell Dev Biol Plant 47(1):110–122. https://doi.org/10.1007/s11627-011-9344-9

Kulak V, Longboat S, Brunet ND, Shukla M, Saxena P (2022) In vitro technology in plant conservation: relevance to biocultural diversity. Plants 11(4):1–20. https://doi.org/10.3390/plants11040503

Li DZ, Pritchard HW (2009) The science and economics of ex situ plant conservation. Trends Plant Sci 14(11):614–621. https://doi.org/10.1016/j.tplants.2009.09.005

Liu LS, Li R, Zhao Y, Wen CL, Ren S, Guo YD (2011) High efficiency regeneration and genetic stability analysis of somatic clones of *Gynura bicolor* DC. Afr J Biotechnol 10(51):10380–10386. https://doi.org/10.5897/ajb10.1790

Loberant B, Altman A (2010) Micropropagation of plants. In: Encyclopedia of industrial biotechnology: bioprocess, bioseparation, and cell technology. Wiley, pp 329–346

Mahendran G, Lavanya Devi K, Narmatha Bai V (2006) Micropropagation of *Spilanthes paniculata* wall ex. DC. Plant Cell Biotechnol Mol Biol 7(1–2):85–88

Maheshwari P, Kumar A (2006) Organogenesis, shoot regeneration, and flowering response of *Vernonia cinerea* to different auxin/cytokinin combinations. In Vitro Cell Dev Biol Plant 42(6):589–595. https://doi.org/10.1079/IVP2006825

Mani M, Kannan N, Priyadarshini S, Shekhawat MS (2020) Effects of growth regulators and gelling agents on ex vitro rooting of Raspberry. In: Proceedings of the national conference on innovations in biological sciences (NCIBS), pp 78–97. https://doi.org/10.2139/ssrn.3554240

Mitra S, Mitra S, Mukherjee SK (2017) Asteraceae of India: its diversity and phytogeographical affinity. CABI, pp 36–70. https://doi.org/10.1079/9781780646947.0036

Murashige T (1977) Plant cell and organ cultures as horticultural practices. In: Acta horticulturae. International Society for Horticultural Science (ISHS), Leuven, pp 17–30

Mylliemngap W, Arya OP (2021) pharmacological potential of ethnomedicinal plants of Asteraceae family from Arunachal Pradesh Northeast India. In: Singh B (ed) Plants for novel drug molecules ethnobotany to ethnopharmacology. New India Publishing Agency, New Delhi, pp 141–168

Nabi N, Saffeullah P, Singh S (2022) Micropropagation using direct and indirect organogenesis in *Artemisia maritima* L.: scanning electron microscopy of somatic embryos and genome size analysis by flow cytometry. In Vitro Cell Dev Biol Plant 58(6):1012–1024. https://doi.org/10.1007/s11627-022-10291-8

Nagel M, Pence V, Ballesteros D, Lambardi M, Popova E, Panis B (2023) Plant cryopreservation: principles, applications, and challenges of banking plant diversity at ultralow temperatures. Annu Rev Plant Biol 16(8):7.1–7.28. https://doi.org/10.1097/00010694-198711000-00013

Namsa ND, Tag H, Mandal M, Kalita P, Das AK (2009) An ethnobotanical study of traditional anti-inflammatory plants used by the Lohit community of Arunachal Pradesh, India. J Ethnopharmacol 125(2):234–245. https://doi.org/10.1016/j.jep.2009.07.004

Nessa F, Ismail Z, Mohamed N, Haris MRHM (2004) Free radical-scavenging activity of organic extracts and of pure flavonoids of *Blumea balsamifera* DC leaves. Food Chem 88(2):243–252. https://doi.org/10.1016/j.foodchem.2004.01.041

Omokhua AG, McGaw LJ, Finnie JF, Van Staden J (2016) *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae) in sub-Saharan Africa: a synthesis and review of its medicinal potential. J Ethnopharmacol 183:112–122. https://doi.org/10.1016/j.jep.2015.04.057

Ou ZQ, Schmierer DM, Rades T, Larsen L, McDowell A (2013) Application of an online post-column derivatization HPLC-DPPH assay to detect compounds responsible for antioxidant activity in *Sonchus oleraceus* L. leaf extracts. J Pharm Pharmacol 65(2):271–279. https://doi.org/10.1111/j.2042-7158.2012.01591.x

Panda RM (2022) Ecology of Eastern Himalaya. In: Panda RM (ed) Plant ecology of Indian Himalaya. Springer, Cham, pp 55–84

Panda SK, Luyten W (2018) Antiparasitic activity in Asteraceae with special attention to ethnobotanical use by the tribes of Odisha, India. Parasite 25(10):1–25. https://doi.org/10.1051/parasite/2018008

Pandey V, Agrawal V (2009) Efficient micropropagation protocol of *Spilanthes acmella* L. possessing strong antimalarial activity. In Vitro Cell Dev Biol Plant 45(4):491–499. https://doi.org/10.1007/s11627-008-9184-4

Pandey V, Sharma G, Shankar V, Agrawal V (2014) Biodiversity and in vitro conservation of three medicinally important herbs: *Spilanthes acmella* L. var. oleraceae Clarke, *S. calva* L., and *S. paniculata* Wall. ex DC. Int J Geogr Inf Syst 20(3):295–318. https://doi.org/10.1080/10496475.2013.869520

Phan TT, Wang L, See P, Grayer RJ, Chan SY, Lee ST (2001) Phenolic compounds of *Chromolaena adorata* protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. Biol Pharm Bull 24(12):1373–1379. https://doi.org/10.1248/bpb.24.1373

Priyanka V, Kumar R, Dhaliwal I, Kaushik P (2021) Germplasm conservation: instrumental in agricultural biodiversity—a review. Sustainability (Switzerland) 13(12):1–18. https://doi.org/10.3390/su13126743

Rajamurugan R, Selvaganabathy N, Kumaravel S, Ramamurthy C, Sujatha V, Suresh Kumar M, Thirunavukkarasu C (2011) Identification, quantification of bioactive constituents, evaluation of antioxidant and in vivo acute toxicity property from the methanol extract of *Vernonia cinerea* leaf extract. Pharm Biol 49(12):1311–1320. https://doi.org/10.3109/13880209.2011.604334

Rajendran R, Chaturvedi R (2017) Screening and optimizing media constituents for enhanced production of medicinal N-alkylamide Deca-2E,6Z,8E-trienoic acid isobutylamide from dedifferentiated in vitro cell lines of *Spilanthes paniculata*. Biocatal Agric Biotechnol 9:95–102. https://doi.org/10.1016/j.bcab.2016.12.002

Rajendran R, Narashimman BS, Trivedi V, Chaturvedi R (2017) Isolation and quantification of antimalarial N-alkylamides from flower-head derived in vitro callus cultures of *Spilanthes paniculata*. J Biosci Bioeng 124(1):99–107. https://doi.org/10.1016/j.jbiosc.2017.02.001

Ramabulana AT, Steenkamp PA, Madala NE, Dubery IA (2021) Application of plant growth regulators modulates the profile of chlorogenic acids in cultured *Bidens pilosa* cells. Plants 10(3):1–13. https://doi.org/10.3390/plants10030437

Rashid S, Rather MA, Shah WA, Bhat BA (2013) Chemical composition, antimicrobial, cytotoxic and antioxidant activities of the essential oil of *Artemisia indica* Willd. Food Chem 138(1):693–700. https://doi.org/10.1016/j.foodchem.2012.10.102

Rathore S, Raj Y, Debnath P, Kumar M, Kumar R (2022) Ethnopharmacology, phytochemistry, agrotechnology, and conservation of *Inula racemosa* Hook f.—a critically endangered medicinal plant of the western Himalaya. J Ethnopharmacol 283:1–21. https://doi.org/10.1016/j.jep.2021.114613

Rawati S, Ginting B, Maulana I, Yahya M, Saidi N, Hasballah K (2023) Phytochemical, antioxidant, and cytotoxicity screenings of n-hexane extract from *Blumea balsamifera* L leaves. Res J Pharm Technol 16(4):1664–1668

Redenbaugh K, Nichol J, Kossler ME, Paasch B (1984) Encapsulation of somatic embryos for artificial seed production. In Vitro 20(2):256–257

Roque-borda CA, Kulus D, de Souza AV, Kaviani B, Vicente EF (2021) Cryopreservation of agronomic plant germplasm using vitrification-based methods: an overview of selected case studies. Int J Mol Sci 22(11):1–33. https://doi.org/10.3390/ijms22116157

Ruffoni B, Pistelli L, Bertoli A, Pistelli L (2010) Plant cell cultures: bioreactors for industrial production. In: Advances in experimental medicine and biology: bio-farms for nutraceuticals functional food and safety control by biosensors. Springer, pp 203–221

Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F (2020) Worldwide research trends on medicinal plants. Int J Environ Res Public Health 17(10):1–20. https://doi.org/10.3390/ijerph17103376

Seal T (2016) Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and Oenanthe linearis of North-Eastern region in India. J Appl Pharm Sci 6(2):157–166. https://doi.org/10.7324/JAPS.2016.60225

Seetharam YN, Rajanna LN, Jyothishwaran G, Aravind B, Sharanabasappa G, Mallikharjun PB (2007) In vitro shoot regeneration from leaf and nodal explants of *Vernonia cinerea* (L.) Less. Indian J Biotechnol 6(3):418–420

Shahzad A, Sahai A, Sharma S (2009) Artificial seeds for propagation and preservation of *Spilanthes acmella* (L.) Murr. a pesticidal plant species Thidiazuron: from urea derivative to plant growth regulator view project artificial seeds for propagation and preservation of *Spilanthes acmella*. Int J Plant Dev Biol 3(1):62–65

Sharma L (2008) Micropropagation of *Ageratum conyzoides* L.—an important medicinal plant. J Phytol Res 21(1):47–52

Sharma S, Shahzad A (2013) Efficient micropropagation of *Spilanthes acmella* (L.) Murr.: a threatened medicinal herb. Br Biotechnol J 3(3):405–415. https://doi.org/10.9734/bbj/2013/3556

Sharma S, Shahzad A, Jan N, Sahai A (2009) In vitro studies on shoot regeneration through various explants and alginate-encapsulated nodal segments of *Spilanthes mauritiana* DC., an endangered medicinal herb. Int J Plant Dev Biol 3(1):56–61

Shinde S, Sebastian JK, Jain JR, Hanamanthagouda MS, Murthy HN (2016) Efficient in vitro propagation of *Artemisia nilagirica* var. nilagirica (Indian wormwood) and assessment of genetic fidelity of micropropagated plants. Physiol Mol Biol Plants 22(4):595–603. https://doi.org/10.1007/s12298-016-0379-6

Singh M, Chaturvedi R (2010) Improved clonal propagation of *Spilanthes acmella* Murr. for production of scopoletin. Plant Cell Tissue Organ Cult 103(2):243–253. https://doi.org/10.1007/s11240-010-9774-9

Singh M, Chaturvedi R (2012) Screening and quantification of an antiseptic alkylamide, spilanthol from in vitro cell and tissue cultures of *Spilanthes acmella* Murr. Ind Crop Prod 36:321–328

Soriano TL, Cangao EC (2009) In vitro regeneration of sambong (*Blumea balsamifera* Linn.). Philipp Agric Sci 92(1):19–24

Sudarshana MS (2013) In vitro regeneration of multiple shoots from encapsulated somatic embryos of *Artemisia vulgaris* L. IOSR J Pharm Biol Sci 6(6):11–15. https://doi.org/10.9790/3008-0661115

Sujatha G, Kumari BDR (2007) Effect of phytohormones on micropropagation of *Artemisia vulgaris* L. Acta Physiol Plant 29(3):189–195. https://doi.org/10.1007/s11738-006-0023-0

Sujatha G, Kumari BDR (2008) Micropropagation, encapsulation and growth of *Artemisia vulgaris* node explants for germplasm preservation. S Afr J Bot 74(1):93–100. https://doi.org/10.1016/j.sajb.2007.09.002

Swaraz AM, Khan Sumi S, Sultana F, Hasan M, Islam MM, Bari MW, Islam MA, Satter MA, Ahmed KS, Hossain MH (2020) Bioactive compound and bioactivity fidelitous micropropagation method of *Blumea lacera* (Burm. f.) DC.: a large scale production potential. Ind Crop Prod 151:1–17. https://doi.org/10.1016/j.indcrop.2020.112370

Tamang S, Singh A, Bussmann RW, Shukla V, Nautiyal MC (2023) Ethno-medicinal plants of tribal people: a case study in Pakyong subdivision of East Sikkim, India. Acta Ecol Sin 43(1):34–46. https://doi.org/10.1016/j.chnaes.2021.08.013

Tangjang S, Namsa ND, Aran C, Litin A (2011) An ethnobotanical survey of medicinal plants in the Eastern Himalayan zone of Arunachal Pradesh, India. J Ethnopharmacol 134(1):18–25. https://doi.org/10.1016/j.jep.2010.11.053

Teoh KH, Weathers PJ, Cheetham RD, Walcerz DB (1996) Cryopreservation of transformed (hairy) roots of *Atemisia annua*. Cryobiology 33(1):106–117. https://doi.org/10.1006/cryo.1996.0011

Teoh WY, Tan HP, Ling SK, Abdul Wahab N, Sim KS (2016) Phytochemical investigation of *Gynura bicolor* leaves and cytotoxicity evaluation of the chemical constituents against HCT 116 cells. Nat Prod Res 30(4):448–451. https://doi.org/10.1080/14786419.2015.1017726

Tiwari KL, Jadhav SK, Joshi V (2011) An updated review on medicinal herb genus *Spilanthes*. J Chin Integr Med 9(11):1170–1178. https://doi.org/10.3736/jcim20111103

Varsha NN, Ghulam MY (2015) Callus culture of *Spilanthes paniculata* (DC) Jansen—an aromatic medicinal plant. Int J Sci Res (IJSR) 4(4):3013–3015

Vujović T, Anđelić T, Marković Z, Gajdošová A, Hunková J (2024) Cryopreservation of highbush blueberry, strawberry, and saskatoon using V and D cryo-plate methods and monitoring of multiplication ability of regenerated shoots. In Vitr Cell Dev Biol Plant 60(1):85–97. https://doi.org/10.1007/s11627-023-10399-5

Wahyuni DK, Purnobasuki H, Kuncoro EP, Ekasari W (2020) Callus induction of *Sonchus arvensis* l. and its antiplasmodial activity. Afr J Infect Dis 14(1):1–7. https://doi.org/10.21010/ajid.v14i1.1

World Health Organization (2002) The world health report 2002: reducing risks, promoting healthy life. World Health Organization

Xu Z, Ji R, Zha X, Zhao H, Zhou S (2023) The aqueous extracts of *Ageratum conyzoides* inhibit inflammation by suppressing NLRP3 inflammasome activation. J Ethnopharmacol 309:1–9. https://doi.org/10.1016/j.jep.2023.116353

Yadav N, Ganie SA, Singh B, Chhillar AK, Yadav SS (2019) Phytochemical constituents and ethnopharmacological properties of *Ageratum conyzoides* L. Phyther Res 33(9):2163–2178. https://doi.org/10.1002/ptr.6405

Zafarullah A, Ilyas S, Naz S, Aslam F, Manzoor F (2013) Effect of culture media and growth regulators on in vitro propagation of *Crysanthemum indicum* L. Pak J Sci 65(4):462–466. https://doi.org/10.1007/978-94-011-3176-6_33

Zaman S, Barkatulllah, Zahoor M, Wadood Ali Shah S, Ullah Z, Ullah R, Alotaibi A (2022) Pharmacognostic evaluation of *Artemisia maritima* L. a highly medicinal specie of genus *Artemisia*. Saudi J Biol Sci 29(10):1–9. https://doi.org/10.1016/j.sjbs.2022.103419

Zhang YX, Wang JH, Bian HW, Zhu MY (2001) Pregrowth-desiccation: a simple and efficient procedure for the cryopreservation of rice (*Oryza sativa* L.) embryogenic suspension cells. Cryo-Letters 22(4):221–228

Zhao M, Zhao Y, Xing F, Bai L (2012) Components from callus cultures of *Sonchus oleraceus* L. and their cytotoxicity in vitro. In: Zhao M, Zhao Y, Xing F, Bai L (eds) Information technology and agricultural engineering. Springer, pp 107–112